Cardiff School of Pharmacy and Pharmaceutical Sciences, Cardiff University

Computer-aided design, synthesis and evaluation of potential anti-HCV agents

A thesis submitted in accordance with the conditions governing candidates for the degree of

Philosophiae Doctor in Cardiff University

Marcella Bassetto

Supervisor: **Dr. Andrea Brancale**

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Abstract

Hepatitis C virus (HCV) is a major cause of chronic liver disease, leading to hepatic steatosis, fibrosis, cirrhosis and hepatocellular carcinoma.

A vaccine is currently not available, while the standard of care is effective in only 50% of treated patients. The first specific anti-HCV drugs have been recently approved, and new classes of targeted agents are under clinical trials/investigation. Nevertheless, improved treatment strategies are needed, in order to bypass the rapid emergence of resistance.

All the viral non-structural proteins are a possible target for the identification of novel and selective antivirals. Among them, the NS3 helicase is still underexploited, with no known inhibitor under pre-clinical or clinical development. This enzyme plays a crucial role in the virus life cycle: it catalyses the separation of double-stranded RNA strands, which is necessary for genome amplification and translation. Due to its essential function, the NS3 helicase was chosen as a target for the identification of new, specific anti-HCV compounds.

Different computer-aided techniques were employed to identify potential small-molecule inhibitors of the enzyme. Two structure-based virtual screenings of commercially available compounds were performed on the main nucleic acid binding site. A series of candidate inhibitors was evaluated in the HCV replicon assay, yielding two primary hits with low μM activity.

Secondly, the model of the one known inhibitor co-crystallised with the enzyme was used as a starting point for a shape-comparison screening of small molecule libraries. A new series of compounds was selected and evaluated for anti-HCV activity, and one of them was found to inhibit the viral replication at a low μM concentration.

Several new derivatives of the initial hits were synthesised, belonging to four main structural families: bis-aromatic piperazine derivatives, symmetrical phenylendiamine compounds, differently substituted thieno-pyrimidines, and triphenyl-pyrrolone analogues.

Inhibition of HCV replication in the replicon assay was evaluated for the new compounds prepared and several structures showed a range of activity from low-μM to nM.

Contents

Chapter 2: Piperazines

Abbreviations and Acronyms

3D	Three-dimensional
Å	Angstrom
ADP	Adenosine diphosphate
Arg	Arginine
Asn	Asparagine
Asp	Aspartic acid
ATP	Adenosine triphosphate
BOC	*tert*-butyloxycarbonyl
CC_{50}	Cytotoxic concentration to observe 50% adverse effect
CDK	Cyclin-dependent kinase
CLDN1	Claudin-1
DCM	Dichloromethane
DIPEA	N,N-Diisopropylethylamine
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
Ds	Double-stranded
EC_{50}	Effective concentration to observe 50% activity
EC_{90}	Effective concentration to observe 90% activity
ER	Endoplasmic reticulum
FDA	Food and Drug Administration
Glu	Glutamic acid
Gly	Glycine
GTP	Guanosine triphosphate
h	Hour
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
Hz	Hertz
kb	Kilobases
IC_{50}	Inhibitory concentration to observe 50% inhibition
IFN	Interferon

IRES	Internal ribosomal entry site
LDL-R	Low density lipoprotein receptor
Lys	Lysine
MOE	Molecular operating environment
MS	Mass Spectroscopy
mRNA	Messenger ribonucleic acid
NMR	Nuclear magnetic resonance
NS	Non-structural protein
OCLN	Occludin
ORF	Open reading frame
PDB	Protein Data Bank
pegIFN	Pegylated interferon
Phe	Phenylalanine
Rb	Retinoblastoma tumour suppressor protein
RdRp	RNA-dependent RNA polymerase
r.t.	Room temperature
Rf	Retention factor
RMSD	Root-mean-square deviation
RNA	Ribonucleic acid
SAR	Structure-activity relationship
SBVS	Structure-Based Virtual Screening
SF2	Super-family 2
SI	Selectivity index
SR-BI	Human scavenger receptor class B type I
Ss	Single-stranded
TBTU	*O*-(Benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium tetrafluoroborate
THF	Tetrahydrofuran
Thr	Threonine
TLC	Thin layer chromatography
Trp	Tryptophan
UTR	Untranslated region
Val	Valine

Chapter 1

Introduction

1.1 Hepatitis C virus

Hepatitis C virus (HCV) is one of the major causes of chronic liver disease, and affects approximately 170 million people worldwide.[1]

This blood-borne infection becomes chronic in 60-85% of patients and leads to the development of hepatic steatosis, fibrosis, cirrhosis and hepatocellular carcinoma.[2-3]

The virus tropism involves both hepatic and lymphatic systems, and chronic infection is therefore associated also to extrahepatic diseases and malignancies such as cryoglobulinemia, lymphoma and thyroid cancer.[4]

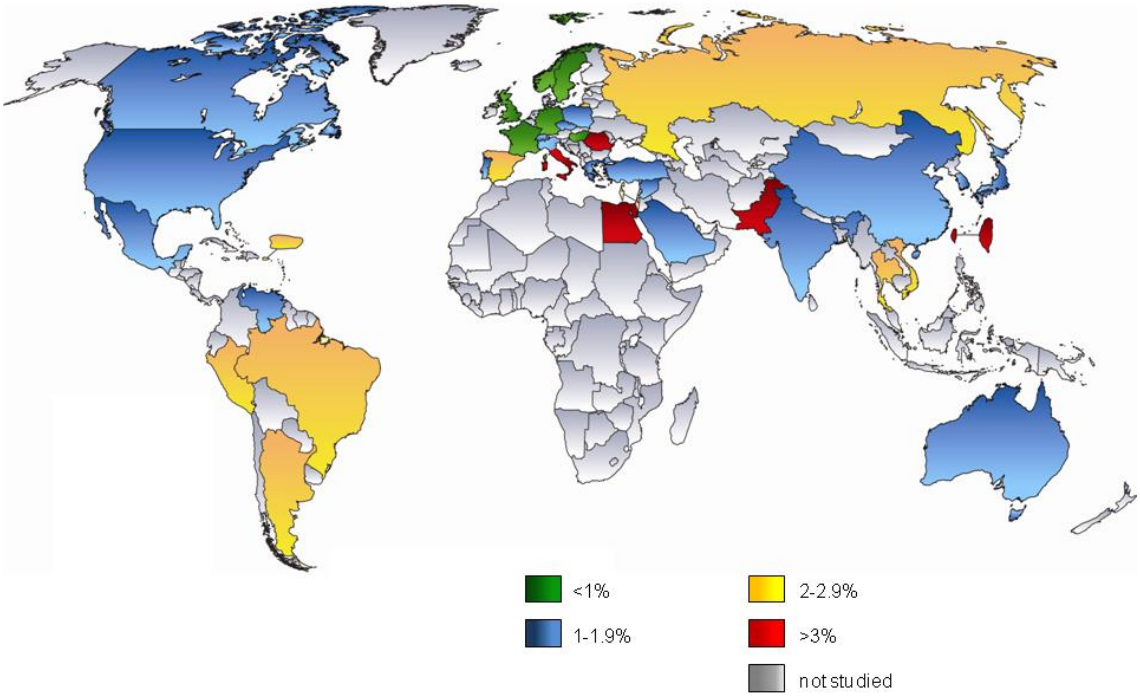


Figure 1.1: HCV prevalence[5]

Patients are generally asymptomatic before development of cirrhosis; the infection is often underdiagnosed, leading people unaware of their disease to spread the virus by body fluids, and causing the loss of treatment opportunities.[4]

HCV modifies the intracellular environment to promote its replication and long-term persistence within the liver. Moreover, it has developed a multi-factorial immune evasion capacity, characterised by disruption of signalling pathways that control innate antiviral responses, evasion of IFN-mediated responses, retardation and impairment of

T-cell responses.[6]

A vaccine against the virus is currently not available and treatment options are still limited. The standard of care is a combination of pegylated interferon (pegIFN) and ribavirin, a therapy that is not specific for HCV and efficient in 50% of treated patients, with many associated side effects.[7] In May 2011 the FDA approved the use of the first two specific HCV NS3/NS4A inhibitors, telaprevir and boceprevir, to treat patients not responding to the standard of care, in association with interferon and ribavirin.[8]

Even if new classes of specific targeted antiviral agents, whose action is mainly directed against the viral non-structural proteins NS5B polymerase and NS3/4A protease, have entered clinical trials, there is still need for improved treatment strategies, which might lead to the cure of all infected patients with an equal effectiveness against all HCV genotypes. A reason for this requirement is the high error rate of the viral RNA-dependent RNA polymerase, which leads to rapid development of drug-resistant viral strains in response to new antiviral administration.[9]

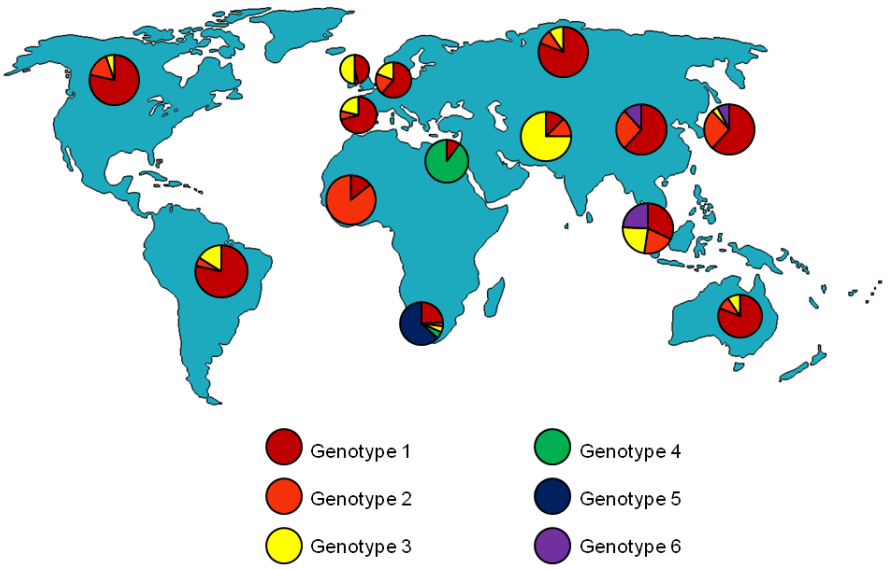


Figure 1.2: HCV genotype distribution

1.1.1 HCV genome organisation

HCV is an enveloped RNA virus that belongs to the *Flaviviridae* family, and is the unique member of the *Hepacivirus* genus.[10] The viral single-stranded, positive-sense, 9.6 kb RNA contains an open reading frame (ORF) that encodes a unique polyprotein precursor of about 3000 residues, and is flanked by an untranslated region (UTR) at

both 5' and 3' termini.[11] The polyprotein is translated under the control of an internal ribosomal entry site (IRES) within the 5'-UTR, and is subsequently cleaved by cellular and viral proteases into four structural proteins (core, E1, E2 and p7) and six non-structural proteins (NS2, NS3, NS4A, NS4B, NS5A and NS5B).[12]

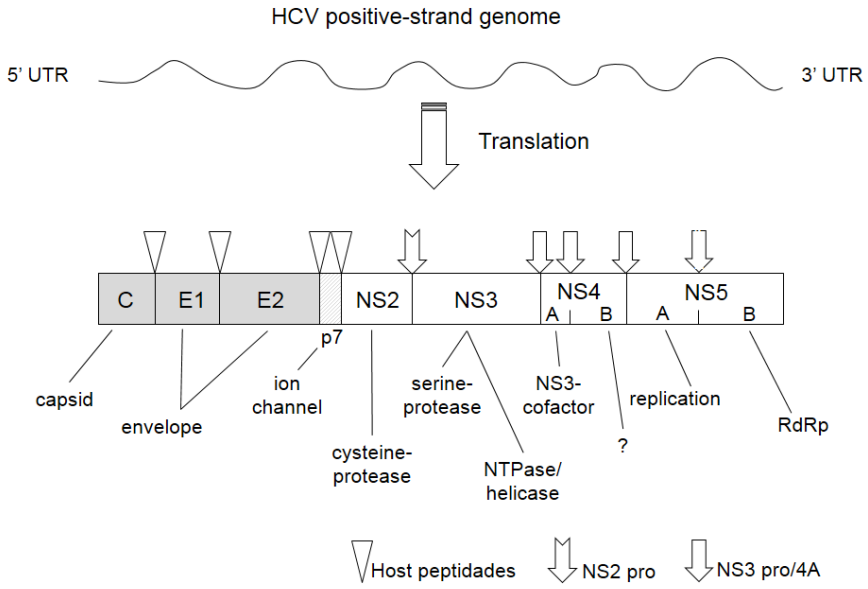


Figure 1.3: Polyprotein precursor encoded in the HCV (+)ssRNA genome[13]

HCV genome is characterised by a high level of genetic variability: it is divided into eleven main genotypes that differ by 31-34% of nucleotide sequences.[14] Moreover, due to the high error rate of the RNA-dependent RNA polymerase (RdRp) function carried by the NS5B protein, HCV is present in a same infected individual as a population of different but closely related variants identified as quasispecies.[9]

1.1.2 Viral proteins

The four structural proteins, cleaved from the N-terminus of the polyproteic precursor, are the core protein, the E1 and E2 envelope proteins, and a small hydrophobic polypeptide, p7. The core protein is believed to form the viral nucleocapsid and shows a high level of conservation among different HCV strains.[15] The E1 and E2 proteins are necessary components for viral entry,[16] while the p7 hydrophobic polypeptide is essential for infectious virions production *in vivo*.[17-18]

The six non-structural proteins (NS) are responsible for polyprotein processing, viral

RNA replication and production of infectious virions; they form a multicomponent RNA replication complex that carries out its functions within virus-induced modified subdomains of the ER, referred to as membranous web.[19]

The NS2 protein is an auto-protease which catalyses the cleavage of the polyproteic precursor on the level of the NS2/NS3.[20]

The NS3-4A complex is a bifunctional molecule; the N-terminal region is a serine protease, non-covalently bound to the NS4A cofactor, which processes the non-structural proteins.[21] The C-terminal portion of NS3 shows a helicase-ATPase domain, whose main function is to unwind double-stranded RNA substrates in the direction 3'-to -5'.[22]

NS4B is responsible for membrane association: its function leads to the formation of a specialised membrane compartment, the membranous web, where viral RNA replication is believed to take place.[19, 23]

NS5A is a phosphoprotein whose function is not completely clear, but it is believed to influence replication efficiency.[24]

The NS5B protein shows a conserved sequence motif that characterises viral RNA-dependent RNA polymerases.[25]

1.1.3 Viral life cycle

Infectious HCV particles circulate in the host serum in association with low-density lipoproteins and very-low-density lipoproteins, forming the so-called lipoviroparticles.[26]

Putative HCV receptors have been identified in CD81, SR-BI, the LDL receptor (LDL-R), claudin-1 (CLDN1) and occludin (OCLN), none of which characterises exclusively liver cells.[27]

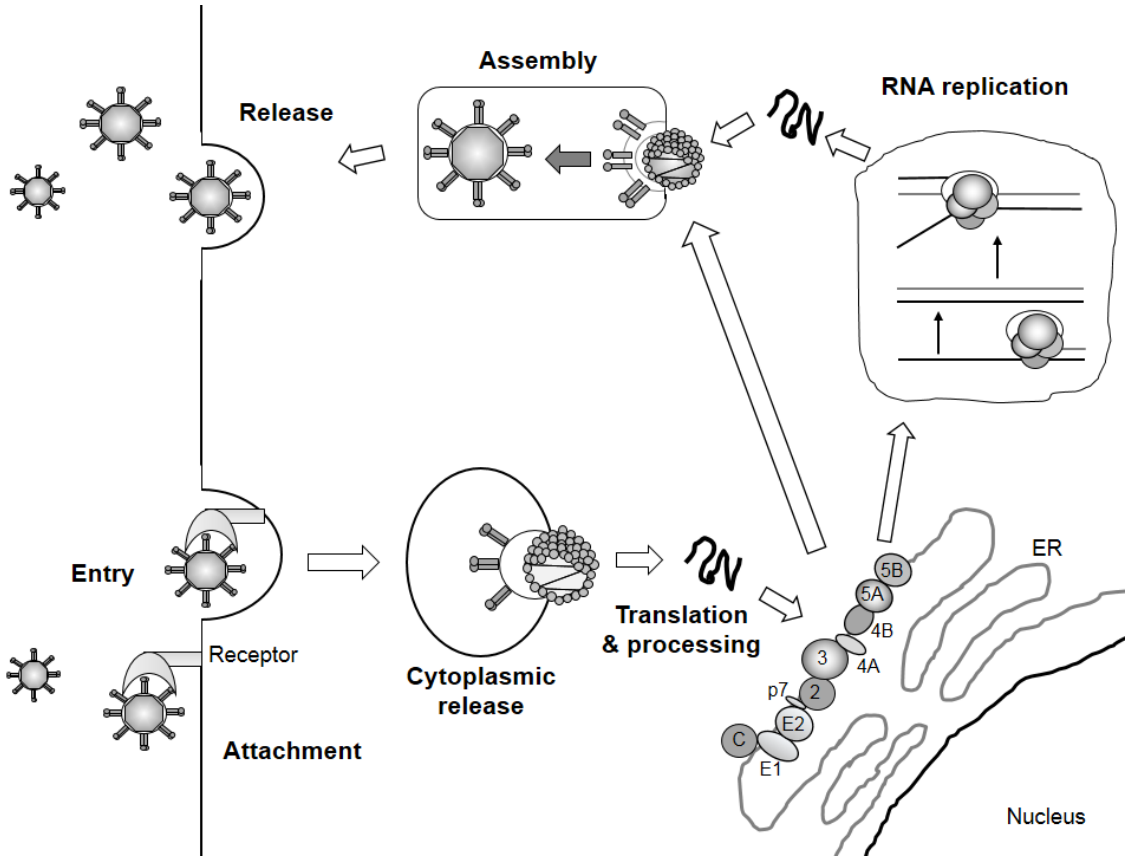


Figure 1.4: HCV life cycle[12]

After receptor-mediated endocytosis, fusion and uncoating events lead to the release of the viral RNA into the host cell cytoplasm. The viral genome undergoes then translation and replication processes by the non-structural protein replication complex.[28-29]

The uncapped viral RNA molecules are translated by a cap-independent IRES-mediated process: the translational product of HCV genome is a large polyprotein precursor processed by cellular and viral proteases into the mature viral proteins.[30]

The structural proteins are processed by host peptidases on the level of the junctions core/E1, E1/E2, E2/p7, p7/NS2.[31] HCV non-structural proteins are processed into their mature forms by two viral proteases: NS2 cystein protease catalyses the cleavage between NS2 and NS3, while the remaining junctions NS3/4A, NS5A/5B, NS4A/4B and NS4B/5B are cleaved by the NS3 serine protease.[32]

HCV genome replication begins with the synthesis of a complementary negative-strand RNA using the positive-strand genomic RNA as a template. The negative-strand RNA is subsequently used as template for the production and amplification of positive-strand RNA. Both these steps are catalysed by the NS5B RdRp.[29]

Mature HCV virions consist of a nucleocapsid surrounded by an outer envelope formed by a lipid membrane and envelope proteins.[33] Nucleocapsid assembly requires oligomerisation of the capsid protein and encapsidation of viral genomic RNA.[34]
Once the nucleocapsid is formed in the cell cytoplasm, it acquires an envelope by budding into the ER lumen, and is released by the Golgi apparatus through exocytosis.[35-37]

1.1.4 Current treatment

The standard of care to treat chronic HCV infection is a combination of pegylated interferon alpha (peg-IFN-α) and ribavirin, both non-specific for HCV. In addition to this treatment, two selective inhibitors of NS3/4A protease, telaprevir and boceprevir, have been approved by the FDA in May 2011 for use in combination with peg-IFN and ribavirin.[8]
Interferon alpha is a natural cytokine produced by leucocytes that allows recognition of viral antigens by the immune system and activates natural killer cells and macrophages. This protein is used as a broad-spectrum antiviral to boost the host immune system against pathogen organisms. Many adverse effects are related to its administration, including depression, autoimmune diseases, nausea, fever, chills, headache, muscle pain, leukopenia and thrombocytopenia.[38-39]
Ribavirin (figure 1.5) is a synthetic nucleoside analogue that exerts activity against a broad spectrum of both RNA and DNA viruses.[40]

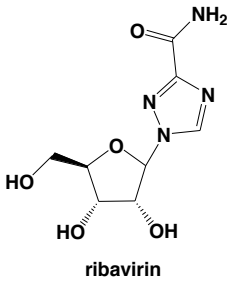


Figure 1.5: Chemical structure of the broad-spectrum antiviral ribavirin

Ribavirin has multiple general mechanisms of action, and in the case of HCV its activity seems to be due to the induction of abnormal viral mutagenesis, that overcomes the threshold of error catastrophe, along with the up-regulation of genes involved in IFN

signalling.[41]

Efficacy of treatment with ribavirin in combination with pegylated interferon alpha is genotype-specific, with genotype 1 and 4 being more resistant than genotypes 2 and 3: viral eradication or a sustained virological response (undetectable HCV RNA six months after the end of treatment) is achieved in 75-85% of cases for genotypes 2 and 3, and in 55% of cases for genotypes 1 and 4.[42] The main adverse effect introduced by the addition of ribavirin to therapy with interferon is haemolytic anaemia.[43]

The significant side effects of the combination treatment, along with its high costs and its limited cross-efficacy against the different viral genotypes, cause the requirement of new, specific anti-HCV agents with a broad spectrum of efficacy across genotypes, reduced adverse effects and unlikeness to develop resistant viral mutants.[44]

Standard treatment has been improved with the approval of the first specific anti-HCV agents, telaprevir (*Incivek*, Vertex)[45] and boceprevir (*Victrelis*, Merck)[46]. They both are NS3/NS4A protease inhibitors, and have been demonstrated to significantly increase a sustained viral response in not-responding patients with genotype 1, in combination with peg-interferon-alpha and ribavirin.

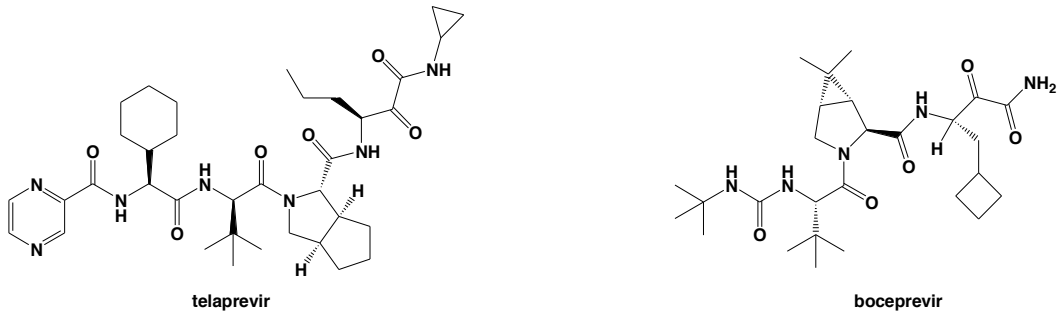


Figure 1.6: Chemical structures of telaprevir and boceprevir

These first-generation direct antiviral agents (DAAs) cannot eradicate HCV infection alone, due to rapid development of resistant viral strains. The two drugs are related to different toxicity profiles: for telaprevir the main associated adverse effect is rash, while boceprevir is related to anaemia, dysgeusia and headache.[47]

1.1.5 New treatments under development

Along with novel types of interferons, several new classes of selective antiviral agents are at different stages of development: the main targets involved are viral NS3/4A protease, NS5B polymerase, binding of NS4B protein to RNA and multifunctional NS5A protein.

Due to the high frequency of mutations that characterises HCV genome and to the fast selection of resistant mutants, a successful therapy with small molecule inhibitors would require the use of a cocktail of antiviral compounds directed against different targets, in order to set a barrier to resistance to multiple separate simultaneous mutations.[48]

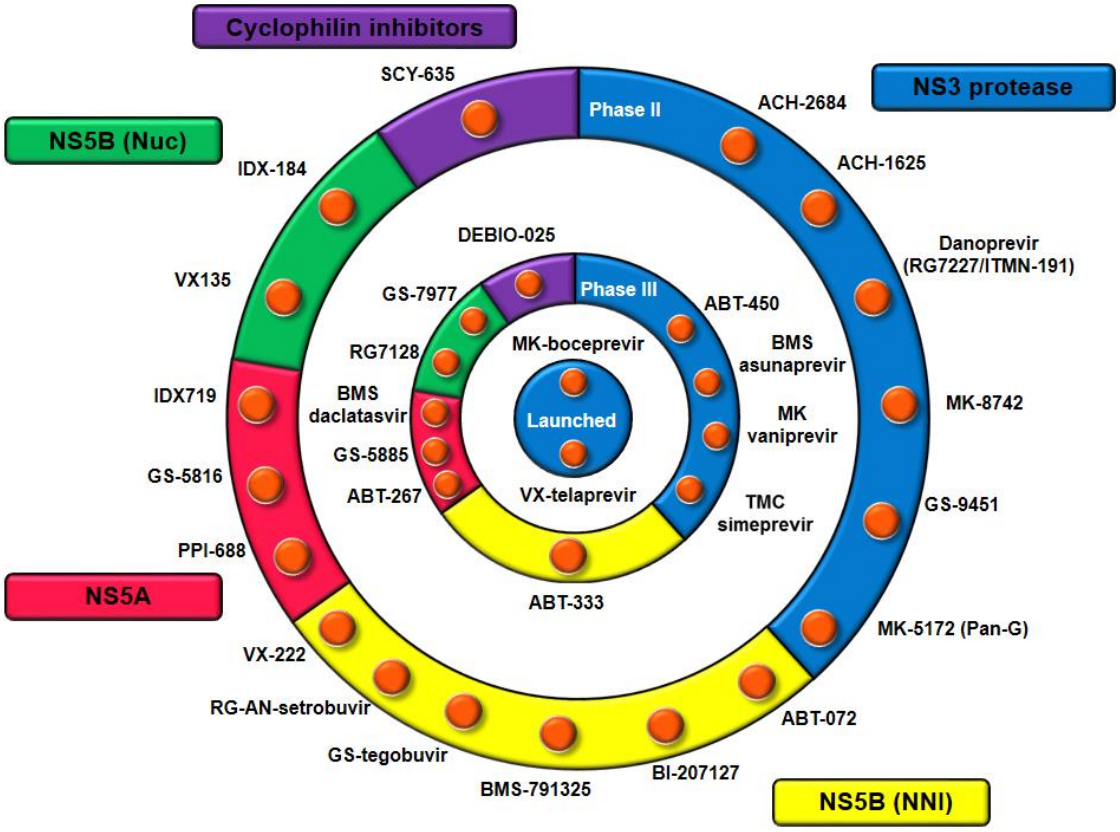


Figure 1.7: HCV inhibitors competitive landscape[5]

NS3/4A protease inhibitors

Several selective inhibitors of NS3/4A protease are at different stages of preclinical and clinical studies: the main candidates in clinical development are summarised in table 1.1.

Name	Company	Phase	IC_{50}(nM)
ACH-2684	Achillion	II	
ABT-450	Abbott/Enanta	III	
ACH-1625 (Sovaprevir)	Achillion	II	
GS-9451	Gilead	II	
RG7227 (Danoprevir)	Intermune/Genentech	II	
MK-5172	Merck	II	
MK-8742	Merck	II	
Vaniprevir (MK-7009)	Merck	III	0.06-1.4
BI-201335	Boehringer Ingelheim	III	
TMC435	Medivir/Tibotec	III	13
BMS-650032 (Asunaprevir)	Bristol-Myers Squibb	III	0.2-0.4

Table 1.1: protease inhibitors in clinical development as of June 2013; IC_{50}, 50% inhibitory concentration in biochemical assays of protease

All protease inhibitors show rapid emergence of viral resistance and significant adverse effects such as severe rash and anaemia, characteristics that make not possible their use as monotherapies.[49]

NS5B polymerase inhibitors

NS5B inhibitors are at various stages of preclinical and clinical trials.[50] These antiviral agents can be classified in nucleosides, targeting the active site of the enzyme, and non-nucleosides, targeting one of the several allosteric sites, and interfering with the enzymatic activity by an induced conformational change. Phase II and III studies include nucleoside and non-nucleoside analogues, as shown in table 1.2.

Name	Class	Company	Phase	Replicon EC_{50} (nM)
MK-3281	NNI	Merck	I	
ABT-333	NNI	Abbott	III	2-7
ANA-598	NNI	Anadys	II	0.5-52
IDX184	Nuc	Idenix	II	
RG7128 (Mericitabine)	Nuc	Genentech	III	610
VX-135 (ALS-2200)	Nuc	Vertex/Alios	II	
GS-7977	Nuc	Gilead	III	
TMC647055	NNI	Tibotec/Janssen	II	

Table 1.2: polymerase inhibitors in clinical development; NNI, non-nucleosides; Nuc, nucleosides

NS4B multifunctional protein inhibitors

The NS4B protein is involved in membranous web formation, interaction with other non-structural proteins in the replication complex, GTP hydrolysis and RNA binding, all essential roles for viral replication.[51-52]

Clemizole hydrochloride, a H1 histamine receptor antagonist widely used as an antipruritic, has been found to inhibit NS4B interaction with the 3' terminus of HCV negative-strand RNA, and has shown a strong synergistic effect *in vitro* with both bocepevir and telaprevir: phase I studies are ongoing, while other small molecule inhibitors are in preclinical development.[53-54]

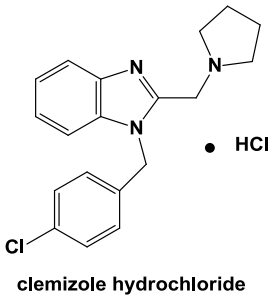


Figure 1.8: Chemical structure of clemizole hydrochloride

NS5A protein inhibitors

Extensive high-throughput screening studies for the identification of HCV replication inhibitors led to the discovery of BMS-790052 (Daclatasvir), which inhibits viral replication at picomolar concentrations and shows a resistance profile addressing its action to NS5A. Synergistic effects were demonstrated with IFN, NS3 inhibitors and NS5B inhibitors, and phase III clinical trials in individuals with genotype 1 infection are now under the way.[55]

Other NS5A inhibitors in clinical development include ABT-267 (Phase III), GS-5885 (Phase III) and PPI688 (Phase II).[56]

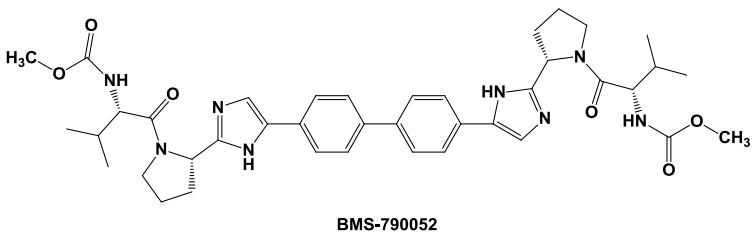
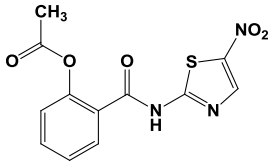


Figure 1.9: Chemical structure of the NS5A inhibitor BMS-790052

Other candidates in preclinical and clinical development

Other therapeutic strategies in preclinical and clinical trials include inhibitors of p7 polypeptide such as amiloride analogues,[57] cyclophilin inhibitors such as cyclosporine A analogues (Alisporivir or Debio 025 is in Phase III, and SCY-635, in Phase II studies),[58] modulators of the host innate immune response like Nitazoxanide (Phase II studies in combination with pegIFN and ribavirin),[59] already approved in the USA for the treatment of specific parasitic gastroenteritides, and modulators of the adaptive immune response.[60]



Nitazoxanide

Figure 1.10: Chemical structure of immune-modulator Nitazoxanide

Approaches in early development

Several new approaches are at early stages of study, including the possibility to rupture HCV virions,[61] interference with host lipid metabolism,[62] inhibition of translation initiation,[63] and the possibility to achieve a therapeutic vaccination.[64]

1.1.6 Alternative strategies

In order to allow oral multitherapies to avoid IFN use and its adverse effects, to have a broad spectrum across genotypes, and to prevent the emergence of viral resistance, targeting different steps of the viral replication with a cocktail of inhibitors is an essential requisite; this goal could be achieved through the exploration of still underexploited viral targets, such as the NS2 protease and the NS3 helicase, which still lack any drug candidate in advanced stages of development.

1.1.7 Experimental techniques

HCV is difficult to grow in laboratory environment and the only non-human animal that the virus can infect is the chimpanzee.[65] The first HCV molecular clones were isolated

from infected blood in 1989,[66] while the first clones to infect chimpanzees were obtained in 1997,[67] and the study of HCV replication in cultured cells, referred to as replicon systems, was possible from 1999.[68] The first replicons used recombinant clones that could associate sequences encoding HCV non-structural proteins with a gene encoding a drug-resistant marker, allowing the selection of cells possessing viral RNA autonomous for its replication.[68] These systems improved with the identification of adaptive viral mutations that allow a more efficient HCV replication in hepatocytes, more permissive cell lines and replicons containing the full-length HCV genome.[69-71]

1.2 HCV NS3 helicase

The NS3 helicase is one of the most underexploited targets in the search for HCV replication inhibitors. The enzyme main actions are duplex RNA separation and displacement of proteins bound to nucleic acids, reactions that are permitted by ATP hydrolysis.[72-74]

This enzyme is formed by three domains and occupies the C-terminal portion of the NS3 protein. It presents multiple ligand-binding regions, the main ones being an ATP binding site in the cleft separating domain 1 from domain 2, a single-stranded nucleic acid binding site at the interface of the three main domains, and a second single-stranded RNA binding site located within the cleft that separates the helicase from the protease portion of NS3.[75]

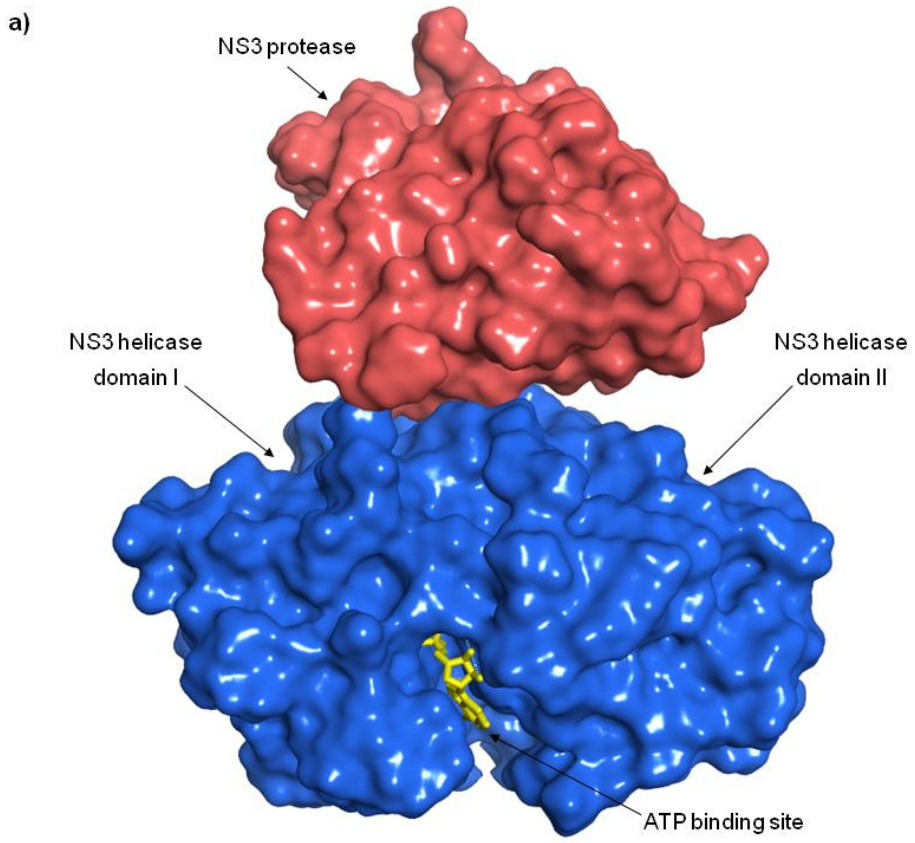


Figure 1.11a: Protease and helicase portions of the NS3 protein

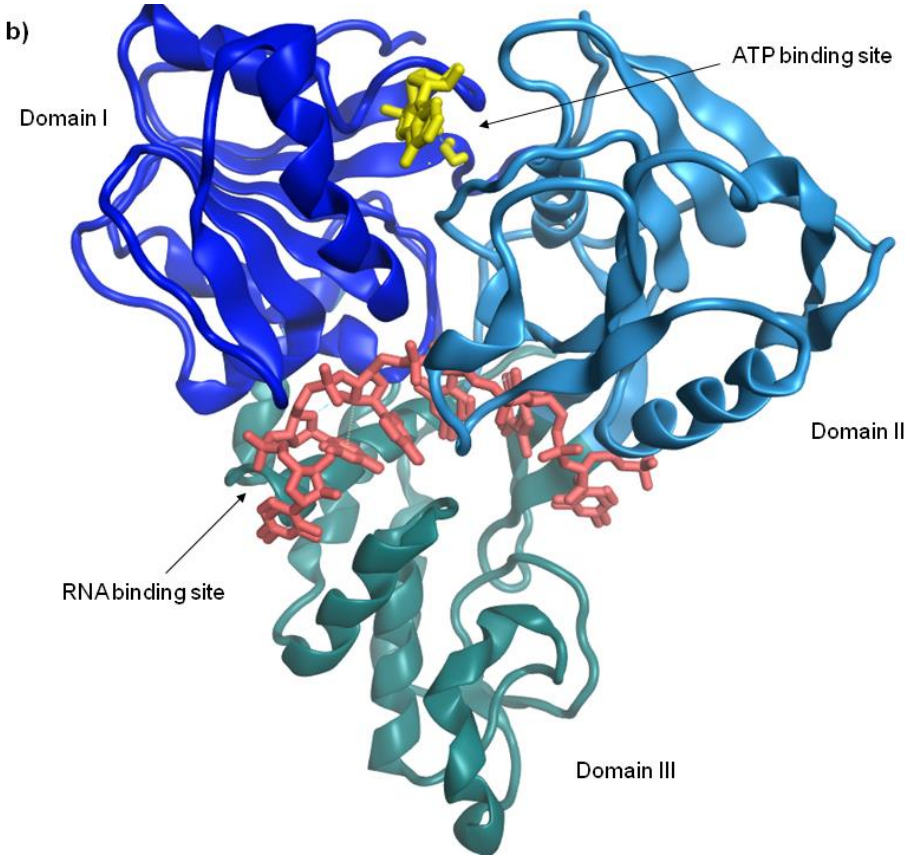


Figure 1.11b: NS3 helicase domains and binding sites

A functional NS3 helicase is essential for viral RNA replication in cells, and HCV with a defective NS3 ATPase function is not infective: evidence validates the NS3 helicase as target to inhibit HCV replication.[76-77]

1.2.1 Roles in HCV replication

For their successful replication, *Flaviviruses* synthesise negative-stranded RNA from genomic positive-stranded RNA, subsequently using the newly-synthesised RNA as a template for the synthesis and the amplification of several copies of positive-stranded RNA genome, which is finally assembled into viral particles. As an intermediate of nucleic acid synthesis, double-stranded RNA sequences are formed between the complementary positive and negative strands, requiring the intervention of the helicase unwinding activity in order to separate RNA strands and terminate viral proliferation, by assisting RNA–dependent RNA replication with a tracking movement along RNA, in correspondence of ss-ds RNA junctions. The unwinding process is unidirectional with

respect to the template strand, moving exclusively in the 3' to 5' direction.[78-79]
By inhibiting the helicase, not only the viral replication cycle could be arrested, but a cellular response against the presence of unnatural double-stranded RNA within the cell could be induced.[80]

1.2.2 Structure, motifs and binding clefts

HCV NS3 helicase is classified as a DExH/D-box motor protein, belonging to the superfamily 2 (SF2) helicases: all enzymes of this family share a nucleic acid remodelling capacity, promoted by ATP hydrolysis, and move in a unidirectional way along a single-stranded nucleic acid, that is identified as the tracking strand.[81-82]
The enzyme has three structural domains, three-dimensionally arranged in the shape of a Y. Domains 1 and 2, known as RecA-like domains, show significant structural similarities with all other helicases, and form a molecular motor that allows the protein to move along nucleic acids. In particular, it is believed that ATP binding and hydrolysis regulate domain 2 conformational changes, that lead to the movement of the protein along RNA.[83]

ATP binding site

The ATP binding cleft has been identified at the interface between the two RecA-like motor domains, 1 and 2. Domain 1 shows a phosphate binding loop known as P-loop, motif I or Walker A motif, and a Mg^{2+} co-factor binding loop, motif II or Walker B motif. ATP is bound to this region via a conserved lysine (Lys210) in the P-loop and an acidic residue (Asp290 in HCV NS3) that interacts with the divalent metal cation cofactor.[84]

Main nucleic acid binding site

The RNA tracking-strand along which the NS3 helicase moves is located on the cleft separating the two RecA-like motor domains from domain 3.[85]
Among the key residues within this site are Val432 and Trp501, which act like bookends between the nucleic acid bases and are critical for unwinding reaction and viral replication.[86] Another important residue is Glu493 (domain 3), that needs protonation for helicase optimal activity: it is conserved in all HCV genotypes, and is characterised by a high pKa value of approximately 7, becoming protonated when the

pH falls to the optimum level for the enzyme, at 6.5.[87] This unusual electrostatic profile is believed to help deriving the driving force for helicase movements along RNA. The residue protonation would help attract the nucleic acid in the binding cleft, while following deprotonation as the pH increases the RNA would be repelled, helping the translocation of the protein along the RNA strand.[87]

The nucleic acid is held in this site by an arginine clamp focused on Arg393, that is proved to rotate in consequence of ATP binding and hydrolysis, allowing the protein to translocate.[88]

1.2.3 Mechanism of action

The structural mechanism by which the NS3 helicase translocates along RNA remains unclear. Nevertheless, the publication of two series of crystal structures where NS3 is bound to a labelled DNA oligonucleotide (PDB IDs 3KQH, 3KQK, 3KQL, 3KQN, 3KQU)[89] and a labelled RNA oligonucleotide (PDB IDs 3O8B, 3O8C, 3O8D and 3O8R),[90] provides insights into the positional changes of nucleic acid and protein during a discrete step of ATP hydrolytic cycle.

ssRNA is bound within the enzyme in an extended conformation, and shows interactions with all three helicase domains. Prior to ATP binding, the enzyme is bound to the nucleic acid substrate in a high RNA-affinity ‘open’ conformation, with five RNA residues bound between the conserved bookends, Val432 and Trp501. Trp501 in particular, which belongs to domain 3, anchors the nucleic acid residue to the protein by a stacking interaction with the base of the last nucleotide at the 3’ end of RNA.

Most specific NS3-RNA interactions consist of hydrogen bonds between polar protein atoms and phosphoryl oxygens of the RNA backbone. These interactions are given by backbone NH groups of protein residues Lys371, Arg393, Val232, Gly255, and the polar side chains of Thr411, Thr416 and Thr 269. The only additional contact between NS3 and a RNA base is a solvent-exposed hydrogen bond between the side chain of Asn556 and the base of the last 5’-terminal residue included in the binding cleft of the ATP-free protein conformation.

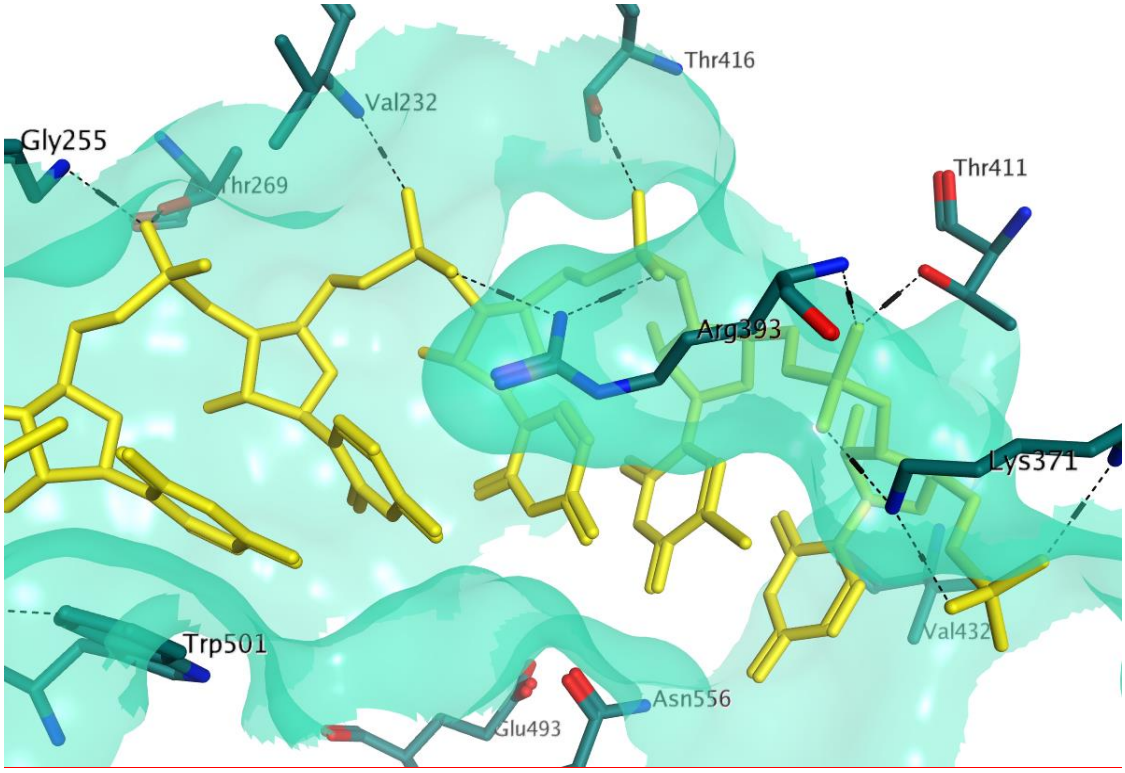


Figure 1.12: RNA binding pocket and interactions within the 3O8C crystal structure

The two series of crystal structures show the three-dimensional conformations of the NS3 helicase in its apo form (3O8B), in complex with an RNA or DNA nucleic acid substrate (3O8C, 3KQH and 3KQK), in a ternary ground-state complex with an oligonucleotide substrate and an ATP-mimicking residue of ADP·BeF_3 (3O8D, 3O8R, 3KQN and 3KQU), and finally in a ternary transition-state complex with a hydrolysed ADP-mimicking residue of ADP·AlF_4^- (3KQL).[89-90]

The ternary complex structures reveal that domain 1 and domain 2 undergo a remarkable conformational change upon ADP·BeF_3 binding, with domain 2 pivoting towards domain 1 in the enzyme ternary complex 'closed' conformation. The number of contacts between NS3 and ssRNA or ssDNA is reduced in the nucleotide-bound state; Thr416 is shifted away from the binding cleft causing the disruption of a hydrogen bond with the phosphodiester backbone of the nucleic acid. The interaction between NS3 and the 5'-end of bound oligonucleotide is weakened, and the ternary complex shows four bases stacked in the nucleic acid binding cleft.[91]

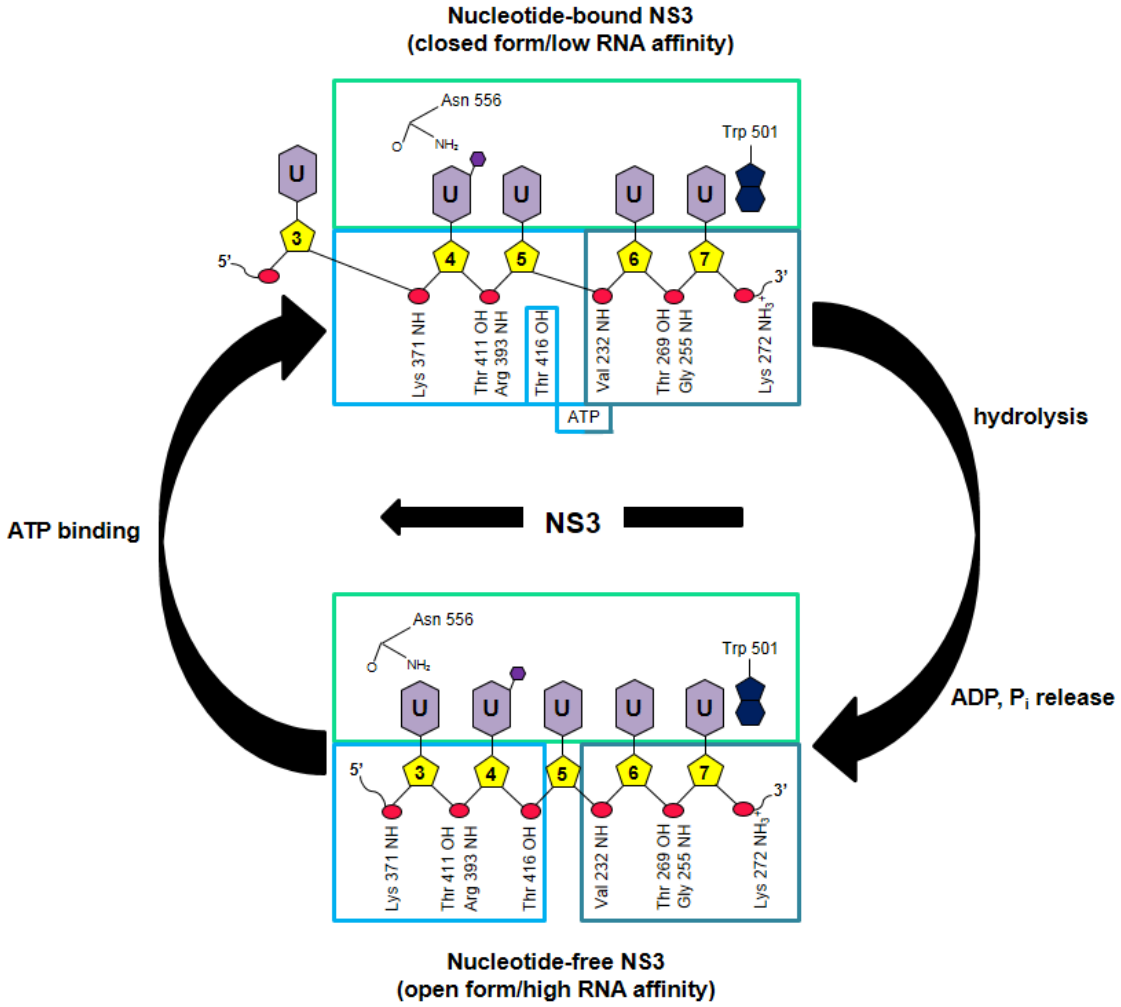


Figure 1.13: ATP-induced changes in RNA binding[90]

Following ATP hydrolysis and ADP release, conformational changes allow the movement of the protein of one base along the oligonucleotide in the direction 3' to 5'. Domains 1 and 2 relax back into the enzyme open conformation, accompanied by the re-exposition of Thr416 to the binding cleft and the restoration of its original interaction, with a shift of one base towards the 5' direction. In its open conformation, the NS3 helicase shows high affinity for the nucleic acid substrate and pulls an additional nucleotide into the binding cleft, resulting in the 3'-5' directed movement of domain 2 along the phosphodiester backbone of the tracking strand. Evidence supports a mechanism of action in which ATPase activity allows the protein to cycle between nucleotide-free high affinity and nucleotide-bound low-affinity conformational states: the enzyme grabs and releases the nucleic acid tracking-strand in correspondence of single-stranded/double-stranded RNA junctions.[92] Moreover, a pair of threonine residues, Thr269 in domain 1 and Thr411 in domain 2, interact with the phosphodiester

backbone in both the nucleotide-bound and the nucleotide-free states, acting as pincers to grip the nucleic acid backbone.[93] Site-directed mutational studies confirm that replacing either Thr269 or Thr411 with alanine binding to nucleic acids is reduced and helicase activity is lost.[94]

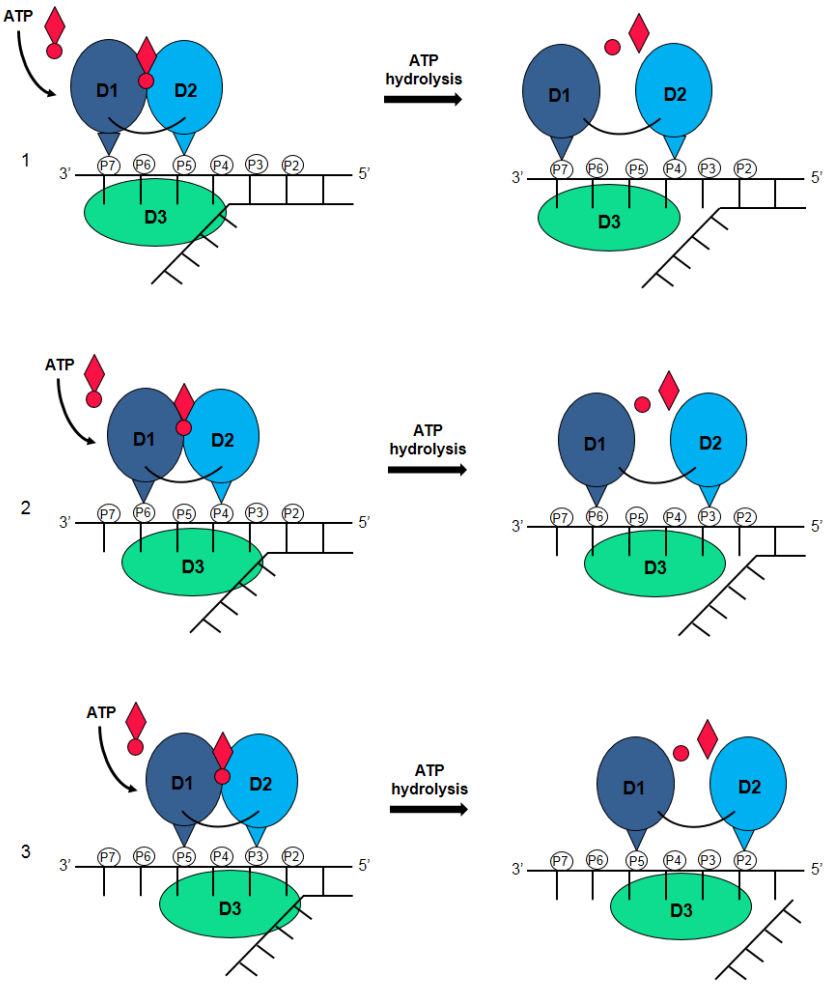


Figure 1.14: Representation of NS3 movements along a RNA tracking strand[102]

1.2.4 HCV helicase inhibitors

Existing helicase inhibitors can be classified into four main categories, on the basis of their mechanism of action: compounds that inhibit helicase activity by interfering with ATP binding, competitive inhibitors of RNA binding, polynucleotide chain intercalators and compounds binding to unknown sites.

Compounds that interfere with ATP binding

To date, only one small molecule has been directly proven to inhibit the helicase by occupying the ATP-binding site: the dye soluble blue HT (compound **1**, figure 1.15), that directly interacts with Lys210 (domain 1), essential for ATP hydrolysis.[95] From its structure, compound **2** was derived: it shows inhibition of helicase activity (IC_{50} 10 μM) and a good inhibition of HCV replication in replicon (EC_{50} 2.7 μM), but it is associated with cytotoxicity (CC_{50} 10 μM).[96]

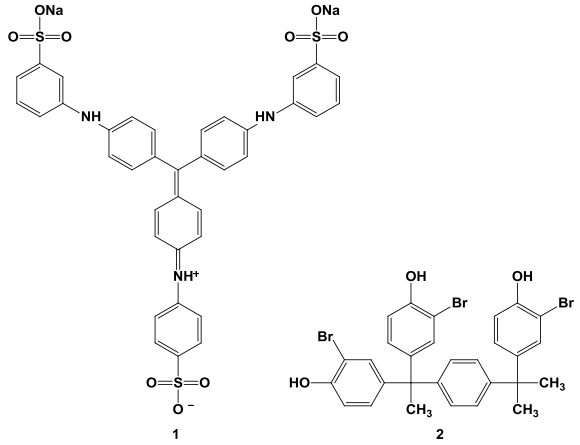


Figure 1.15: Chemical structures of compounds **1** and **2**

Nucleotides and derivatives are believed to competitively occupy the ATP-binding site (figure 1.16): this is the case for ribavirin 5'-triphosphate (RTP, IC_{50} 180 μM) and ribavirin 5'-diphosphate (RDP, IC_{50} 250 μM), that demonstrate good ATPase inhibition but are weak inhibitors of unwinding activity.[97] Other nucleoside or base-analogue inhibitors are dichloro(ribofuranosyl) benzotriazole DRBT (IC_{50} 1.5 μM) and tetrabromo-benzotriazole TBBT (IC_{50} 20 μM), that both inhibit as well HCV RNA replication in cells.[98]

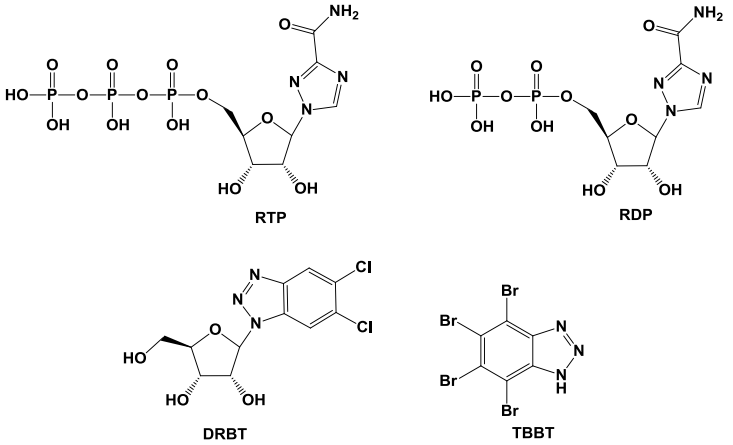


Figure 1.16: Chemical structures of RTP, RDP, DRBT and TBBT

Another category of compounds shown to inhibit HCV helicase-catalysed reactions *in vitro* are ring-expanded 'fat' nucleosides such as compound **3** (IC_{50} 5.5 μM).[99]

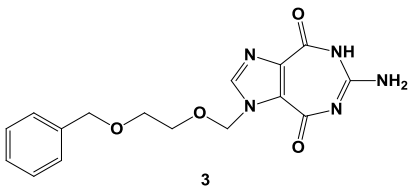


Figure 1.17: Chemical structure of compound **3**

Finally, the natural sesterterpene manoalide, isolated from marine sponge extracts, has been shown to inhibit HCV NS3 helicase activity (IC_{50} 15 μM), by both inhibiting ssRNA binding and ATPase activity (IC_{50} 70 μM).[100]

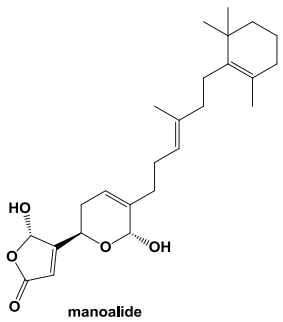


Figure 1.18: Chemical structure of manoalide

Competitive inhibitors of RNA binding

A small number of compounds are known to inhibit HCV helicase by acting on the known RNA binding cleft. Small molecule inhibitors include symmetrical aminophenylbenzimidazole and piperidinylbenzimidazole derivatives such as $(BIP)_2B$ (figure 1.19) and compounds **4-6** (figure 1.20), patented by ViroPharma in 1997, the most active of which being compounds **4** (IC_{50} 0.7 μM) and **5a-e** (IC_{50} 0.7 μM).[101]

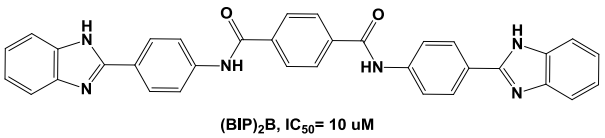


Figure 1.19: Chemical structure and activity of $(BIP)_2B$

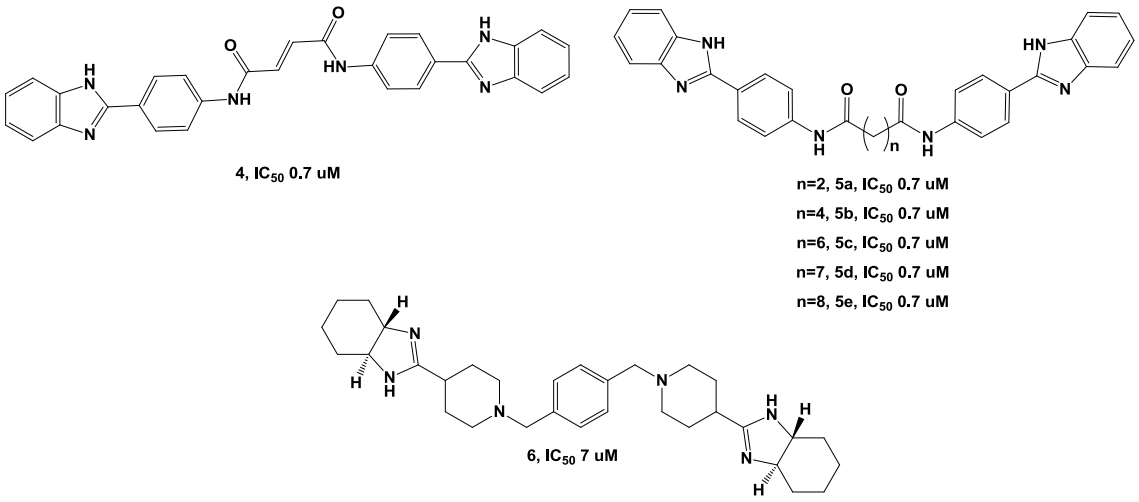


Figure 1.20: Chemical structure and activity of compounds **4-6**

Another series of symmetrical inhibitors has been recently reported, with the most active compounds being DB2 and DB11 (IC_{50} 0.7 μM), both sharing the presence of a dimeric benzimidazole system (figure 1.21).[102]

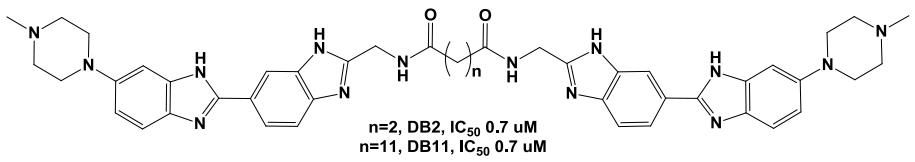


Figure 1.21: Chemical structure and activity of DB2 and DB11

Structure optimisation of the nucleotide-mimicking inhibitor QU663 (IC_{50} 0.75 μM) has led to the identification of more potent derivatives, the most effective in enzymatic assays being compound **7** (IC_{50} 20 nM) (figure 1.22).[103-104]

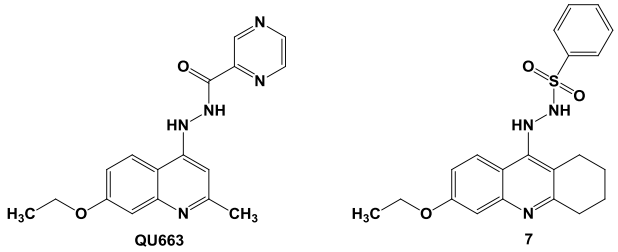


Figure 1.22: Chemical structures of QU663 and compound **7**

Finally, a 14 amino acid-long peptide inhibitor, p14, has proven to inhibit both helicase-catalysed DNA unwinding (IC_{50} 200 nM) and replication of HCV replicons in cells (EC_{50} 83 μM). This peptide shows basic characteristic that could suggest both a potential activity of sequestration of the nucleic acid substrate and binding in correspondence of the P-loop in domain 1, twisting along it and occupying the RNA-binding cleft surrounding Trp501.[105-106]

Compounds that inhibit unwinding by intercalating the polynucleotide chain

DNA and RNA duplexes are stabilised by the presence of a bound or intercalated agent, increasing the energy required for duplex unwinding: as a consequence, intercalating compounds become potential helicase inhibitors.[107]

The two anthracyclines epirubicin and nogalamycin show a good activity profile against HCV helicase unwinding reaction, with IC_{50} values of 0.75 μM and 0.1 μM respectively.[108] Both are associated with a high cytotoxicity and weak penetration into cells, their potential as antiviral agents is limited and the identification of less toxic and more selective derivatives is required.

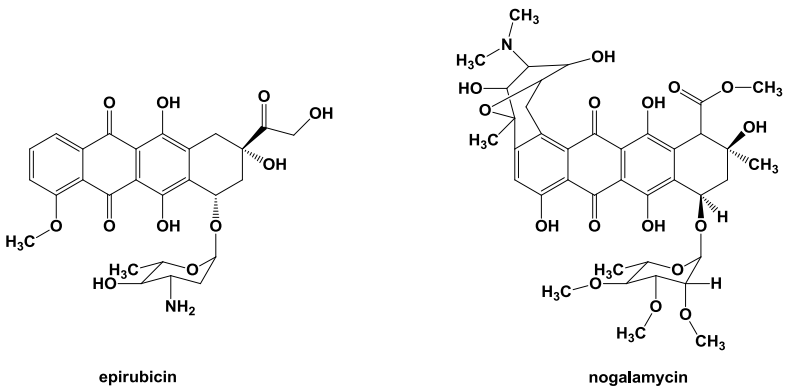


Figure 1.23: Structures of intercalating agents epirubicin and nogalamycin

Compounds binding to unknown sites

A small number of compounds have been found to inhibit HCV helicase activity with a binding mechanism that has not been clarified so far.

This group includes a series of tropolone derivatives, the most potent being 3,7-dibromo-5-morpholinomethyltropolone DBMTr, that shows an IC_{50} value of 17.6 μM and is not related to high cytotoxicity (figure 1.24).[109]

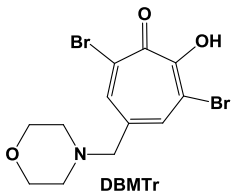


Figure 1.24: Structure of DBMTr

Some acridone compounds have been identified as potential HCV helicase-inhibitors, with two of the most potent derivatives, compounds **8** and **9** in figure 1.25, showing IC_{50} values of respectively 9 and 4 μM. Interestingly, these compounds also inhibit HCV replication in replicon systems, with EC_{50} values of approximately 10 μM, and are not cytotoxic.[110]

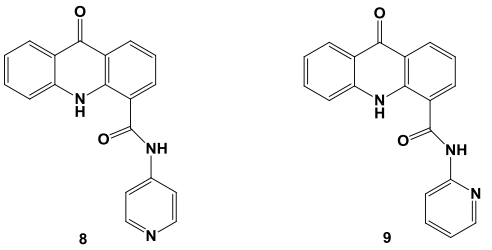


Figure 1.25: Structures of compounds **8** and **9**

Application of a *de novo* drug design approach to the structure of HCV NS3 helicase led to the identification of potent inhibitor compound **10** (figure 1.26), which shows activity in both enzymatic (IC_{50} 0.26 μM) and replicon assay (EC_{50} 9 μM), but is relatively toxic to cells (CC_{50} 30 μM).[111]

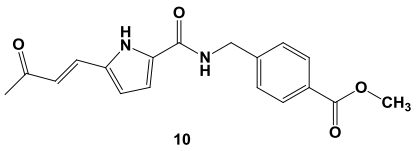


Figure 1.26: Structure of compound **10**

Finally, from the isolation of different components of the yellow dyes thioflavine S and primuline, a new series of compounds was found to show both helicase unwinding and viral replication inhibition potential, with the most active compound being P4 (IC_{50} 0.9 +/- 0.4 μM, ~45% HCV inhibition at 10 μM) (figure 1.27).[112]

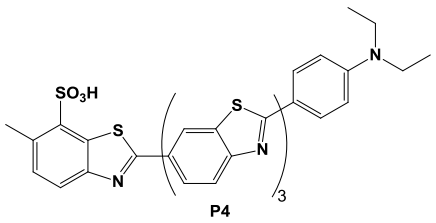
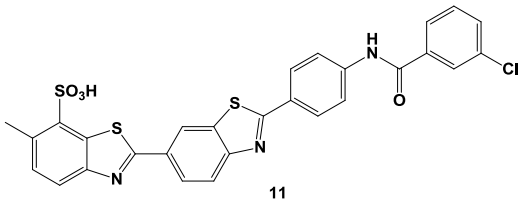


Figure 1.27: Structure of P4

From a first series of synthetically modified analogues of the dyes components, compound **11** was found as the most potent (IC_{50} 2.6+/- 1 μM, 54+/- 10% HCV replication inhibition at 10 μM).[112]



11

Figure 1.28: Structure of compound **11**

1.2.5 HCV helicase assays

A variety of assays are available to monitor HCV-catalysed DNA or RNA unwinding activity, in order to test the ability of small molecules to inhibit this reaction. The most recent and widely used techniques are based on helicase substrates labelled with fluorescent moieties. In a first generation assay, one nucleic acid strand is labelled on the 5'-end with a fuorescent probe annealed to a complementary strand with a quenching molecule at the 3'-end, and the increase of fluorescence as the substrate is incubated with HCV helicase is measured: it will be proportional to the amount of DNA or RNA unwound.[113-114] In a new generation assays, dual-labelled single stranded nucleic acid moieties that can form stem loop structures are used: one end of these moieties, called molecular beacons, is attached to a fluorescent molecule, while the other end is linked to a quencher. Molecular beacons are annealed to a complementary DNA or RNA oligonucleotide to form helicase substrates and, once incubated with the enzyme and upon strand separation, the stem-loop structure, or intramolecular hairpin, is irreversibly formed, and fluorescence is quenched as a consequence. The measured decrease in fluorescence will be proportional to the amount of substrate unwound.[115-116] Despite the abundance of structural information available, only few specific inhibitors of the enzyme have been reported so far: this might be due to the fact that high throughput screenings yield few hits.[116] The reason for such a low success rate could rely on the nature of the assays: the measurement of strand separation upon helicase activity is relatively complex, and inhibitors could act through different mechanisms, which might involve required cofactors not included in the assay. Moreover, the protein conformational changes during the monitoring of its motor action in the assays could interfere with the identification of potential inhibitors. Furthermore, fluorescent compounds or compounds that absorb the light emitted by fluorescent probes can interfere with the assay and give false leads or skipped hit compounds, while the results

of these assays have been proven to be difficult to reproduce.[112, 117]

These limitations in the enzymatic assay could be an explanation for the lack of specific helicase inhibitors identified so far.

1.3 Molecular modelling

Molecular modelling techniques are widely used in pharmaceutical research.
Most drugs are effective due to specific interactions with a biological target: many of them have significant shape complementarity with their binding site, and can form hydrogen bonds with the target, while some targets have hydrophobic pockets where the ligand can place a hydrophobic group with the right size. In a drug discovery programme, the first step is usually to identify hit molecules, and then to produce lead series. Finding novel lead series among chemical compounds can be hard, and it becomes often necessary to identify subsets of potential ligands for a given target with the aid of computational techniques, that can be applied also for lead optimisation and identification of structure-activity relationships among series of compounds. Computer-aided techniques can be used to speed up different steps of drug discovery and optimisation processes.
On the basis of how the system energy is calculated, algorithms used in molecular modelling calculations belong to three main categories: programs to perform *ab initio* quantum mechanics, programs for semi-empirical quantum mechanics and programs for molecular mechanics.[118-119]

1.3.1 Molecular mechanics: empirical force field models

Molecular mechanics or force field methods ignore the electronic motions and calculate the energy of a system as a function of the nuclear positions only; they can be used for calculations on systems containing large numbers of atoms. Their principal limitation is that it is not possible to calculate properties that depend on electronic distribution.[120]
Molecular mechanics can be interpreted in terms of a four-component ensemble of the intra- and inter-molecular forces within the system: energetic penalties associated with the deviation of bonds and angles from their equilibrium values (stretching and bending), energetic changes that follow bond rotations (torsional terms), interactions between non-bonded parts of the system (electrostatic and Van der Waals interactions). To these four basic elements, additional terms can be added in more sophisticated elaborations; force fields used in molecular modelling are mainly designed to reproduce certain structural properties and are therefore parameterised accordingly.[121]

These methods are empirical: there is not an absolute correct form for them, and many share a similar structure that usually tends to conform to the interactions present in a system, and is often the result of a compromise between accuracy and computational efficiency.

A common concept to most force fields is atom type: the atom type for each atom in the system is necessary to be assigned, and it contains not only the atomic number but also its hybridisation state and sometimes the local environment. Often atom types reflect the neighbouring environment and can be extensive for some atoms, making the force field they are included suitable for different categories of molecules: for example, MMFF94 is a force field widely used for small molecules,[122] while AMBER force fields are commonly used for proteins and nucleic acids. [123]

1.3.2 Energy minimisation

Except for the simplest systems, potential energy tends to be a complicated multidimensional function of the system coordinates. The way in which energy varies with the coordinates of the atoms in a system is usually referred to as potential energy surface.[124] In molecular modelling, the interest is mainly in minimum points of the energy surface: minimum energy arrangements of atoms correspond to stable states of the system, and any movement away from a minimum gives a configuration with a higher energy level.

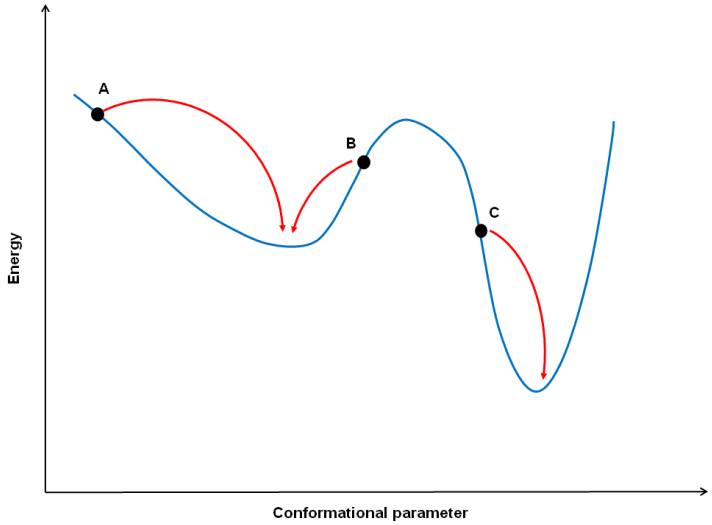


Figure 1.29: Example of one-dimension energy surface. Minimisation algorithms move downhill to the closest minimum

In order to identify those geometries of a system that correspond to minimum points on the energy surface, minimisation algorithms are commonly used: such methods go downhill on the energy surface to locate the nearest minimum for a given system.[125] These analyses are almost always used in molecular modelling and related techniques like conformational search procedures, to prepare a system for other types of calculation, in order to identify and avoid any unfavourable interaction in the system initial configuration.

1.3.3 Conformational analysis

Physical-chemical and biological properties of a molecule can dramatically depend on the three-dimensional structures, or conformations, that it can adopt: a conformational analysis is the evaluation of the conformations of a molecule and their influence on its properties.[126] The different conformations of a molecule are identified as the arrangements of its atoms that can be interconverted by rotation about single bonds. The purpose of a conformational analysis is to identify the preferred conformations of a molecule, which determine its chemical and biological behaviour. These conformations usually correspond to minimum points on the energy surface: energy minimisation plays therefore an important part in conformational analysis.

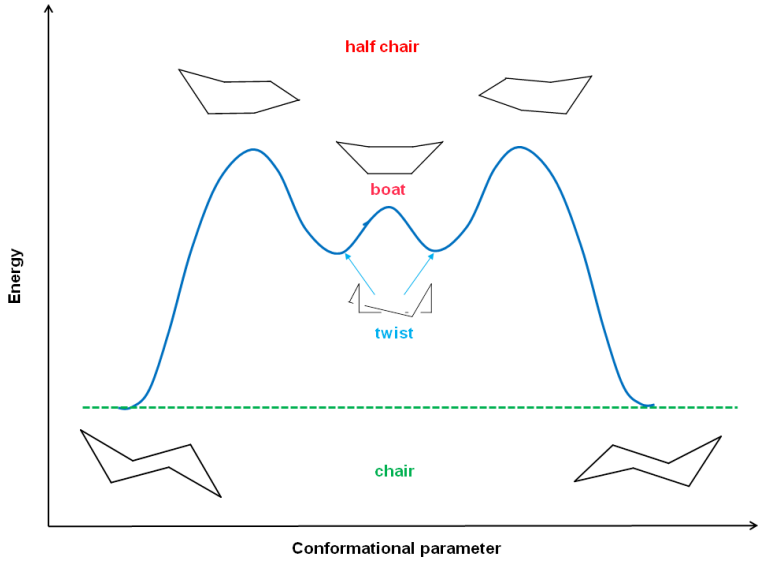


Figure 1.30: Potential energy diagram for cyclohexane conformations

1.3.4 Database searching

Substructure searching is the simplest way to identify compounds of interest. Many databases of chemical compounds can be used as a research source, and some of them are of public access. A quick way to find all the molecules in a database that contain specific required features is a two-dimensional substructure search.[127] Nevertheless, biological targets recognise three-dimensional stereoelectronic features of a molecule, and to search for compounds that satisfy the chemical and geometrical requirements of a given target a three-dimensional database searching method is required. One method that can be used also when detailed information about the target structure is not available is a pharmacophoric model, which indicates the key characteristics of a set of active molecules.[128]

1.3.5 3D Pharmacophores and conformational databases

Typical features of a pharmacophore are hydrogen-bond donors and acceptors, positively and negatively charged groups, and hydrophobic regions, which can be all referred to as pharmacophoric groups: they can be seen as an extension of the concept of bioisosteres. A three-dimensional pharmacophore embodies the spatial relationship between these groups, generally expressed in terms of distance ranges, angles and planes.[128]

Once a pharmacophore has been developed, it can be used to find other active molecules. The key point to create a good pharmacophoric model is to deduce which features are required for activity, and to determine conformations in which pharmacophoric groups are in the same relative position. Pharmacophores are often expressed in terms of location constraints, which are points in the space surrounded by a spherical region: a molecule must be able to place the relevant features within the appropriate spheres. The presence of the receptor can also be included in terms of exclusion spheres, which indicate regions where the ligand is not permitted to position any of its parts.

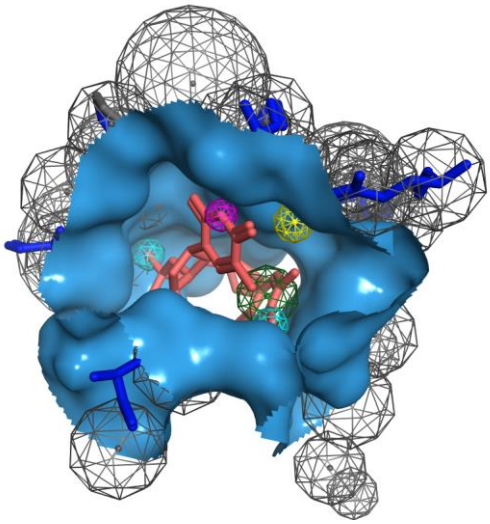


Figure 1.31: Example of pharmacophoric model based on the 3KQN crystal structure

In a three-dimensional database search the three-dimensional structures of the molecules to screen need to be considered: for most of them a well-defined crystal structure is not available, therefore structure generation programs are often used to perform a conformational analysis, in order to find low-energy conformations for the compounds to analyse. In order to take conformational flexibility into account during a database search, many conformations, not only the lowest-energy one, are usually stored for each input structure.

1.3.6 Molecular docking

In the search for new drug-like entities, it is often useful to have a prediction of the potential binding mode of a set of chemical compounds to the biological target of interest. The aim of molecular docking is to predict the structure of the intermolecular complex formed between two or more molecules, thus suggesting the binding modes of ligands to the biological target. Docking algorithms usually generate a large number of possible conformations, each of which needs to be scored in order to identify the most interesting ones. The docking problem is the generation and evaluation of potential structures for intermolecular complexes. It involves many degrees of freedom: six degrees of translational and rotational freedom of one molecule relative to the other, and the conformational degree of freedom of each molecule itself.[129]

Most methods to perform conformationally flexible docking include the conformational degrees of freedom of the ligand only, while the receptor is assumed to be rigid.

In order to take into account both ligand and receptor conformational space, the most common solution is to perform a molecular dynamics simulation of the ligand-receptor complex. Nevertheless, such calculations are computationally highly demanding, and are practically useful only to refine structures produced by other docking methods, since they do not explore the range of possible binding modes, except for very small and mobile ligands.

1.3.7 Scoring functions

Most docking algorithms generate a large number of possible solutions: some of these can be immediately rejected because of high-energy clashes with the protein, but the remaining ones have to be assessed with mathematical functions, the scoring functions, used to identify the docked orientation that corresponds to the most probable structure of an intermolecular complex. When docking a database of compounds, the scoring function should not only identify the best docking mode of a given ligand, but also allow the ranking of one ligand relative to another.

Many scoring functions approximate the binding free energy for the ligand to the receptor, dividing it in different components that reflect the various contributions to binding:[130]

$$\Delta G_{bind} = \Delta G_{solvent} + \Delta G_{conf} + \Delta G_{int} + \Delta G_{rot} + \Delta G_{t/r} + \Delta G_{vib}$$

$\Delta G_{solvent}$ is the contribution due to solvent effects, ΔG_{conf} arises from conformational changes in the protein and the ligand, ΔG_{int} is the free energy variation due to specific protein-ligand interactions, ΔG_{rot} is the free energy loss associated with freezing internal rotations of the protein and the ligand, most due to entropic contribution, $\Delta G_{t/r}$ corresponds to the loss in translational and rotational free energy due to the association of the two structures to give a single body, and ΔG_{vib} is the free energy variation due to changes in vibrational modes.

Many scoring functions also consider the relationship between binding free energy and a series of parameters like hydrogen bonding, ionic and lipophilic interactions, introducing also specific penalty scores that account for large deviations from the ideal geometry of each type of interaction. These parameters are derived from multiple linear regression analyses of experimental binding data on protein-ligand complexes. One

limitation is that they are derived from ligands that bind very tightly to their receptors, while docking is usually used to identify ligands of modest potency from a large database. For this and other reasons, comparing the results of different scoring functions shows better results than the use of a single scoring function: this approach is referred to as consensus scoring.[131, 132]

1.3.8 Drug discovery strategies

When facing a new biological target or searching for a better treatment for a certain pathology, molecular modelling techniques can provide a variety of useful tools to obtain hit compounds and lead molecules.

Two different approaches can be adopted: attention can be focused on the molecular target, if known, or on the chemical structure of known ligands.

If the starting point is the biological target, the first question to be asked is whether its crystal structure has been experimentally determined or not. If the target structure is available, a structure-based virtual screening or a structure-based drug design study can be performed.[133]

Structure-based virtual screenings (SBVS) usually start with a database search, in order to find already known compounds that can fit the binding site or show particular features for binding to the protein. This initial phase is often followed by molecular docking analyses, which allow to evaluate the binding free energy associated with each ligand-target complex. The same approach can be applied if a crystal structure is not available for the given target, starting from its amino acid sequence and creating a homology model on the basis of the 3D structures of homologous proteins. A different strategy is to search for new structures to interact within the target binding site: in this case, a structure-based *de novo* design (SBDD, structure-based drug design) method can be used.

If the structure of one or more active ligands is known, a ligand-based drug design or a ligand-based virtual screening study can be performed. In ligand-based drug design the starting point is the ligand structure(s), from which new compounds are derived on the basis of similarity and pharmacophore concepts, or of QSAR, quantitative structure-activity relationship, analyses.[134]

In ligand-based virtual screening the structures of known ligands are taken as the starting point to perform database searching such as 2D similarity and pharmacophore

matching. Virtual screening studies based on the structure of known active ligands are proven to give better results, in terms of activity, than screenings based on the target structure.[135]

Once a series of hit compounds has been determined, the next step is lead optimisation, whose aim is to find chemical modifications to the hit structures that can improve their activity or chemical-physical properties.[135] This study can be performed on the basis of the target structure, in structure-based lead optimisation, or on the basis of the ligand structure, in ligand-based lead optimisation.

1.4 Project aims

Due to the essential role of a functional NS3 helicase for HCV replication, and the lack of selective inhibitors under development as drug candidates, this enzyme was chosen as a target for the identification of viral replication inhibitors, through the potential interference with its biological activity on the level of the known RNA binding cleft.

This purpose will be pursued with the application of different molecular modelling techniques to the study of the enzyme and its known inhibitors structures, in order to identify and synthesise small molecule compounds as potential antiviral agents.

All selected compounds will be evaluated for their activity against the viral replication in HCV replicon assays, and their biological evaluation will be considered to direct further studies.

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Chapter 2

Piperazines

2.1 Structure-based Virtual Screening

Given the abundance of crystal structure information available for the HCV NS3 helicase, different structure-based *in silico* evaluations were performed to identify potential inhibitors of this enzyme.

A recent series of HCV NS3 helicase structures in complex with a single-stranded DNA substrate was used for all the studies carried out.[1] Among the five ssDNA-NS3 helicase complexes, two were chosen for their high resolution and their conformational characteristics, representative respectively of the low-affinity closed conformation (PDB ID 3KQN) and the high-affinity open conformation of the complex (PDB ID 3KQH).[1]

The target site selected was the known nucleic acid binding cleft, in order to inhibit natural substrate binding. Due to the essential role demonstrated for Thr269, Arg393, Thr411 and Trp501, the aim was to identify compounds likely to bind the region including these residues, situated approximately at the interface between the enzyme main domains 1, 2 and 3.[2]

The highest-resolution crystal structure of the NS3 helicase complex with a ssDNA substrate in the closed conformation corresponds to PDB ID 3KQN (2.05 Å). An ATP-mimicking residue, ADP-Be·F_3, is present in the ATP binding pocket, domain 2 is shifted towards domain 1 and four nucleotide residues are spanned between the two bookends, Val432 and Trp501. In this low-affinity conformation of the enzyme the three main domains are close together, and the target residues are all located within a narrow space. In particular, a possible interaction with both Trp501 (domain 3) and Arg393 (domain 2) was evaluated in the search for inhibitors, to ideally block the enzyme in the closed conformation.

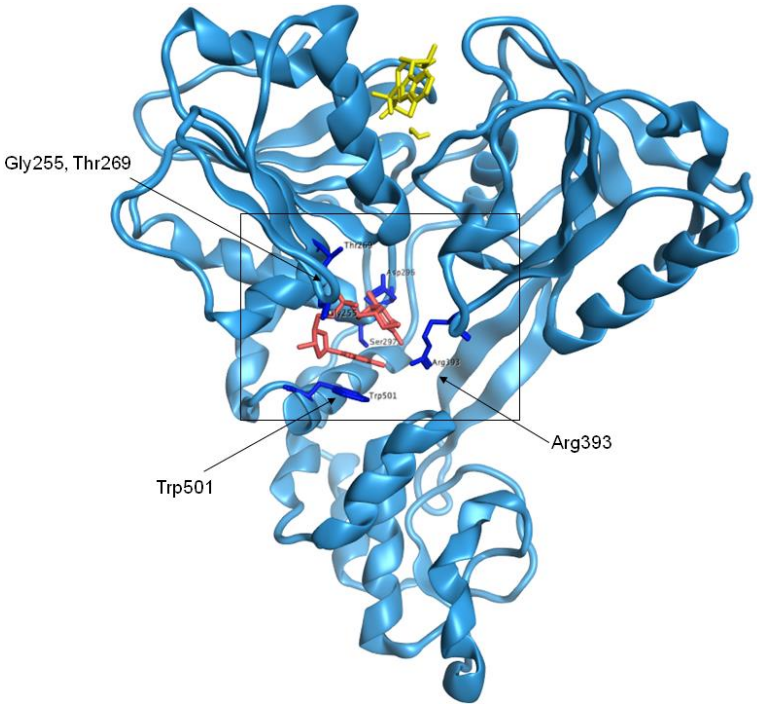


Figure 2.1: 3KQN crystal structure

2.1.1 Database conformational search and pharmacophoric filter

The site defined by the position of Trp501 and Arg393 in the 3KQN crystal structure was used to perform a structure-based virtual screening (SBVS) of small molecule libraries: the SPECS database was chosen for this search,[3] and pre-screened with a pharmacophoric filter. The approximately 450000 structures available were downloaded and analysed with MOE 2010.10 conformational search tool; 500 low-energy conformations were kept for each input molecule.[4]

The main interactions between the target residues and the co-crystallised substrate were considered to build the pharmacophoric query, and the selection was restricted to five features. A hydrophobic/aromatic group to interact with Trp501 (F1:Hyd|Aro in figure 2.2) and a hydrogen-bond acceptor or anion group to interact with Gly255 and Thr269 (F2:Acc|Ani) were kept as essential elements, while a H-bond donor to target Asp296 (F3:Don), a H-bond donor and acceptor to interact with Thr298 and Ser297 (F4:Acc&Don) and a hydrogen-bond acceptor to target Arg393 (F5:Acc) were added as alternative features, requiring a partial match of four elements.

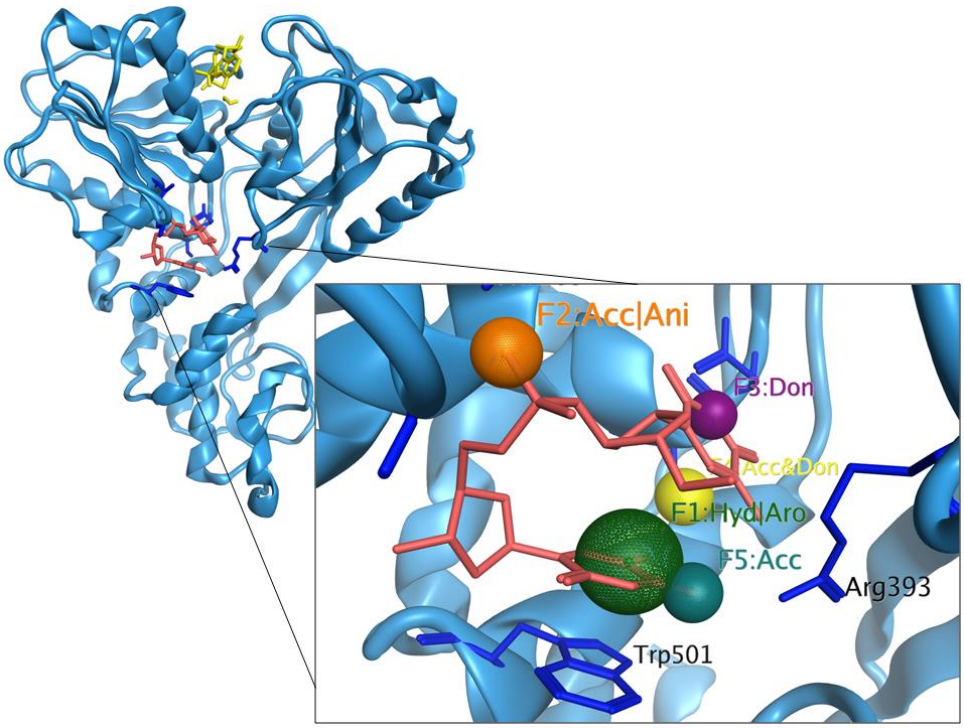


Figure 2.2: 3KQN-based pharmacophoric model

Exclusion volumes corresponding to the protein occupational space were added, and the model was used to screen the conformational database of the SPECS compounds, obtaining approximately 3000 molecules matching the search criteria.

2.1.2 Molecular docking and consensus scoring

The 3000 hit structures were evaluated within the RNA binding site with a docking analysis, to predict which of them could best fit the target pocket. Three different docking programs were used in parallel; they were chosen for their validated ability to predict binding capacities of different compounds against different target structures:[5] Glide,[6] FlexX[7] and Plants.[8] One conformational database of docked poses was obtained for each algorithm, while the next step was the evaluation of the docking results to select those structures assessed as the best to bind the target pocket.

Each docking program evaluates the output poses by applying its own scoring function, assigning an internal score to each generated conformation. Nevertheless, the use of a single scoring function is not recommended in virtual screening,[9] since for their own definition different scoring functions typically give different importance, for the binding free energy calculation, to diverse aspects, that may or may not be the most significant

to evaluate the effective binding potential of a specific compound. To overcome these limitations, a combination of multiple scoring functions was used to evaluate the docking results.[10] The three docking outputs were re-scored with the scoring functions implemented in each docking program: Glide XP, Plants ChemPLP and FlexX scoring function, all belonging to the most widely used and best validated scoring algorithms.[11-12]

Prior to the application of the rescoring procedure, all docking solutions were minimised within the RNA binding site of the 3KQN structure: the Glide Refinement tool was used to perform such conformational smoothing. Once refined, the three conformational databases deriving from docking were rescored with the three scoring functions, and the results belonging to a same docking output were combined together for each docked pose. A consensus scoring function was elaborated to equally give the same importance to the three scoring algorithms, and to select the structures belonging to the best 25% according to all functions at the same time (Chapter 6.1).

The selected poses were visually inspected in the context of the 3KQN target site in MOE 2010.10,[4] in order to identify a small number of candidates to purchase and test against HCV replication in the replicon assay.[13] The main criterion that guided the identification of potential NS3 inhibitors was the interaction with Trp501 and Arg393, and with the surrounding residues important for nucleic acid binding.

A final selection of fourteen molecules was made (Appendix I). Among them, compound **12** (figure 2.3) showed a good activity profile, without relevant toxicity against cells: its EC_{50} value for HCV replication inhibition was 8 μM, with a CC_{50} greater than 90 μM. Due to these results, its structure was chosen as the starting point for the synthesis of a series of new derivatives.

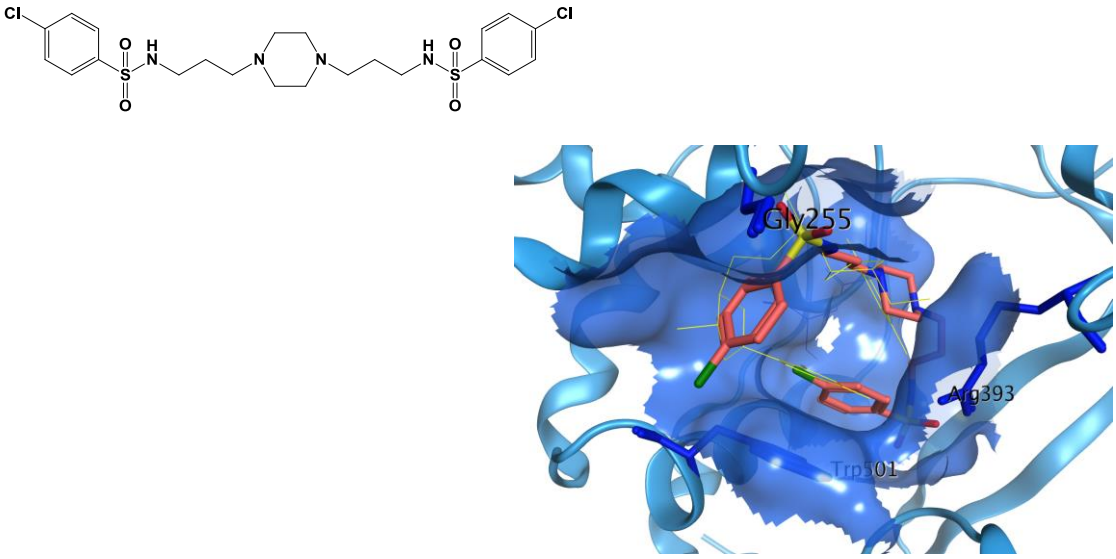


Figure 2.3: structure and predicted binding mode for compound **12**

2.2 Synthesis of symmetrical piperazines

Compound **12** has a symmetrical structure, with a central piperazine ring and two aliphatic linkers connected by a sulfonamide group to two equal aromatic systems (figure 2.4).

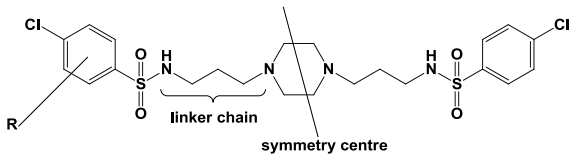
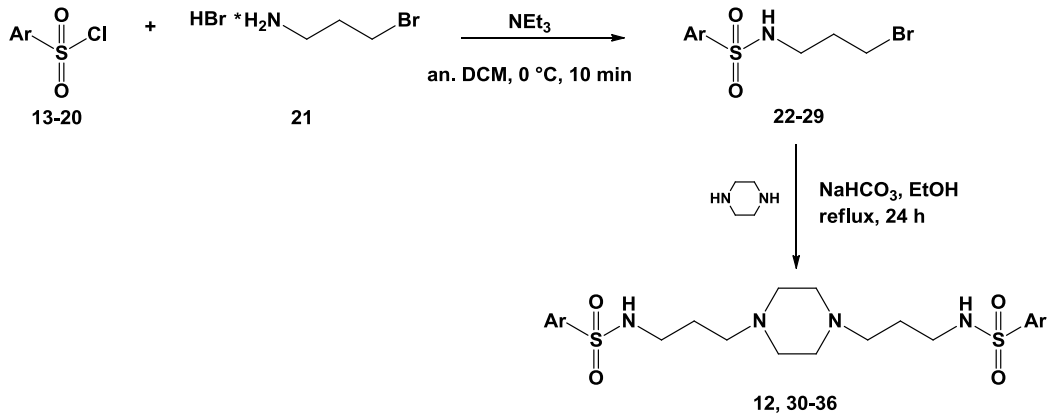


Figure 2.4: Structure and features of compound **12**

The first modifications designed included different aromatic substitutions, linker chain variations and disruption of the molecule symmetry.

2.2.1 *N,N'*-(3,3'-(Piperazine-1,4-*diyl*)bis(propane-3,1-*diyl*))diaryl sulfonamides (12, 30-36)

A first series of new derivatives was designed to explore the effect of the aromatic substituent on the original structure, by varying the substitutions on the phenyl rings while keeping the central piperazine nucleus and the three-carbon saturated linker.
Some commercially available aromatic sulfonyl chlorides (**13-20**) were chosen as starting materials to obtain piperazine derivatives **12**, **30-36** according to a two-step synthetic pathway (scheme 2.1).



Starting Sulfonyl Chloride	Ar	Bromide intermediate	Yield %	Product	Yield %
13	4-Chlorophenyl	**22**	94	**12**	64
14	Phenyl	**23**	97	**30**	64
15	4-Methylphenyl	**24**	89	**31**	68
16	4-Methoxyphenyl	**25**	86	**32**	60
17	4-Nitrophenyl	**26**	73	**33**	43
18	3,4-Dimethoxyphenyl	**27**	61	**34**	65
19	2,5-Dimethoxyphenyl	**28**	70	**35**	71
20	2-Naphthyl	**29**	89	**36**	79

Scheme 2.1: Pathway for the synthesis of compounds **12**, **30-36**

Synthesis of *N*-(3-bromopropyl)arylsulfonamides (22-29)

Intermediates **22-29** were obtained with a nucleophilic displacement by the amine group of 3-bromopropylamine on the electrophilic site of the sulfonylchloride.[14] In order to avoid a self-reaction between two molecules of the nucleophile, the amine group of 3-bromopropylamine hydrobromide (**21**) was slowly released by the dropwise addition of the base, triethylamine, at 0°C, to favour the reaction towards the sulfonylchloride.

Synthesis of *N,N'*-(3,3'-(piperazine-1,4-di*yl*)bis(propane-3,1-di*yl*))diaryl sulfonamides (12, 30-36)

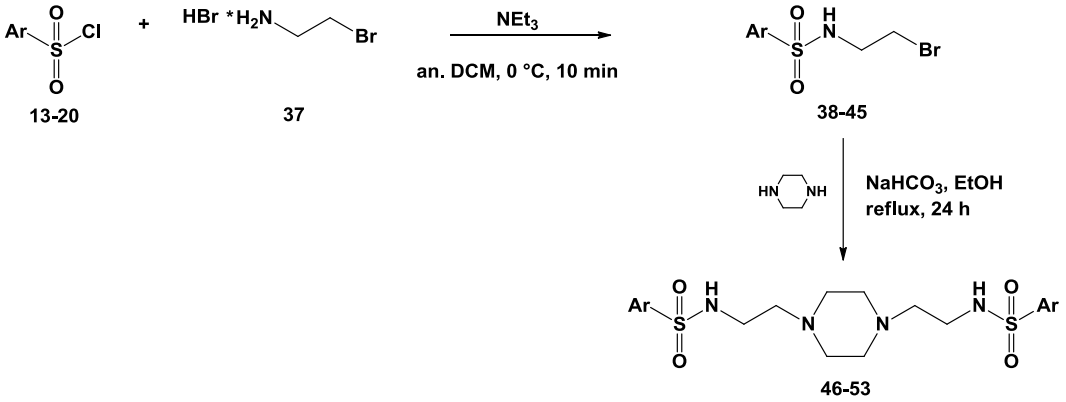
Compound **12** and its derivatives **30-36** were obtained with a second nucleophilic substitution, with the displacement of the bromide leaving group of intermediates **22-29** by the two secondary amine groups of piperazine, thus obtaining the final symmetrical derivatives.

Two good methods in terms of yield (approximately 60% after purification) were identified for the synthesis of compound **12**: the first consisted of stirring the two

reagents with triethylamine in THF for 48 h,[15] the second using $NaHCO_3$/ethanol as base/solvent system, and refluxing the reaction mixture for 24 h.[16] Due to the shorter time required by the second procedure, it was generally adopted for the preparation of all remaining compounds of this series.

2.2.2 *N,N'*-(2,2'-(Piperazine-1,4-diyl)bis(ethane-2,1-diyl)) diaryl sulfonamides (46-53)

A second series of derivatives was planned to modify, along with the aromatic substituent, the length of the aliphatic linker, by shortening it from a three-carbon to a two-carbon saturated chain, while maintaining the symmetry of the molecule with the central piperazine ring. The previous two-step reaction strategy was applied (scheme 2.2).



Starting Sulfonyl Chloride	Ar	Bromide intermediate	Yield %	Product	Yield %
13	4-Chlorophenyl	**38**	87	**46**	45
14	Phenyl	**39**	80	**47**	52
15	4-Methylphenyl	**40**	83	**48**	89
16	4-Methoxyphenyl	**41**	75	**49**	78
17	4-Nitrophenyl	**42**	65	**50**	63
18	3,4-Dimethoxyphenyl	**43**	83	**51**	74
19	2,5-Dimethoxyphenyl	**44**	81	**52**	77
20	2-Naphthyl	**45**	87	**53**	82

Scheme 2.2: Pathway for the synthesis of compounds **46-53**

Synthesis of *N*-(2-bromoethyl)arylsulfonamides (38-45)

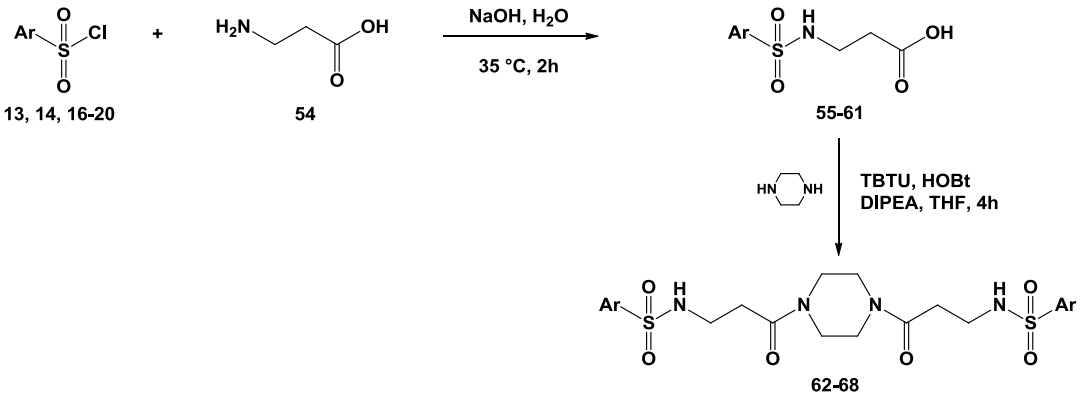
The same nucleophilic displacement strategy adopted for compounds **22-29** was followed for the synthesis of compounds **38-45**, using 2-bromoethylamine hydrobromide (**37**) to obtain a two-carbon linker, to be subsequently functionalised by piperazine secondary amine groups.

Synthesis of *N,N'*-(2,2'-(piperazine-1,4-di*yl*)bis(ethane-2,1-di*yl*))diaryl sulfonamides (46-53)

Final symmetrical piperazine derivatives with a shorter linker **46-53** were prepared in the system $NaHCO_3$/ethanol under reflux for 24 h, to allow the nucleophilic displacement by piperazine secondary amine groups on the level of the alkyl bromide leaving group.

2.2.3 *N,N'*-(3,3'-(Piperazine-1,4-di*yl*)bis(3-oxopropane-3,1-di*yl*))diaryl sulfonamides (74-80)

A third series of derivatives was designed to rigidify the three-carbon linker and study the importance of the two positive charges/H-bond acceptors of the piperazine amine groups. For this reason, it was planned to insert an amide group in correspondence of piperazine nitrogen atoms, obtaining an oxypropylic linker in the symmetrical structure: to achieve this result, the scaffold of β-alanine (**54**) was chosen as the new linker, and inserted in the two-step synthetic pathway shown in scheme 2.3.



Starting Sulfonyl Chloride	Ar	Acid intermediate	Yield %	Product	Yield %
13	4-Chlorophenyl	**55**	64	**62**	41
14	Phenyl	**56**	52	**63**	57
16	4-Methoxyphenyl	**57**	72	**64**	46
17	4-Nitrophenyl	**58**	51	**65**	35
18	3,4-Dimethoxyphenyl	**59**	58	**66**	47
19	2,5-Dimethoxyphenyl	**60**	64	**67**	52
20	2-Naphthyl	**61**	61	**68**	54

Scheme 2.3: Pathway for the synthesis of compounds **62-68**

Synthesis of 3-arylsulfonylamine propionic acids (55-61)

Intermediate acids **55-61** were obtained through a nucleophilic substitution by β-alanine (**54**) amine group on the level of the sulfonyl chloride, according to procedures reported for glycine.[17] By following the reaction with monitoring of the pH to maintain basic conditions with 1M NaOH, the corresponding carboxylates were obtained, while the free acids were subsequently precipitated out by acidification of the reaction mixture.

Synthesis of *N,N'*-(3,3'-(piperazine-1,4-di*yl*)bis(3-oxopropane-3,1-di*yl*))diaryl sulfonamides (62-68)

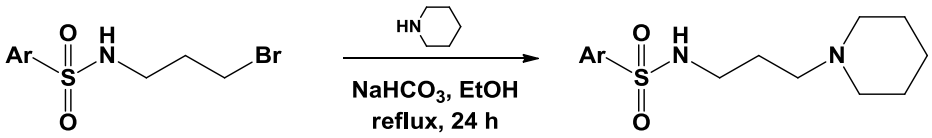
Final symmetrical amide derivatives **62-68** were obtained with a coupling reaction between propionic acids **55-61** and piperazine, using TBTU as coupling agent.[18]

TBTU, *O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium tetrafluoroborate, belongs to a family of coupling agents called uronium compounds, used for efficient amide bond formation under mild reaction conditions. Uronium reagents, like most coupling agents, are used to enhance the electrophilic characteristic of the carbonyl carbon towards nucleophilic substitution, by the formation of a highly reactive intermediate ester.[19]

This type of reaction can be carried out efficiently at r.t. for a few hours or overnight.

2.2.4 *N*-(3-(Piperidin-1-*yl*)propyl)arylsulfonamides (69, 70)

Aiming to evaluate the role of the length and the symmetry of the molecule, two shorter unsymmetrical derivatives were obtained by reacting intermediates **22** and **29** with piperidine. This was used to replace the piperazine central core with a single nucleophilic substitution by piperidine secondary amine on the alkyl bromide leaving group (scheme 2.4).



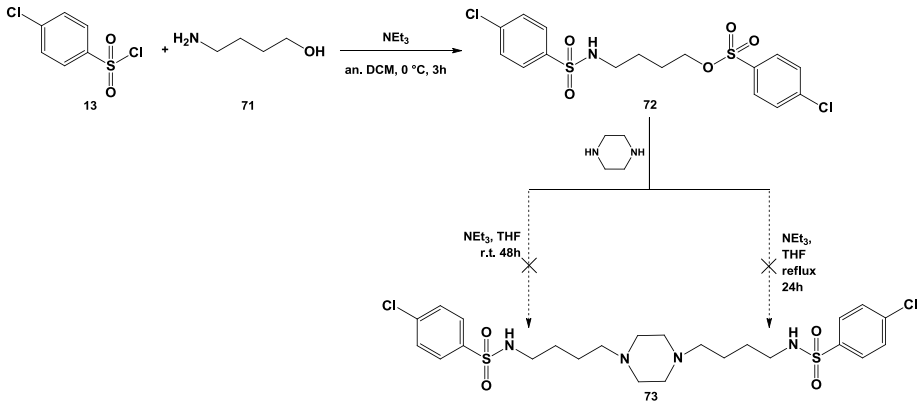
22, 29 **69, 70**

Bromide intermediate	Ar	Product	Yield %
22	4-Chlorophenyl	**69**	75
29	2-Naphthyl	**70**	71

Scheme 2.4: Synthesis of compounds **69** and **70**

2.2.5 *N,N'*-(4,4'-(Piperazine-1,4-diyl)bis(butane-4,1-diyl))bis(4 chlorobenzenesulfonamide) (73)

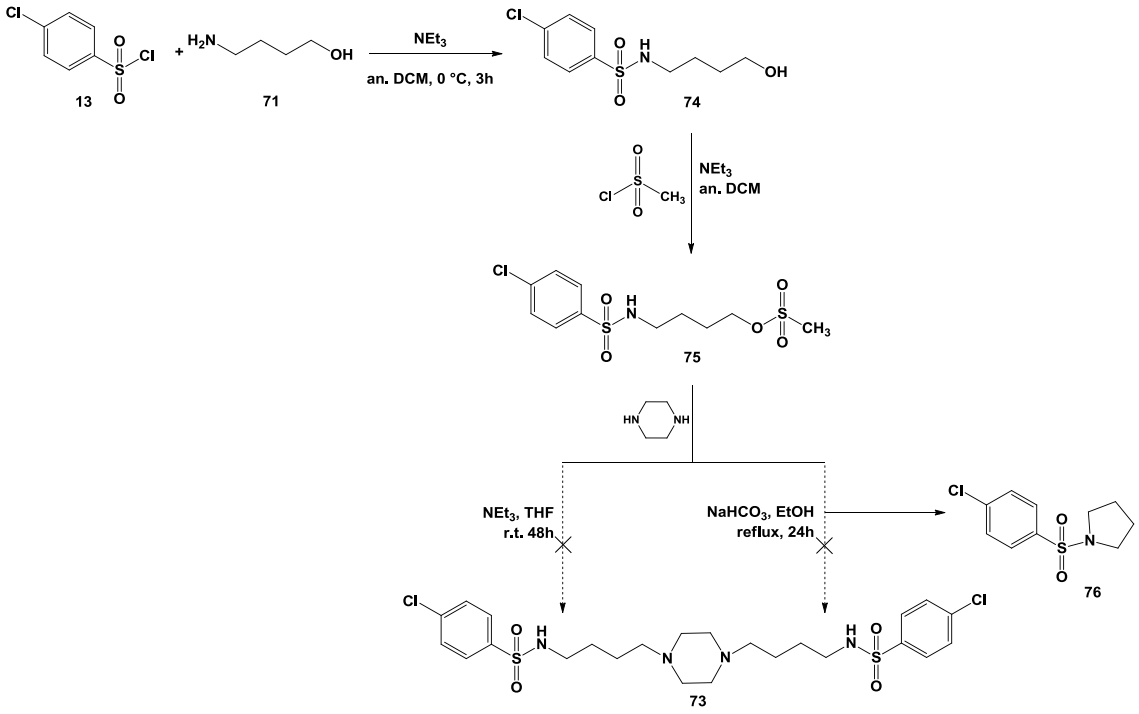
Several attempts were made in order to synthesise one symmetrical derivative with a longer aliphatic linker of four carbons. The first strategy designed for the preparation of the desired analogue, compound **73**, is shown in scheme 2.5.



Scheme 2.5: First strategy for the synthesis of compound **73**

The two-step synthetic pathway started with the formation of intermediate **72**, 4-chloro-benzenesulfonic acid 4-(4-chloro-benzenesulfonylamine)-butyl ester, through a double nucleophilic displacement on 4-chlorobenzenesulfonyl chloride (**13**) by both the amine and the hydroxyl groups of 4-aminebutanol (**71**), following procedures previously reported for tosyl chloride.[20] This intermediate was isolated and reacted with piperazine in THF and triethylamine at r.t., in order to displace the good leaving group of the sulfonate ester with the two amine groups of piperazine. The desired reaction did not proceed at r.t., and only starting material **72** was isolated after 48 h. The reaction mixture was then heated under reflux for 24 h, and even though the formation of a new species was observed by T.L.C, it was not possible to purify the new compound via column chromatography or recrystallisation.

To overcome purification difficulties, a second synthetic strategy, shown in scheme 2.6, was attempted.



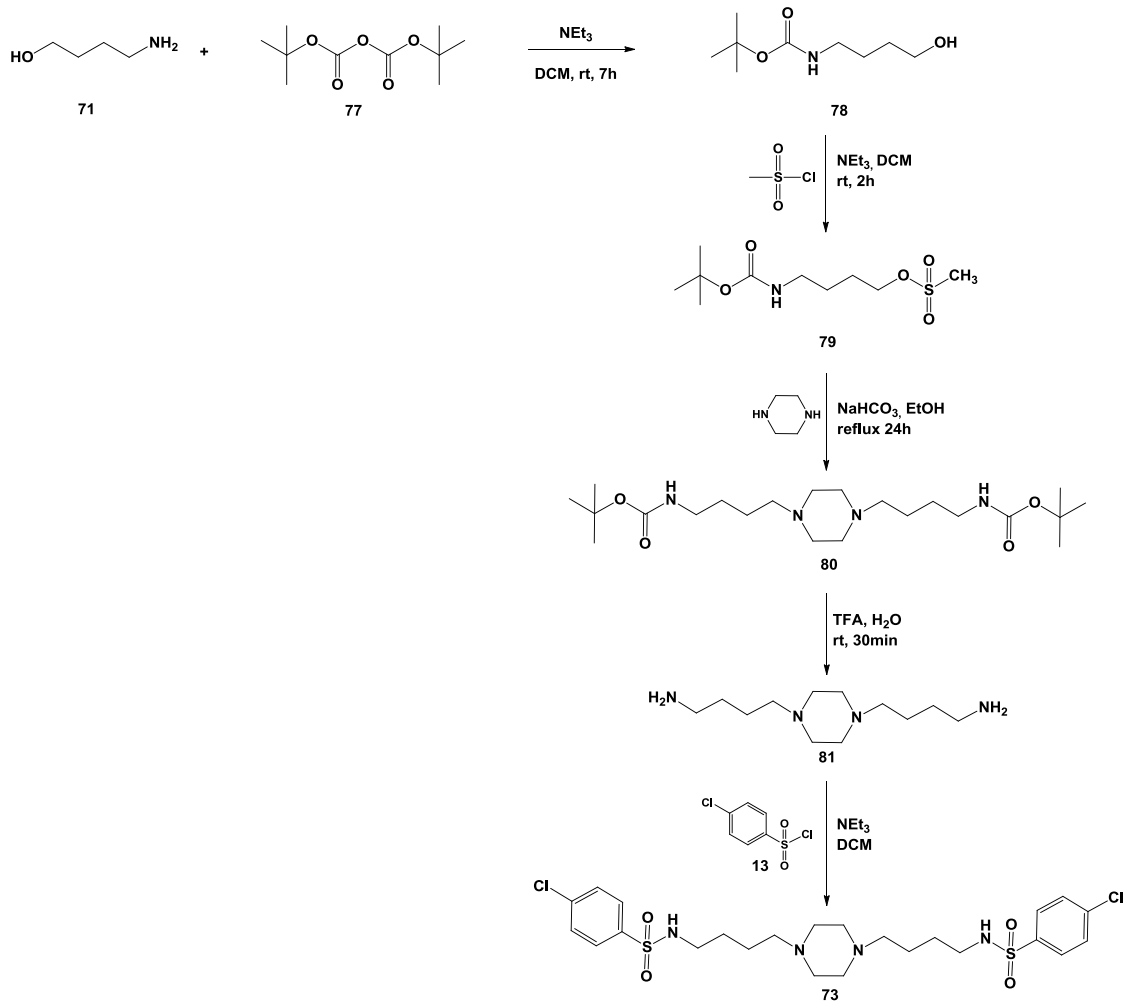
Scheme 2.6: Second strategy designed for the synthesis of compound **73**

The amine group of 4-aminebutanol was first substituted with 4-chlorobenzenesulfonyl chloride to give compound **74**, 4-chloro-*N*-(4-hydroxy-butyl)-benzenesulfonamide. Next a good leaving group was created on the free hydroxy function in **74** by conversion to mesylate ester, through a nucleophilic displacement on mesyl chloride.

Intermediate **75**, methanesulfonic acid 4-(4-chloro-benzenesulfonylamine)-butyl ester, was obtained and purified, but different attempts to displace the mesylate ester with piperazine were unsuccessful. Only starting material **75** was recovered after reacting it with piperazine in THF and triethylamine for 48 h at r.t. Reaction conditions were then changed to reflux in EtOH for 24 h, using sodium bicarbonate as base. In this second reaction system, all starting material **75** was converted into its ring-closure derivative on the sulfonamide nitrogen, compound **76** (1-(4-chloro-benzenesulfonyl)-pyrrolidine). The reason of the formation of this side product could be explained by the reflux conditions, where the energy given by heating could enhance the intramolecular displacement of the mesylate leaving group by the sulfonamide group.

Due to these unsuccessful attempts, it was decided to avoid any possible interference of the sulfonamide group by freezing its residual reactivity towards nucleophilic displacement through amine protection of 4-aminebutanol **71** with BOC, using di-*tert*-butyl dicarbonate $(BOC)_2O$, stable in basic environment and easily removed under acidic conditions.

The new synthetic pathway is shown in scheme 2.7.



Scheme 2.7: Third synthetic pathway designed for compound **73**

In this third strategy, the free amine in 4-aminebutanol was first protected with BOC to give **78**, and then a good leaving group was obtained by converting the hydroxy function to mesylate ester in compound **79**. Once substituted the two amine groups of piperazine by nucleophilic displacement of the mesylate group (**80**), under reflux in ethanol and sodium bicarbonate, it was designed to deprotect the two terminal amine groups by hydrolysis of the carbamate ester with trifluoroacetic acid in compound **81**. This fourth intermediate was not isolated, but precipitated as the trifluoroacetate salt from the reaction mixture, and directly treated with an excess of 4-chloro-benzenesulfonyl chloride (**13**) in basic conditions, in order to obtain the desired final product **73**. This third synthetic approach was successful and compound **73** was finally isolated after purification by flash column chromatography. Each step of this successful procedure will now be discussed.

Synthesis of (4-hydroxy-butyl)-carbamic acid *tert*-butyl ester (78)[21]

The amine group of 4-aminebutanol **71** was protected by conversion to carbamic ester using the BOC protecting group, which was chosen due to its stability under basic conditions, required in the following reaction steps where a nucleophilic displacement was to be performed.[21] Desired protected amine **78** was obtained in high yield (93%) after stirring the reagents at r.t. for 7 h in DCM and NEt_3.

Synthesis of methanesulfonic acid 4-*tert*-butoxycarbonylamine-butyl ester (79)

The free hydroxy group of compound **78** was converted to a good leaving group, for the subsequent nucleophilic displacement with piperazine, by using mesyl chloride, in order to obtain a methanesulfonic ester function, easily replaced by nucleophiles, such as the secondary amine groups of piperazine.[22] Also this second intermediate was obtained in high yield (86%) and purity.

Synthesis of {4-[4-(4-*tert*-butoxycarbonylamine-butyl)-piperazin-1-*yl*]-butyl}-carbamic acid *tert*-butyl ester (80)

In order to achieve a double nucleophilic displacement by the piperazine amine groups on mesylate ester **79**, the two starting materials were reacted for 24 h under reflux in ethanol, using sodium bicarbonate as base, in the same reaction conditions that had been previously adopted. Symmetrical piperazine intermediate **80** was obtained in 70 % yield and used for the following step without purification.

Synthesis of *N,N'*-(4,4'-(piperazine-1,4-di*yl*)bis(butane-4,1-di*yl*))bis(4-chlorobenzene-sulfonamide) (73)

The two protected carbamic ester functions of compound **80** were converted to free amine groups by removal of the BOC protecting group, using trifluoroacetic acid. In the acidic reaction conditions, symmetrical tetra-amine **81**, 4,4'-(piperazine-1,4-di*yl*)bis(butan-1-amine), was present as a trifluoroacetate salt, that was precipitated after concentrating the reaction mixture under vacuum and pouring the residue into diethyl ether. The white precipitate formed was washed with diethyl ether and directly treated with an excess of 4-chlorobenzenesulfonyl chloride (**13**) and triethylamine.

Compound **73** was finally obtained following this five-step synthetic route, after purification by flash column chromatography.

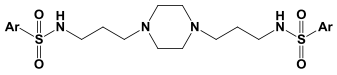
2.2.6 Biological evaluation in the HCV replicon and cytostatic assay

HCV replicon systems, genetic elements that can replicate under their own control in a cell, are powerful tools to study HCV replication and evaluate the potential interference of chemical compounds.[13] The most recent HCV subgenomic replicons are genetically modified to replace the region encoding the structural proteins with a reporter gene, such as firefly luciferase, that allows the evaluation of HCV replication inhibition through the measurement of the reduction of luciferase signal.

For all compounds prepared, the subgenomic replicon assay in the human hepatoma cell line Huh-7 and the cytostatic assay were kindly performed in the Rega Institute for Medical Research, KU Leuven, Leuven, Belgium, under the supervision of Professor Johan Neyts.[23]

Activity is expressed for each compound in terms of EC_{50}, that is the effective concentration (μM) of compound required to reduce HCV replication by 50%, and eventually EC_{90}, that is the effective concentration (μM) of compound required to reduce HCV replication by 90%. Cytotoxicity is evaluated in terms of CC_{50}, that is the cytostatic/cytotoxic concentration (μM) required to observe 50% of adverse effect on the host cell. The selectivity index, SI, is evaluated by calculating the ratio CC_{50}/EC_{50}.

***N,N'*-(2,2'-(Piperazine-1,4-diyl)bis(propyl-2,1-diyl)) diaryl sulfonamides (12, 30-36)**



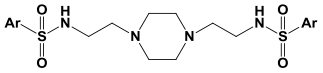
Compound	Ar-	EC_{50}(μM)	CC_{50}(μM)	EC_{90}(μM)	SI
12	4-Chlorophenyl	13	>182	-	>14
30	Phenyl	58.3	>104	-	>1.7
31	4-Methylphenyl	19	>197	>197	>10.4
32	4-Methoxyphenyl	>92.5	>92.5	>92.5	-
33	4-Nitrophenyl	17.5	77.4	61.1	4.4
34	3,4-Dimethoxyphenyl	82	>166	>166	2
35	2,5-Dimethoxyphenyl	>83	>83	>83	-
36	2-Naphthyl	4.9	49.9	-	10.2

Table 2.1: activity and cytotoxicity data for compounds **12**, **30-36**

As can be inferred from table 2.1, compound **12** confirmed the activity potential

previously found, while a hydrophobic substituent in the *para* position in the aromatic rings was related to activity retention (compound **31**). Compounds **33** and **36**, with *para*-nitrophenyl and 2-naphthyl aromatic moieties, respectively, were associated with cytotoxicity, while the removal of the substituent or its replacement with methoxy groups led to loss of activity.

N,N'-(2,2'-(Piperazine-1,4-di*yl*)bis(ethane-2,1-di*yl*)) diaryl sulfonamides (46-53)

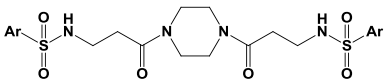


Compound	Ar-	EC_{50}(μM)	CC_{50}(μM)	EC_{90}(μM)	SI
46	4-Chlorophenyl	>95.9	>95.9	-	-
47	Phenyl	>110	>110	-	-
48	4-Methylphenyl	>104	>104	>104	-
49	4-Methoxyphenyl	>97.5	>97.5	>97.5	-
50	4-Nitrophenyl	17.4	22.3	-	1.3
51	3,4-Dimethoxyphenyl	85.8	>87.3	>87.3	>1
52	2,5-Dimethoxyphenyl	>87	>87	-	-
53	2-Naphthyl	10.6	>90.5	-	>8.5

Table 2.2: activity and cytotoxicity data for compounds **46-53**

Symmetrical shortening of the aliphatic linker was associated with loss of activity, indicating an important role played by the length of the linker for viral replication inhibition. The only exception to this trend was compound **53**, where the presence of the 2-naphthyl ring resulted in activity retention, while the shortening of the linker appears to reduce the toxic effect found for its three-carbon analogue, compound **36**.

N,N'-(3,3'-(piperazine-1,4-di*yl*)bis(3-oxopropane-3,1-di*yl*))diarylsulfonamides (62-68)

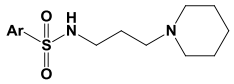


Compound	Ar-	EC_{50}(μM)	CC_{50}(μM)	EC_{90}(μM)	SI
62	4-Chlorophenyl	-	>86.6	-	-
63	Phenyl	>197	>197	>197	-
64	4-Methoxyphenyl	-	>87.9	-	-
65	4-Nitrophenyl	84	>167	>167	>1.9
66	3,4-Dimethoxyphenyl	>159	>159	>159	-
67	2,5-Dimethoxyphenyl	>79.5	>79.5	-	-
68	2-Naphthyl	12.4	40.8	78.3	3.3

Table 2.3: activity and cytotoxicity data for compounds 62-68

For the third series of symmetrical derivatives prepared, biological results indicate that either the presence of the positive charge on the two amine groups or the flexibility of the two linkers are important for viral replication inhibition, since this modification is correlated to loss of activity. The only exception is represented by compound **68**, where the 2-naphthyl group is associated with retained activity, but also shows increased toxicity against the cell.

N-(3-(Piperidin-1-yl)propyl)arylsulfonamides (69, 70)



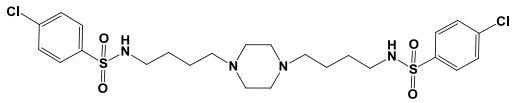
Compound	Ar-	EC_{50}(μM)	CC_{50}(μM)	EC_{90}(μM)	SI
69	4-Chlorophenyl	61	>157	-	2.5
70	2-Naphthyl	12.7	70.4	-	5.5

Table 2.4: activity and cytotoxicity data for compounds **69**, **70**

Biological results for the two shorter unsymmetrical derivatives where the piperazine central core is replaced with a piperidine ring, compounds **69** and **70**, confirmed the importance of the overall length of the molecule, since this last modification is associated with loss of activity for compound **69**, while the values for the 2-naphthyl

analogue confirmed the cytotoxic effect associated with this substitution.

***N,N'*-(4,4'-(Piperazine-1,4-diyl)bis(butane-4,1-diyl))bis(4-chlorobenzene sulfonamide) (73)**



Compound	EC_{50}(μM)	CC_{50}(μM)	EC_{90}(μM)	SI
73	69	>173	164	>2.5

Table 2.5: activity and cytotoxicity data for compound **73**

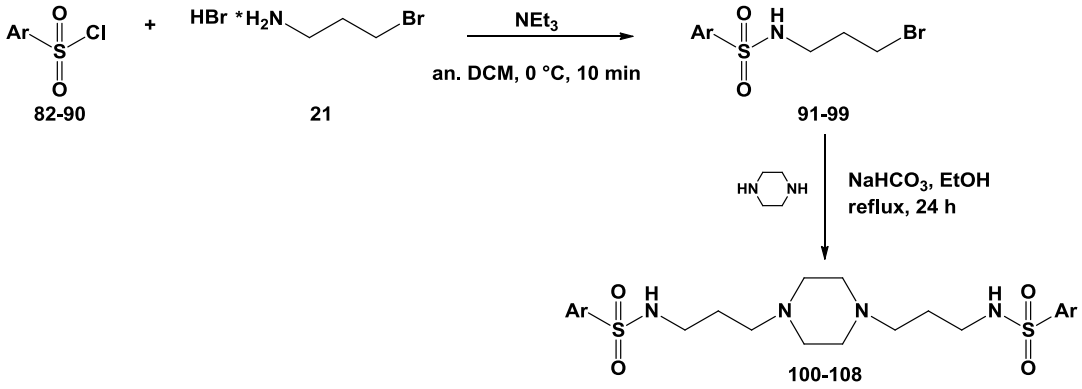
Loss of activity associated with a longer aliphatic linker of four methylene groups, in compound **73**, confirmed that the length of the original molecule **12** plays an important role for the viral replication inhibition.

2.2.7 Design of a new series of symmetrical derivatives

Following the biological results obtained for the first series of analogues tested, a new series of compounds was designed, and for most of them the original three-carbon aliphatic linker was maintained. The new modifications planned aimed to explore the effect of the hydrophobic substituent in the aromatic system, by changing the position of the original chlorine to *meta* and *ortho*, and by replacing the hydrophobic group in the position *para* with a *tert*-butyl, trifluoromethyl and phenyl functions. In order to investigate the residual activity shown for the 4-nitro analogue **33**, it was decided to insert a carboxylic function in the *para* position of the aromatic system, and also the carboxylate ethyl ester was chosen for preparation and biological evaluation. Moreover, the aromatic system in the original scaffold was replaced with heteroaromatic rings (pyridine, thiophene and quinoline), and the 2-naphthyl system in compound **33** was substituted with a 1-naphthyl group. In order to assess the importance of the two sulfonamide groups, it was decided to replace them with an amide group. Finally, the first non-piperazine derivative was designed, aiming to rigidify the original structure and to remove its double positive charge, while maintaining the opportunity of hydrogen-bond formation. To achieve this result, the scaffold of *para*-phenylendiamine was chosen to replace piperazine.

2.2.8 *N,N'*-(3,3'-(piperazine-1,4-di*yl*)bis(propane-3,1-di*yl*))diarylsulfonamides (100-108, 111, 116)

Nine new derivatives with a linear three-carbon linker were prepared following the previously applied two-step synthetic pathway, starting from sulfonyl chlorides **82-90**, preparing intermediate alkyl bromides **91-99**, and finally alkylating piperazine amine groups to obtain symmetrical products **100-108**, as shown in scheme 2.8.

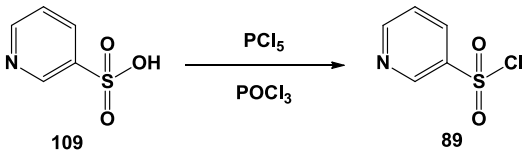


Starting Sulfonyl Chloride	Ar	Bromide intermediate	Yield %	Product	Yield %
82	3-Chlorophenyl	**91**	75	**100**	79
83	4-*tert*-Butylphenyl	**92**	77	**101**	82
84	4-Trifluoromethylphenyl	**93**	80	**102**	61
85	4-Biphenyl	**94**	67	**103**	87
86	1-Naphthyl	**95**	95	**104**	91
87	8-Quinoline	**96**	73	**105**	63
88	1-Thiophene	**97**	89	**106**	83
89	3-Pyridine	**98**	56	**107**	63
90	4-Ethylcarboxyphenyl	**99**	66	**108**	41

Scheme 2.8: Synthesis of compounds **100-108**

Sulfonyl chlorides **82-88** were commercially available, while 3-pyridine sulfonyl chloride (**89**) and ethyl 4-(chlorosulfonyl)benzoate (**90**) were synthesised starting from pyridine-3-sulfonic acid (**109**) and 4-chlorosulfonyl benzoic acid (**110**), respectively.

Synthesis of pyridine-3-sulfonyl chloride (89)[24]

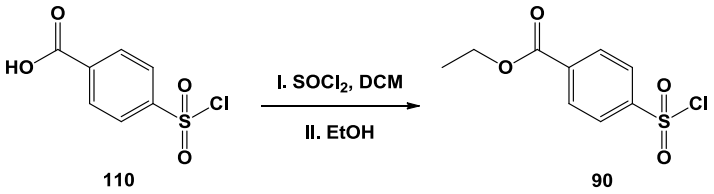


Scheme 2.9: Synthesis of sulfonyl chloride **89**

Following reported procedures, compound **89** was obtained by refluxing over night pyridine-3-sulfonic acid (**109**) with phosphorus pentachloride and phosphorus oxychloride, in order to perform a substitutive chlorination of the sulfonic acid function to sulfonyl chloride.[24]

Synthesis of 4-chlorosulfonyl-benzoic acid ethyl ester (90)

The carboxylic function of 4-chlorosulfonyl benzoic acid (**110**) was converted to its ethyl ester derivative following procedures reported for the preparation of the methyl ester (scheme 2.9).[25]

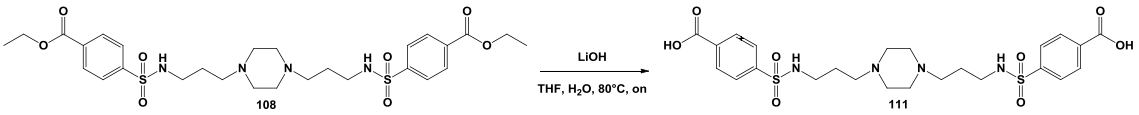


Scheme 2.9: Synthesis of sulfonyl chloride **90**

Starting material **110** was first treated with thionyl chloride in DCM, refluxing the reaction mixture for 2 h prior removal of the solvent at reduced pressure, thus obtaining the acyl chloride. This intermediate was immediately treated with cold ethanol at 0°C, allowing the nucleophilic substitution of the chloride leaving group with the formation of the ethyl ester derivative, compound **90**.

3-(*N*-(3-(4-(3-(4-Carboxyphenylensulfonamide)propyl)piperazin-1-*yl*)propyl) sulfamoyl)benzoic acid (111)

The designed derivative **111** with a carboxylic substituent in the *para* position of the aromatic group in the lateral chain was obtained through a base-catalysed hydrolysis of the two ester functional groups of compound **108**, as shown in scheme 2.10.

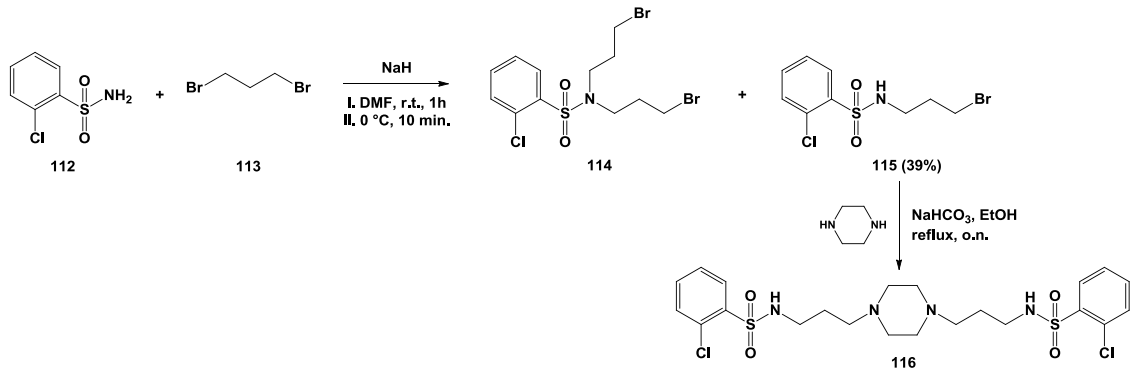


Scheme 2.10: Synthesis of compound **111**

Ethyl ester groups in compound **108** were hydrolysed into the free carboxylic acids with lithium hydroxide, stirring the reaction mixture at 80°C over night. The final product **111** was precipitated after removal of the organic solvent and subsequent acidification of the water residue to pH 5.

N,N'-(3,3'-(Piperazine-1,4-di*yl*)bis(propane-3,1-di*yl*))bis(2-chlorobenzene-sulfonamide) (116)

In order to move the original 4-chloro aromatic substituent into the *ortho* position, 2-chlorobenzenesulfonamide (**112**) was the only starting material available. The first step of the usual synthetic pathway had therefore to be changed to obtain this derivative (scheme 2.11).



Scheme 2.11: Synthesis of compound **116**

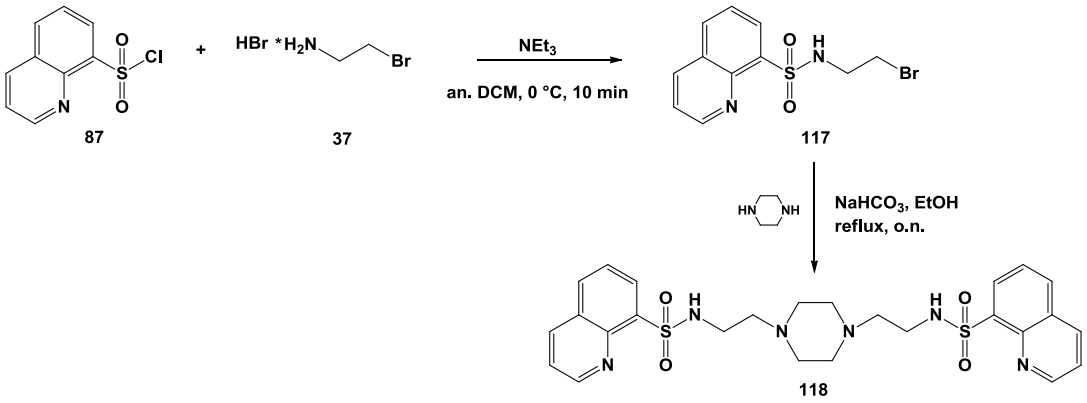
Synthesis of *N*-(3-bromo-propyl)-2-chloro-benzenesulfonamide (115)

Intermediate alkyl bromide **115** was obtained by reacting 2-chlorobenzene sulfonamide (**112**) with 1,3-dibromopropane (**113**). One proton of the sulfonamide nitrogen was first removed by treating with 1 equivalent of NaH in anhydrous DMF, followed by the addition of compound **113** to the mixture, in order to obtain the displacement of one of the bromides by the nitrogen lone pair.[26]

Desired intermediate **115** was not the main product formed: most of the starting material was converted into the product of the double substitution on the sulfonamide nitrogen, to give compound **114**, while compound **116** was obtained in 39% yield.

2.2.9 *N,N'*-(2,2'-(Piperazine-1,4-diyl)bis(ethane-2,1-diyl)diquinoline-8-sulfonamide (118)

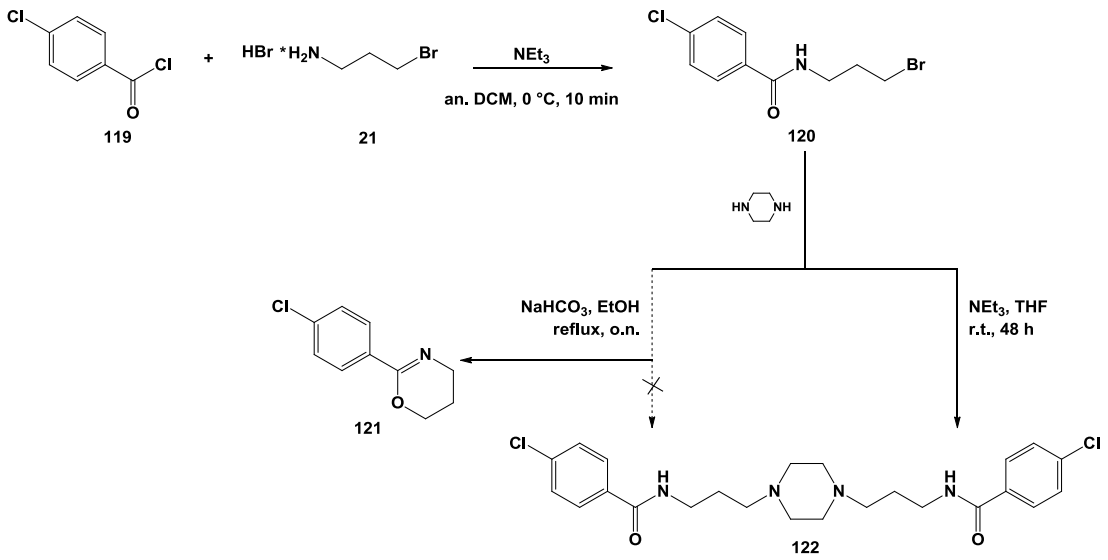
Due to the activity potential revealed for compound **53**, which shows a shorter two-carbon linker and a 2-naphthalene aromatic system, it was thought that the insertion of another bicyclic aromatic system could maintain a certain activity in the shorter scaffold. One new symmetrical derivative was therefore prepared with a two-carbon linker, through the usual synthetic approach, starting from 8-quinoline sulfonyl chloride (**87**) (scheme 2.12).



Scheme 2.12: Synthesis of compound **118**

2.2.10 *N,N'*-(3,3'-(Piperazine-1,4-diyl)bis(propane-3,1-diyl))bis(4-chloro benzamide) (122)

In order to investigate the role of the sulfonamide group for antiviral activity, it was replaced with an amide group. The synthetic pathway carried out was the same previously used (scheme 2.13).



Scheme 2.13: Synthesis of compound **122**

Synthesis of *N*-(3-bromopropyl)-4-chlorobenzamide (120)

As was done for the synthesis of previous intermediate alkyl bromides, 4-chlorobenzoyl chloride (**119**) was converted into the corresponding benzamide **120** through a nucleophilic substitution with 3-bromopropylamine hydrobromide (**21**) in the presence of triethylamine.

Synthesis of *N,N'*-(3,3'-(piperazine-1,4-di*yl*)bis(propane-3,1-di*yl*))bis(4-chloro-benzamide) (122)

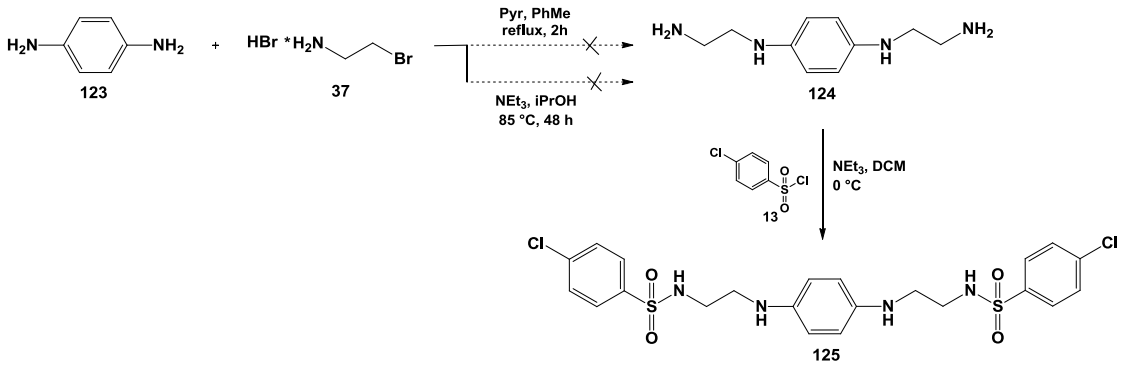
A first attempt to obtain final product **122** was made by heating compound **120** at reflux with piperazine in ethanol and sodium bicarbonate, following the usual strategy previously used for sulfonamide products. Nevertheless, under these conditions all of the starting material was converted into its internal closure product, compound **121**: the energy given by heating could enhance an intramolecular reaction with the formation of a cyclic side-product. Before changing the synthetic strategy, in was decided to repeat the reaction without heating, using triethylamine and THF at r.t. for 48 h: this second attempt was successful and the desired product **122** was obtained in a 36% yield.

2.2.11 *N,N'*-(2,2'-(1,4-Phenylenebis(azanedi*yl*))bis(ethane-2,1-di*yl*))bis(4-chlorobenzenesulfonamide) (125)

At this point of the study, a modification of the piperazine central nucleus was designed, in order to rigidify the molecule and remove the positive charge associated with the two

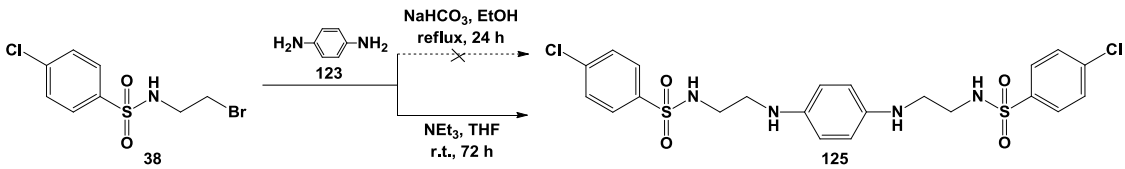
amine groups, while keeping the opportunity of hydrogen-bond formation. The structure of 4-phenylendiamine (**123**) was chosen as a new scaffold, separated by two methylene groups from the two terminal arylsulfonamide groups, in order to maintain the original length of compound **12**. Different strategies were attempted to obtain one derivative with the new central system.

As shown in scheme 2.14, a first attempt began with the formation of *N*,*N*'-bis-(2-amine-ethyl)-benzene-1,4-diamine **124** as the first intermediate. Subsequent substitution of the free amine groups with 4-chlorobenzenesulfonyl chloride was planned to obtain compound **125**.[27-28]



Scheme 2.14: First synthetic pathway designed for compound **125**

The first step was attempted by stirring of 4-phenylendiamine (**123**) with 2-bromoethylamine hydrobromide (**37**) for half of the reaction time in the appropriate solvent before adding the base, pyridine or triethylamine. These two attempts to obtain intermediate **124**, using different base/solvent systems, were unsuccessful. A different strategy was therefore planned, starting from intermediate alkyl bromide **38** and trying to obtain a double nucleophilic displacement by the two aromatic amine groups of compound **123** (scheme 2.15).



Scheme 2.15: Second strategy attempted for the synthesis of compound **125**

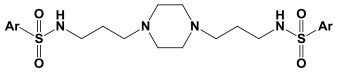
The reaction was first tried by heating under reflux in ethanol and sodium bicarbonate. This attempt gave a complex mixture of products that was not possible to purify

successfully.

The reaction system was therefore changed, and the reaction was repeated at room temperature for 72 h, using triethylamine and THF as base/solvent. Also in this case the formation of a complex mixture of products was observed, but after a series of chromatographic purifications, which reduced the yield of the reaction to 17%, it was possible to isolate desired pure product **125**.

2.2.12 Biological evaluation

***N,N'*-(3,3'-(Piperazine-1,4-di*yl*)bis(propane-3,1-di*yl*))diaryl sulfonamides (100-108, 111, 116)**



Compound	Ar-	EC_{50}(μM)	CC_{50}(μM)	EC_{90}(μM)	SI
100	3-Chlorophenyl	76.9	>182	173	>2.4
101	4-*tert*-Butylphenyl-	4.9	91.5	42.9	18.6
102	4-Trifluoromethylphenyl	6.5	>162	-	>24.9
103	4-Biphenyl	<1.2	4.25	-	>3.5
104	1-Naphthyl	>172	>172	>172	-
105	8-Quinoline	42.5	>182	173	>4.3
106	1-Thiophene	62	>203	156	>3.3
107	3-Pyridine	>207	>207	>207	-
108	4-Ethylcarboxyphenyl	56.2	>160	-	>2.8
111	4-Carboxyphenyl	>176	>176	-	-
116	2-Chlorophenyl	39.6	>182	>182	>4.6

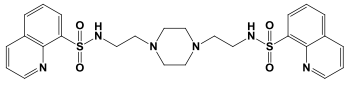
Table 2.5: activity and cytotoxicity data for compounds **100-108**, **111**, **116**

As can be inferred from table 2.5, the presence of a *para*-hydrophobic aromatic substituent in the original scaffold seems necessary for retention of activity: moving the 4-chloro group to position 2 and 3 is associated with loss of activity, while the presence of a bulkier lipophilic substituent in the *para* position, such as a *tert*-butyl in compound **101** and a trifluoromethyl in compound **102**, results in retained activity. The lowest EC_{50} value is reached when a second phenyl group replaced the original 4-chloro (compound **103**): however, this last modification also shows an increased toxic effect.

Replacement of the two phenyl rings with heteroaromatic ones leads to loss of activity, and the same effect can be noticed for the substitution of the previous 2-naphthyl group (compound **36**) with 1-naphthyl: cytotoxicity is reduced in compound **104**, but activity potential against viral replication is lost as well.

Trying to mimic the effect of a 4-nitro group (compound **33**), a 4-carboxylate function was introduced as aromatic substituent (compound **111**): with this last modification, toxic effect is lost, but the same can be said for antiviral activity. A mild inhibition of viral replication is observed for ethyl ester derivative **108**, but antiviral potential is reduced in comparison with compound **12**.

***N,N'*-(Piperazine-1,4-diylbis(ethane-2,1-diyl))bis(quinoline-8-sulfonamide)**

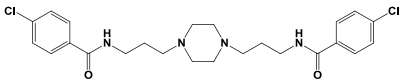


Compound	EC_{50}(μM)	CC_{50}(μM)	EC_{90}(μM)	SI
118	>180	>180	>180	-

Table 2.6: activity and cytotoxicity data for compound **118**

The same trend shown in the three-carbon linker series, where replacement of the 2-naphthyl group with a 1-naphthyl or a 8-quinoline substituent led to loss of activity, can be observed in the two-carbon linker series: activity previously found for compound **53** is lost when a 8-quinoline moiety replaces the 2-naphthyl.

***N,N'*-(3,3'-(Piperazine-1,4-diyl)bis(propane-3,1-diyl))bis(4-chlorobenzamide) (122)**

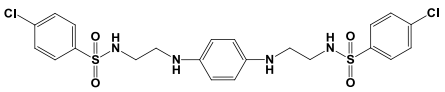


Compound	EC_{50}(μM)	CC_{50}(μM)	EC_{90}(μM)	SI
122	>209	>209	>209	-

Table 2.7: activity and cytotoxicity data for compound **122**

Along with the presence of a 4-hydrophobic aromatic substituent, the two sulfonamide groups seem to play an essential role for antiviral activity: their replacement with an amide group in compound **122** is associated with a dramatic loss of antiviral potential.

***N,N'*-(2,2'-(1,4-Phenylenebis(azanedi*yl*))bis(ethane-2,1-di*yl*))bis(4-chlorobenzene sulfonamide) (124)**



Compound	EC_{50}(μM)	CC_{50}(μM)	EC_{90}(μM)	SI
124	12.1	22	-	1.8

Table 2.8: activity and cytotoxicity data for compound **124**

Biological results for compound **124**, with which the structure of compound **12** was rigidified and the positive charges removed with the introduction of a new 4-phenylendiamine central scaffold, show that this modification does not seem to be successful for cytotoxicity reasons: even though activity is retained from an EC_{50} point of view, compound **124** is mainly associated with an adverse effect against cells.

2.3 Synthesis of unsymmetrical piperazines

In order to explore the importance of symmetry in the original scaffold, three series of new derivatives were designed to introduce small asymmetry elements in the structure, without dramatically modifying its overall length or chemical nature, and considering the biological results obtained for the previous series of derivatives.

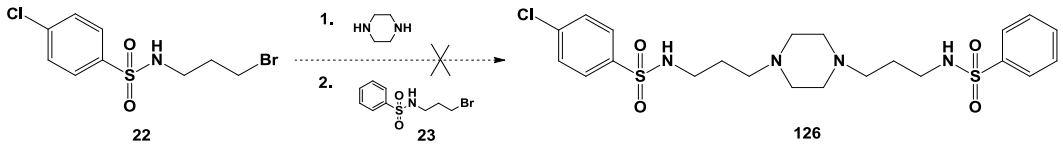
In all new compounds designed, the presence of two aromatic sulfonamide groups was to be maintained, along with a *para* hydrophobic substituent in at least one phenyl ring. Another element to be kept was the central piperazine nucleus and one three-carbon linker.

2.3.1 Unsymmetrical *N*-{3-[4-(3-arylsulfonylamine-propyl)-piperazin-1-*yl*]-propyl}-arylsulfonamides (126-138)

A first series of unsymmetrical derivatives was designed to maintain the original scaffold while introducing different *para* hydrophobic substituents in the two aromatic rings, combining together the most successful substitutions previously obtained (4-chloro, 4-methyl, 4-*tert*-butyl and 4-trifluoromethyl), along with the unsubstituted phenyl moiety and the biphenyl one (for which the lowest EC_{50} value had been found, but was also associated with a toxic effect in compound **103**).

To achieve the desired results, the two amine groups of the central piperazine core had to be functionalised with two different *N*-(3-bromopropyl)arylsulfonamide intermediates.

Starting from alkyl bromide intermediates **22-24**, **92-94**, a first strategy was attempted to obtain the final products in a one-pot manner, as shown in scheme 2.16.



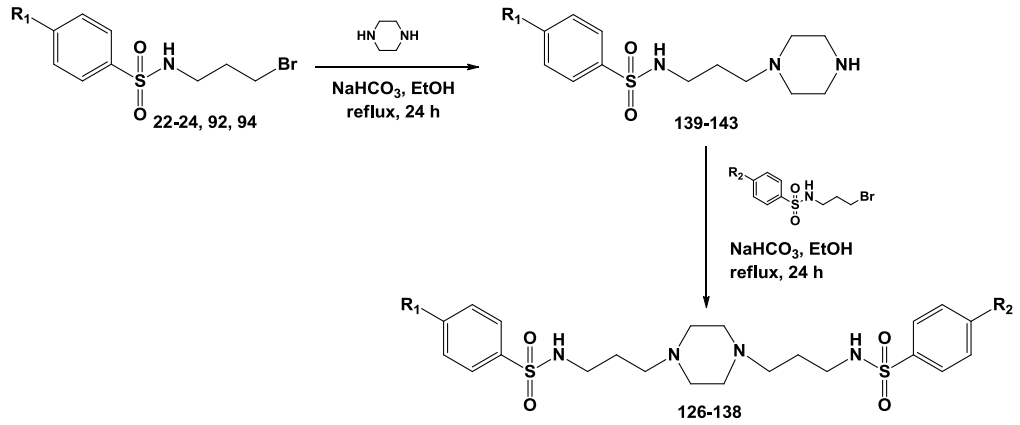
Scheme 2.16: First strategy attempted for the synthesis of compounds **126-138**. Conditions: 1. $NaHCO_3$, EtOH, reflux, o.n.; 2. Reflux, 24h

In this attempt, the first alkyl bromide (**22**) was refluxed in ethanol o.n. with an equimolar quantity of piperazine, in the presence of $NaHCO_3$, in order to allow the

formation of the product of the nucleophilic displacement by one of piperazine amine groups, monitoring the presence of this intermediate by TLC. The second alkyl bromide (**23**) was subsequently added in small excess to the reaction mixture, leaving it stirring under reflux for a further 24 h. Formation of the desired product **126** was confirmed by both NMR and MS experiments, but it was not possible to purify, due to the simultaneous formation of both the two symmetrical products, confirmed by MS.

Several attempts were made in terms of molar ratio of the starting materials and reaction conditions, in order to enhance the formation of the unsymmetrical product, but it was not possible to purify it by either chromatographic techniques or re-crystallisation.

In order to overcome purification difficulties, it was decided to first isolate the pure products of monosubstitution on the level of piperazine, and then react these intermediates with the second alkyl bromide, thus obtaining unsymmetrical products **126-138** after chromatographic purification (scheme 2.17).



Alkyl bromide 1	R_1	Mono-intermediate	Yield %	Alkyl bromide 2	R_2	Product	Yield %
22	-Cl	**139**	56	**23**	-H	**126**	68
22	-Cl	**139**	-	**24**	-Me	**127**	86
22	-Cl	**139**	-	**92**	-tBu	**128**	60
22	-Cl	**139**	-	**93**	$-CF_3$	**129**	85
22	-Cl	**139**	-	**94**	-Ph	**130**	71
92	-tBu	**142**	71	**23**	-H	**131**	54
92	-tBu	**142**	-	**24**	-Me	**132**	83
92	-tBu	**142**	-	**93**	$-CF_3$	**133**	70
92	-tBu	**142**	-	**94**	-Ph	**134**	58
24	-Me	**141**	59	**23**	-H	**135**	52
24	-Me	**141**	-	**94**	-Ph	**136**	67
94	-Ph	**143**	41	**23**	-H	**137**	71
23	-H	**140**	71	**93**	CF_3	**138**	50

Scheme 2.17: Final pathway for the synthesis of compounds **126-138**

Synthesis of *N*-(3-piperazin-1-*yl*-propyl)-arylsulfonamides (139-143)

Pure mono-substituted piperazine intermediates **139-143** were obtained with a nucleophilic displacement by one of the piperazine amine groups on the bromide leaving group. In order to reduce the formation of double substitution products, which was always noticed with different molar ratios and reaction systems attempted, the starting alkyl bromide was treated with a large excess of piperazine, and the mixture was heated at reflux in ethanol for 24 h. Pure intermediates were isolated by flash column chromatography before being used for the final step.

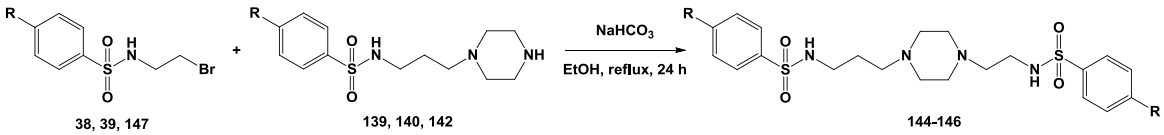
Synthesis of *N*-{3-[4-(3-arylsulfonylamine-propyl)-piperazin-1-*yl*]-propyl}-arylsulfonamides (126-138)

Final unsymmetrical products **126-138** were prepared by treating mono-substituted intermediates **139-143** with a small excess of the second alkyl bromide, in order to obtain a further nucleophilic displacement by the piperazine secondary amine while refluxing the mixture in ethanol for 24 h, in the presence of $NaHCO_3$, added to neutralise the hydrobromic acid formed.

2.3.2 Unsymmetrical *N*-(3-(4-(2-(arylsulfonamide)ethyl)piperazin-1*yl*)propyl)aryl sulfonamides (144-146)

A second small series of unsymmetrical derivatives was designed to maintain the same *para* hydrophobic substituent in the two aromatic rings, while reducing the length of one of the two linkers from three to two methylene groups. According to biological results obtained for the previous series of compounds, it was chosen to synthesise corresponding derivatives with a 4-chloro and a 4-*tert*-butyl substituent respectively, along with the unsubstituted phenyl derivative.

The synthetic strategy applied was the same designed for compounds **126-138**: final products **144-146** were obtained by reacting mono-substituted intermediates **139**, **140**, **142** with *N*-(2-bromoethyl)arylsulfonamides **38**, **39**, **147**, as shown in scheme 2.18.



Ethyl bromide	Mono-intermediate	R	Product	Yield %
38	**139**	-Cl	**144**	72
39	**140**	-H	**145**	47
147	**142**	-tBu	**146**	53

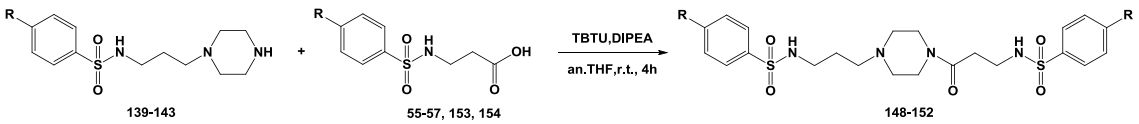
Scheme 2.18: Synthetic pathway for compounds **144-146**

The previous system $NaHCO_3$/ethanol under reflux conditions was used to obtain final products **144-146** from mono-substituted intermediates **139**, **140** and **142**, reacting them with *N*-(2-bromoethyl)arylsulfonamides in order to displace the bromide leaving group with the secondary amine group of piperazine. *N*-(2-Bromoethyl)-4-(*tert*-butyl)

benzenesulfonamide **147** was prepared by reacting 4-*tert*-butylbenzene sulfonyl chloride **83** with 2-bromopropylamine, following the procedure previously applied for the synthesis of ethyl bromide intermediates **38-45**.

2.3.3 Unsymmetrical *N*-(3-(4-((arylsulfonamide)propanoyl)piperazin-1-*yl*)-3-oxo propyl)aryl sulfonamides (148-152)

A third and final series of unsymmetrical derivatives was designed to functionalise one amine group in the original structure to amide, thus removing one positive charge and partially rigidifying the scaffold, while keeping the overall length of the molecule and the same hydrophobic substituent in the *para* position of the two aromatic rings. A derivative with an unsubstituted phenyl moiety was chosen as well for preparation, to potentially confirm the importance of a 4-hydrophobic substituent for antiviral activity.
Starting from mono-substituted intermediates **139-143**, it was planned to convert the piperazine secondary amine group to amide by inserting an oxypropylic spacer in the structure, using the scaffold of β-alanine (**54**), following a TBTU-assisted coupling reaction (scheme 2.19), as had been done previously for symmetrical compounds **62-68**.



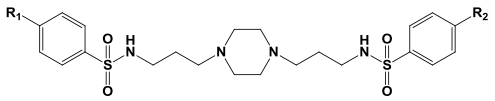
Propionic acid	Mono-intermediate	R	Product	Yield %
55	**139**	-Cl	**148**	46
56	**140**	-H	**149**	37
57a	**141**	-Me	**150**	34
153	**142**	-tBu	**151**	47
154	**143**	-Ph	**152**	47

Scheme 2.19: Pathway for the synthesis of compounds **148-152**

Intermediate propanoic acids **153** and **154** were prepared by reacting 4-*tert*-butylbenzene sulfonyl chloride **83** and 4-biphenyl sulfonyl chloride **85**, respectively, with β-alanine, according to the same procedure previously followed for compounds **55-61**.

2.3.4 Biological evaluation

_N_-{3-[4-(3-Arylsulfonylamine-propyl)-piperazin-1-_yl_]-propyl}-arylsulfonamides

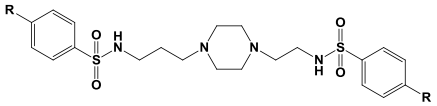


Compound	R_1	R_2	EC_{50}(μM)	CC_{50}(μM)	EC_{90}(μM)	SI
126	-Cl	-H	18.6	81.5	>64.1	4.4
127	-Cl	$-CH_3$	8.7	48.8	36.2	5.6
128	-Cl	-tBu	1.9	8.7	4.9	4.6
129	-Cl	$-CF_3$	10.1	68.7	33.5	6.8
130	-Cl	-Ph	2.1	3.9	-	1.9
131	-tBu	-H	3.8	18.8	-	4.9
132	-tBu	$-CH_3$	3.8	14.3	9.4	3.8
133	-tBu	$-CF_3$	7.4	25.5	13.8	3.4
134	-tBu	-Ph	1.5	4.1	3.1	2.7
135	$-CH_3$	-H	26.4	138	>66.7	5.2
136	$-CH_3$	-Ph	2.1	6.1	-	2.9
137	-Ph	-H	2.9	12.1	-	4.2
138	-H	$-CF_3$	11.9	70	-	5.9

Table 2.9: activity and cytotoxicity data for *N*-{3-[4-(3-arylsulfonylamine-propyl)-piperazin-1-*yl*]-propyl}-arylsulfonamides

Biological data for compounds **126-138** suggest that a different *para*-hydrophobic substitution in the two aromatic rings of the original scaffold is tolerated and does not decrease viral replication inhibition: the EC_{50} values for most compounds tested are in the range of 1-10 μM. Nevertheless, the small modifications carried out on the original structures seem to strongly affect cytotoxicity: for all newly synthesised compounds the selectivity index is below 10, and an increased toxic effect against cells appears to be the most evident trend for this series of compounds.

N-(3-(4-(2-(Arylsulfonamide)ethyl)piperazin-1-*yl*)propyl)arylsulfonamides

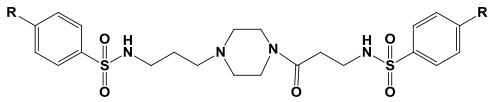


Compound	R	EC_{50}(μM)	CC_{50}(μM)	EC_{90}(μM)	SI
144	-Cl	5.6	32.5	-	5.8
145	-H	69.2	>214	212	>3.1
146	-tBu	1.7	5.09	-	3

Table 2.10: activity and cytotoxicity data for *N*-(3-(4-(2-(arylsulfonamide)ethyl)piperazin-1-*yl*)propyl)arylsulfonamides

Except for compound **145**, which does not show relevant activity nor cytotoxicity, unsymmetrical shortening of one aliphatic linker is associated with a retained antiviral effect, suggesting that symmetry is not an essential requirement for activity. Nevertheless, also in this second series of unsymmetrical compounds the small structural modification seems to be related to an increased cytotoxic effect.

N-(3-(4-(3-(Arylsulfonamide)propanoyl)piperazin-1-*yl*)-3-oxopropyl)aryl sulfonamides



Compound	R	EC_{50}(μM)	CC_{50}(μM)	EC_{90}(μM)	SI
148	-Cl	8.5	69.1	25.9	8.1
149	-H	>202	>202	>202	-
150	-Me	64.3	>191	173	3
151	-tBu	4.1	28	>4.9	6.8
152	-Ph	3.0	7.52	-	2.5

Table 2.11: activity and cytotoxicity data for *N*-(3-(4-(3-(arylsulfonamide)propanoyl) piperazin-1-*yl*)-3-oxopropyl)arylsulfonamides

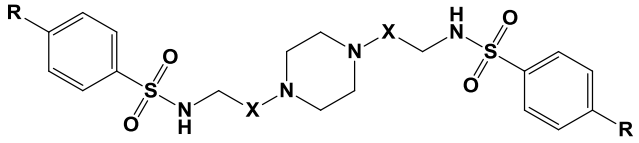
Biological results for the final family of unsymmetrical derivatives highlight the same trend found for the previous two series of compounds: antiviral potential seems to be retained by functionalising one of the piperazine nitrogens to amide, but toxic effect

against cells is enhanced, since the selectivity index for all new compounds is below 10. The only exception to this trend is again represented by the unsubstituted phenyl-derivative, compound **149**: as expected, it does not show antiviral activity, confirming the essential role played by a *para*-hydrophobic aromatic group for viral replication inhibition, but represents at the same time the unique analogue for which cytotoxic effect is not observed.

2.4 Conclusions

In the course of this first part of the study, the application of computer-aided techniques led to the identification of compound **12**, which showed antiviral potential against HCV replication in the replicon assay.

Starting from its structure, a series of modifications was designed to explore the potential activity associated with this molecule, and 61 new compounds were subsequently synthesised and sent for biological evaluation. The antiviral activity originally found for compound **12** was confirmed, and with the evaluation of its derivatives it was possible to identify several structural analogues with a retained antiviral effect against HCV replication.



Different structural modifications were explored in order to understand the role of aromatic substituents, sulfonamide groups, linker chains, piperazine central nucleus and symmetry of the original molecule. Two equal phenyl rings with a hydrophobic substituent in the *para* position are essential for antiviral activity, along with the presence of the two sulfonamide groups and the central piperazine ring. The length and nature of the two linker chains is also important for activity retention: a three-carbon aliphatic linker is required on both sides of the structure, while shortening or elongating either one or two of them is associated with loss of activity. The same effect can be observed with the functionalization of piperazine amine groups to amide, by inserting a carbonyl group in the terminal methylene group of the linker either by one side of the structure or both. Replacement of the piperazine central ring with the ones of phenylendiamine or piperidine is associated with loss of activity. The overall symmetry of the structure plays as well an important role for activity retention: the insertion of small asymmetry elements in the structure has a negative impact on the cytotoxicity, and most unsymmetrical derivatives prepared show low values of CC_{50} and SI.

Even if a clear trend was identified for the antiviral potential of this family of compounds, the modifications attempted so far did not lead to a significant improvement in terms of activity. For this reason, a further modification of the structure

of compound **12** was planned: a ligand-based *in silico* comparative analysis against known symmetrical inhibitors of the HCV NS3 helicase was performed, in order to replace and expand the central piperazine linker while maintaining the two sulfonamide groups and the symmetry of the molecule.

2.5 References

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Chapter 3

p-Phenylendiamines

3.1 Ligand-based optimisation of compound 12

With the aim to further modify the structure of compound **12**, its symmetrical scaffold was the object of a computer-based comparative analysis against the structures of known symmetrical HCV NS3 helicase inhibitors, in order to evaluate possible similarities in their chemical features.

Symmetrical known inhibitors considered are shown in figure 3.1.

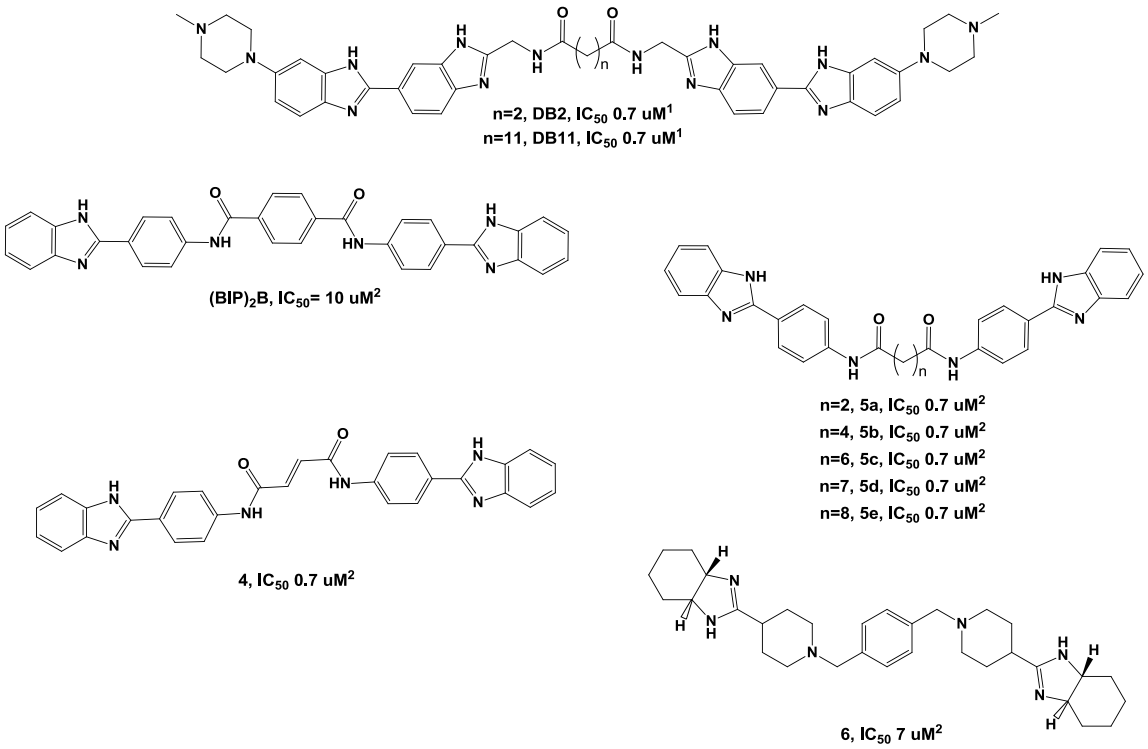


Figure 3.1: Chemical structures and inhibitory activities of known symmetrical inhibitors considered[1,2]

3.1.1 Flexible alignment of compound 12 and known symmetrical inhibitors

In order to identify a common structural pattern between the known inhibitors and compound **12**, the Flexible Alignment application available in MOE 2010.10 was used:[3] its main function is to flexibly align (or superimpose) small molecules by maximising steric and feature overlap while minimising internal ligand strain.[4]

For each alignment performed, the average internal strain energy U, the total mutual similarity score F (the lower value it assumes, the more similar two structures appear to

be), and the value of the objective function S (corresponding to the sum of U and F values for a given alignment, the lower the better) are evaluated. Alignments with a low S value usually correspond to the best alignments. Nevertheless, since the application balances intramolecular strain forces and intermolecular pharmacophoric features, pharmacophoric forces can overpower internal strain energies in a molecular system, giving an alignment with large similarity scores, associated with large positive dU values. A good alignment should present a dU value lower than 1 kcal/mol.

Once flexibly aligned the structure of compound **12** (in pink) to the ones of the known symmetrical inhibitors (in green), alignments with a dU value up to 3 kcal/mol were analysed, in order to evaluate potential structural overlapping that could be achieved by chemically modifying compound **12** (figures 3.2-3.9).

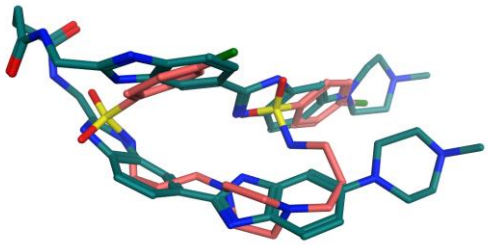


Figure 3.2: Aligned compound **12** and DB2

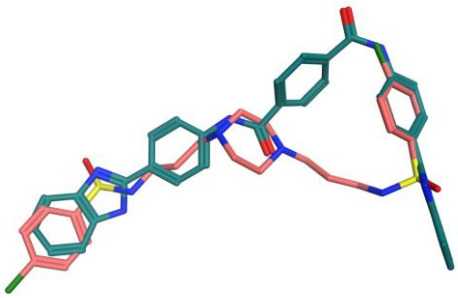


Figure 3.3: Aligned compound **12** and $(BIP)_2B$

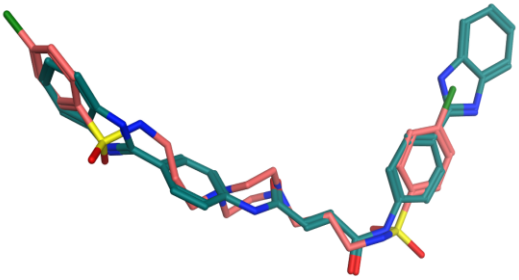


Figure 3.4: Aligned compounds **12** and **4**

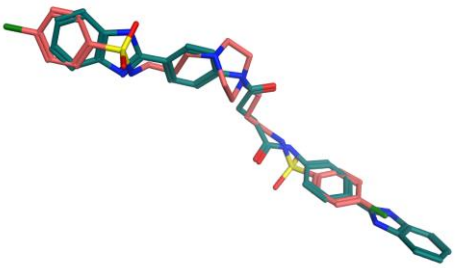


Figure 3.5: Aligned compounds **12** and **5a**

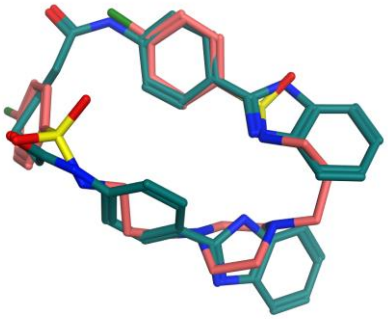


Figure 3.6: Aligned compounds **12** and **5b**

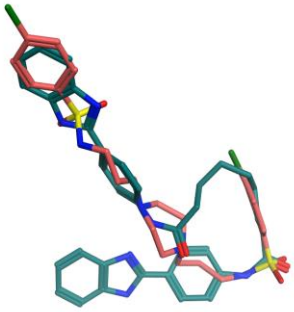


Figure 3.7: Aligned compounds **12** and **5d**

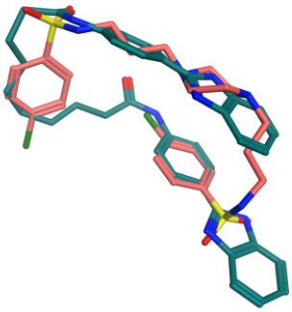


Figure 3.8: Aligned compounds **12** and **5e**

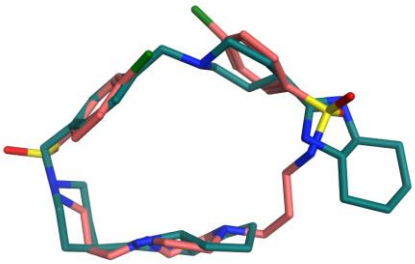


Figure 3.9: Aligned compounds **12** and **6**

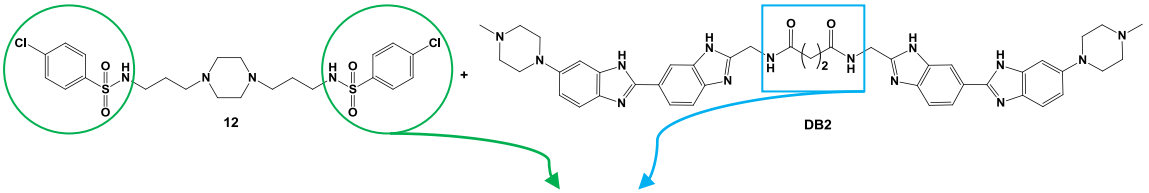
From the alignment results, the structure of compound **12** appears to be too short to allow a good spatial superimposition with the known inhibitors, but a common overlapping feature can be identified: the aromatic sulfonamide group of compound **12** shows good superimposition with the benzimidazole ring of the known inhibitors, in particular with its NH group, present in most of the structures analysed.
This overlapping feature was taken as the starting point to modify the scaffold of compound **12**.

3.1.2 Design of new derivatives of compound 12

The structures of four inhibitors, BD2, $(BIP)_2B$, compounds **4** and **5a**, were chosen as reference scaffolds for the optimisation of compound **12**. New derivatives were designed with the aim to maintain the two equal aromatic sulfonamide groups, while varying the linker between them, according to the evaluation of the conformational overlap with the corresponding known inhibitor. Both structures analysed in each pair

(reference known inhibitor and newly designed derivative) were kept as flexible in MOE's Flexible Alignment, as had previously been done for compound **12**.

Trying to combine the structures of compound **12** and DB2 in order for the sulfonamide groups to better overlap with the internal benzimidazole cycles, three new scaffolds were designed and analysed for their alignment with the reference inhibitor. Their structures and alignments are shown in table 3.1.



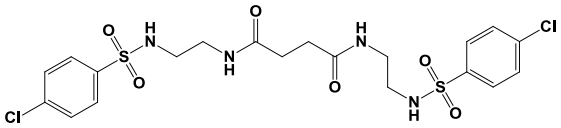
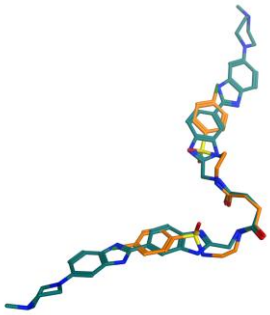
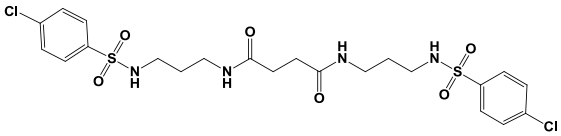
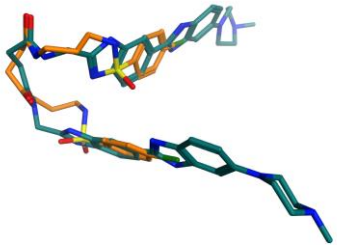
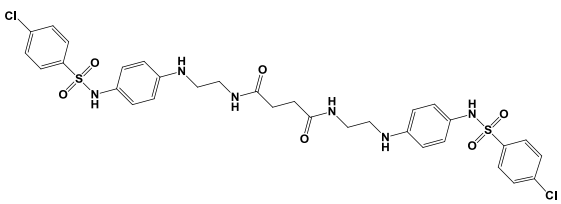
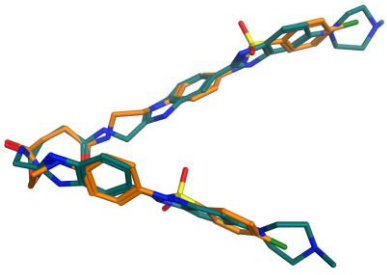
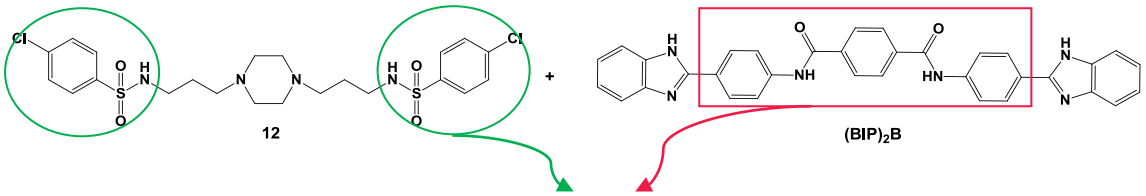
 Compound **155**: dU= 0 kcal/mol	
 Compound **156**: dU= 4 kcal/mol	
 Compound **157**: dU= 7 kcal/mol	

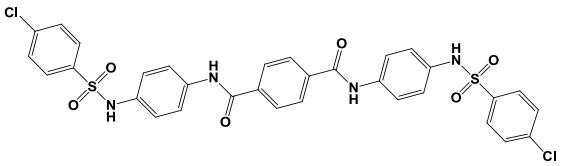
Table 3.1: Structures and alignment results of designed compounds **155-157**

All three new derivatives present an improved overlapping with the known inhibitor

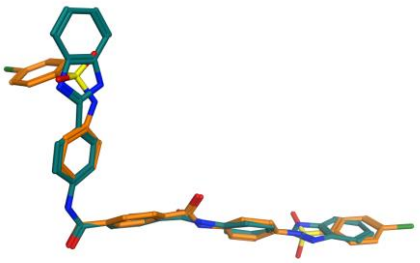
DB2, with the sulfonamide groups superimposing well with the internal benzimidazole portions of the reference compound. Among them, the scaffold of compound **155** was chosen to be further developed, since it corresponds to the only alignment with a dU value lower than 1 kcal/mol.

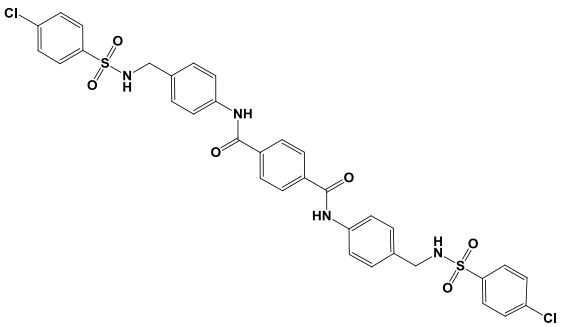
The same approach was applied for the design of new derivatives starting from the structural combination of compound **12** and $(BIP)_2B$, compound **4** and **5a**.





Compound **158**: dU= 0 kcal/mol





Compound **159**: dU= 0 kcal/mol

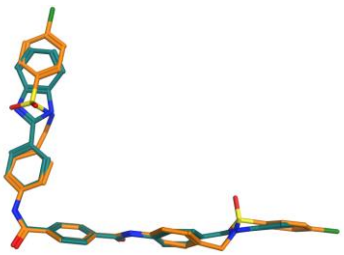
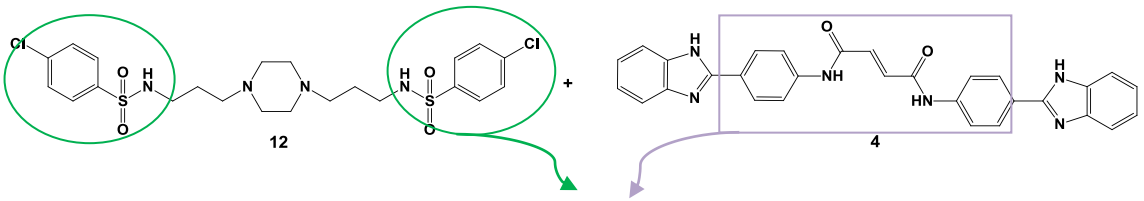


Table 3.2: Structures and alignment results of designed compounds **158**, **159**

Both new derivatives present a dU value equal to zero kcal/mol, but only the scaffold of compound **158** was chosen for further evaluation, since it was considered more accessible from a synthetic point of view.



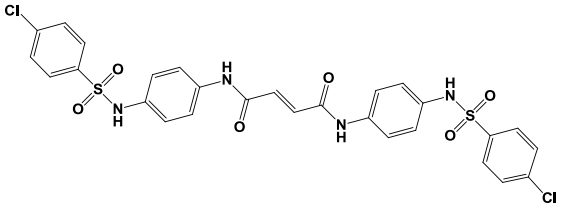
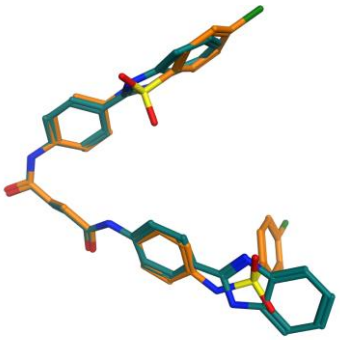
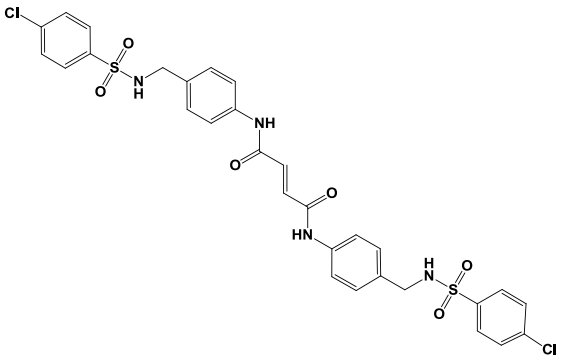
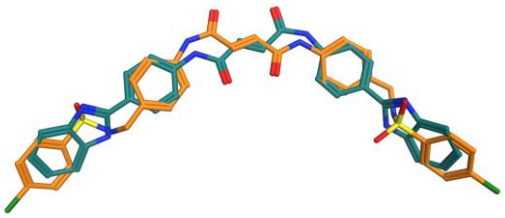
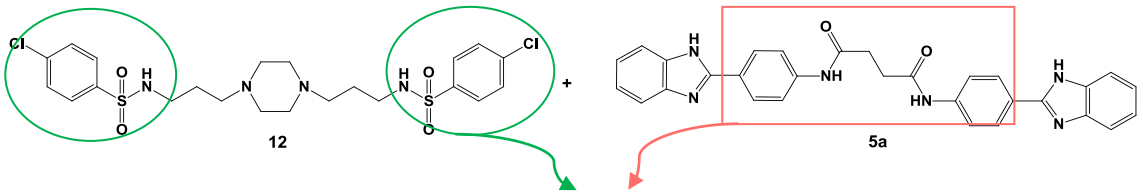
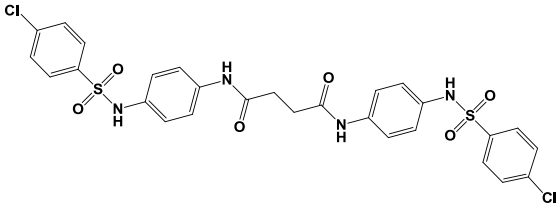
 Compound **160**: dU= 0 kcal/mol	
 Compound **161**: dU= 5.1 kcal/mol	

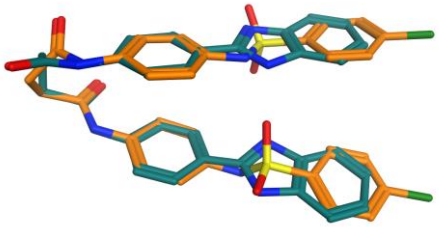
Table 3.3: Structures and alignment results of designed compounds **160**, **161**

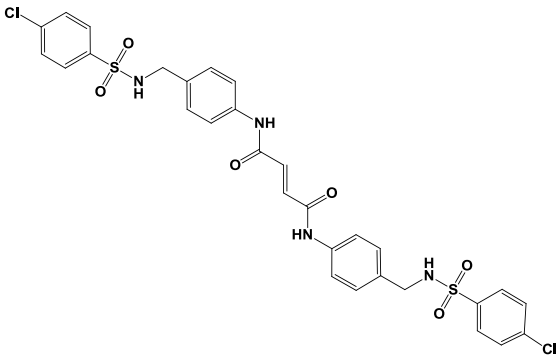
Between the two structures designed to achieve a good overlapping with compound **4**, only compound **160** shows a dU <1 kcal/mol: its structure was selected for further developement.





Compound **162**: dU= 0 kcal/mol





Compound **163**: dU= 0.7 kcal/mol

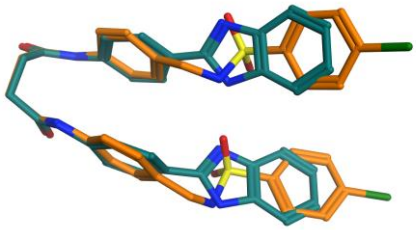


Table 3.4: Structures and alignment results of designed compounds **162**, **163**

Designed to achieve a better overlapping with compound **5a**, both compounds **162** and **163** are associated with dU values <1 kcal/mol. Compound **162** was more accessible synthetically, and its scaffold was chosen to be further developed.

In order to better validate the selected scaffolds of compounds **155**, **158**, **161** and **162**, their structures were analysed with a molecular docking study on the enzyme open conformation. The GlideSP docking algorithm was chosen for this purpose, using a 16 Å binding site grid derived from the 3KQH crystal structure.[5]

All the structures analysed demonstrate a good fitting of the RNA binding pocket, with a good spatial occupation of the region at the interface of the three main domains, and all show probable hydrophobic and hydrogen-bond interactions with several target residues, including Trp501, Arg393, Glu493, Thr411, Ser287, Asn556 and Phe557

(figure 3.10 a-d).

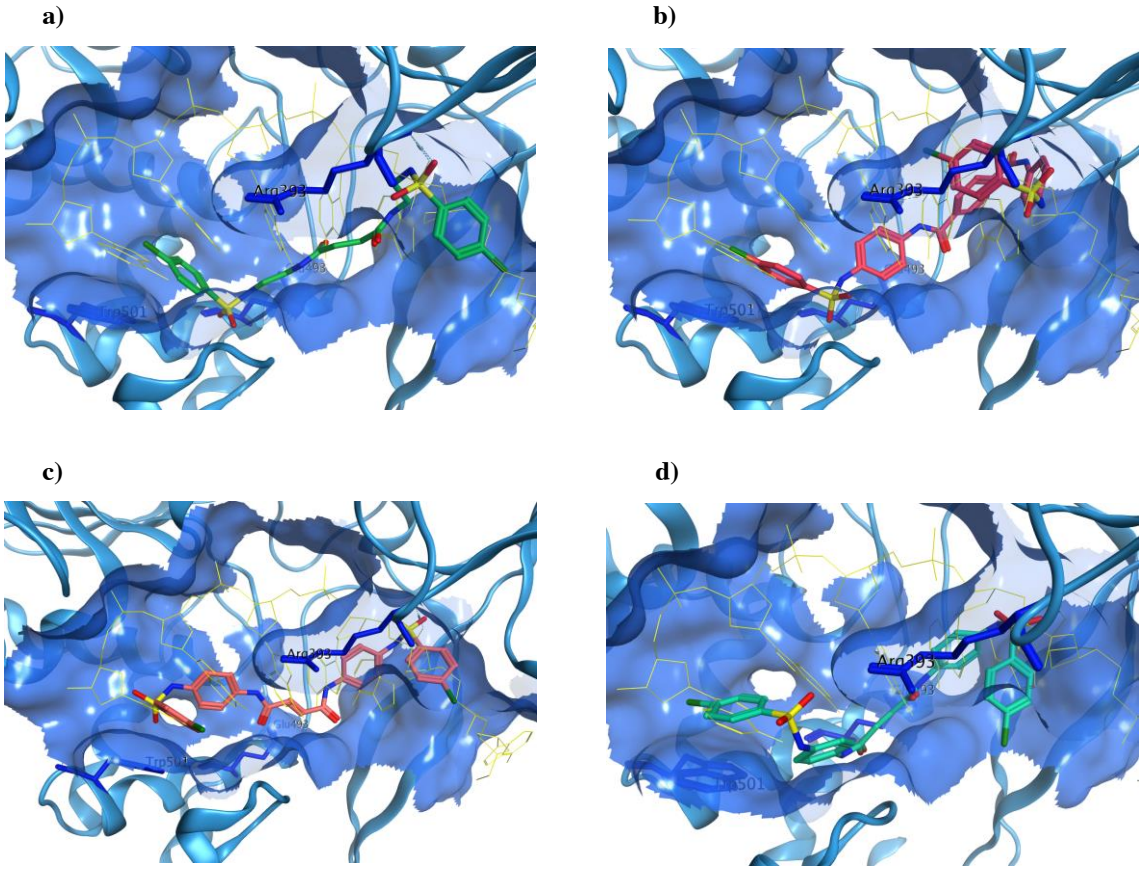


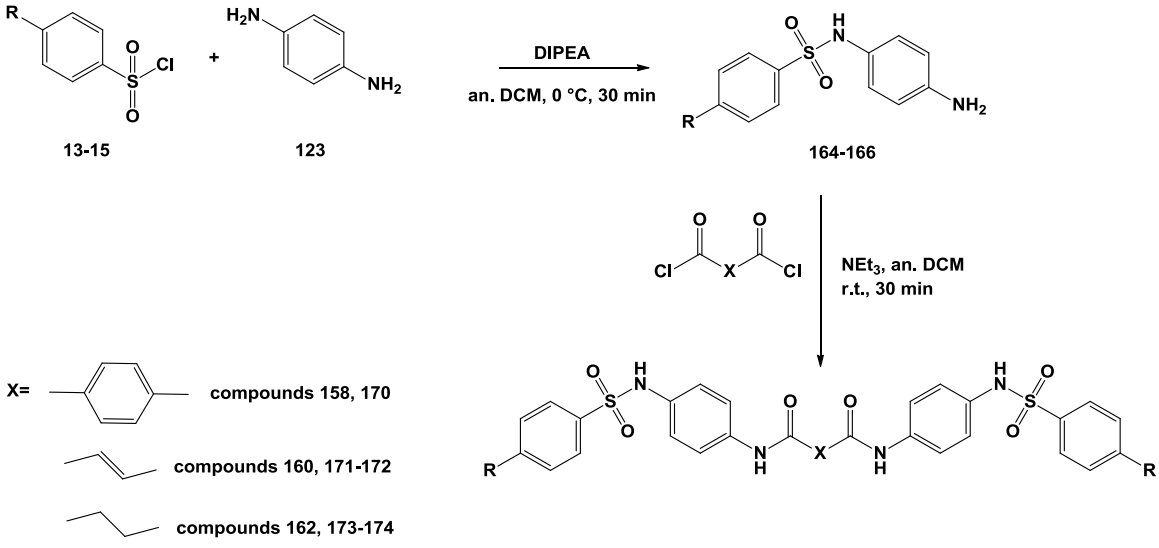
Figure 3.10 a-d: Predicted binding mode for compounds **155**, **158**, **160**, **162**, respectively

As their potential as helicase inhibitors was confirmed by a structure-based docking analysis, all four new scaffolds were chosen for synthetic development.

3.2 Synthesis of phenylendiamine- and ethylendiamine-based structures

The four new symmetrical scaffolds were synthesised according to two different procedures, a general one for compounds **158**, **160** and **162**, and a second one for compound **155**. Moreover, in the case of molecular structures **158**, **160** and **162**, different substituents in the *para* position of the sulfonamide aromatic rings were explored, in order to follow the initial SAR data obtained for compound **12**, where a hydrophobic group in this position had proven to be important for antiviral activity. In particular, a 4-chloro, 4-methyl and eventually an unsubstituted aromatic ring were chosen for a first series of new compounds.

The common synthetic pathway applied for the scaffolds of compounds **158**, **160** and **162** is shown in scheme 3.1.



Scheme 3.1: Common synthetic strategy applied for compounds **158**, **170**, **160**, **171-172**, **162**, **173-174**

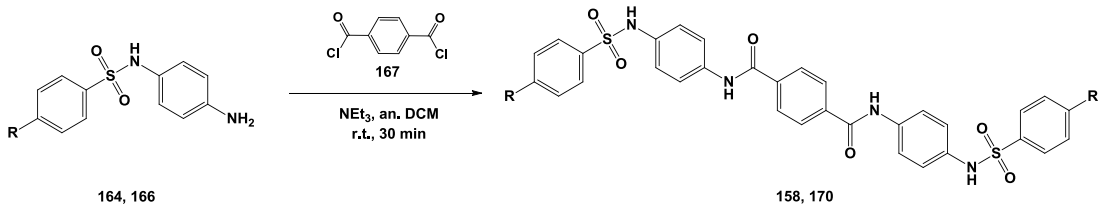
Synthesis of *N*-(4-amino-phenyl)-arylsulfonamides (164-166)

Two possible strategies were evaluated for the synthesis of common intermediates **164-166**. One option could have been to first react arylsulfonyl chlorides with 4-nitroaniline, in order to obtain the product of nucleophilic displacement of the chloride leaving group by the free aromatic amino group, and subsequently reduce the nitro function to amine to isolate the desired intermediate. Comparing yields and reaction times of reported

procedures, it was decided to follow a one-step synthetic pathway, and react the starting sulfonyl chloride with an excess of 4-phenylendiamine (**123**), in anhydrous conditions, using DIPEA to neutralise the hydrochloric acid formed.[6] The desired intermediates, product of the monosubstitution of one free amino group of compound **124**, were then purified by flash column chromatography.

3.2.1 *N,N'*-Bis-(4-arylsulfonylamino-phenyl)-terephthalamides (158, 170)

A first small series of two compounds was prepared by reacting intermediates **164** and **165** with terephthaloyl chloride (**167**), in order to obtain a double nucleophilic displacement of the two chloride leaving groups by the free aromatic amine group of the intermediates (scheme 3.2).

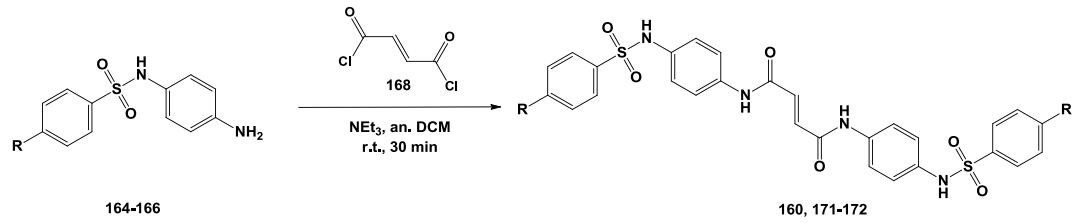


Intermediate	R	Yield %	Product	Yield %
164	-Cl	75	**158**	36
165	-Me	73	**170**	69

Scheme 3.2: Final step for the synthesis of compounds **158**, **170**

3.2.2 *N,N'*-Bis-(4-arylsulfonylamino-phenyl)-fumaramides (160, 171-172)

Three new derivatives were obtained with the scaffold of compound **160** by reacting an excess of intermediates **164-165** with fumaryl chloride (**168**) (scheme 3.3).

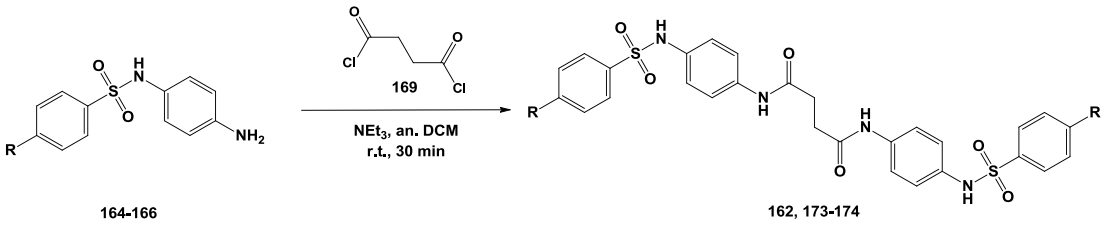


Intermediate	R	Yield %	Product	Yield %
164	-Cl	-	**160**	61
165	-Me	-	**171**	47
166	-H	77	**172**	39

Scheme 3.3: Final step for the synthesis of compounds **160**, **171-172**

3.2.3 *N,N'*-Bis-(4-(arylsulfonamido)phenyl)-succinamides (162, 173-174)

The same strategy applied for the previous compounds was followed to obtain a small series of new derivatives with the structural scaffold of compound **162**, by reacting an excess of intermediates **164-166** with succinyl chloride (**169**) (scheme 3.4).

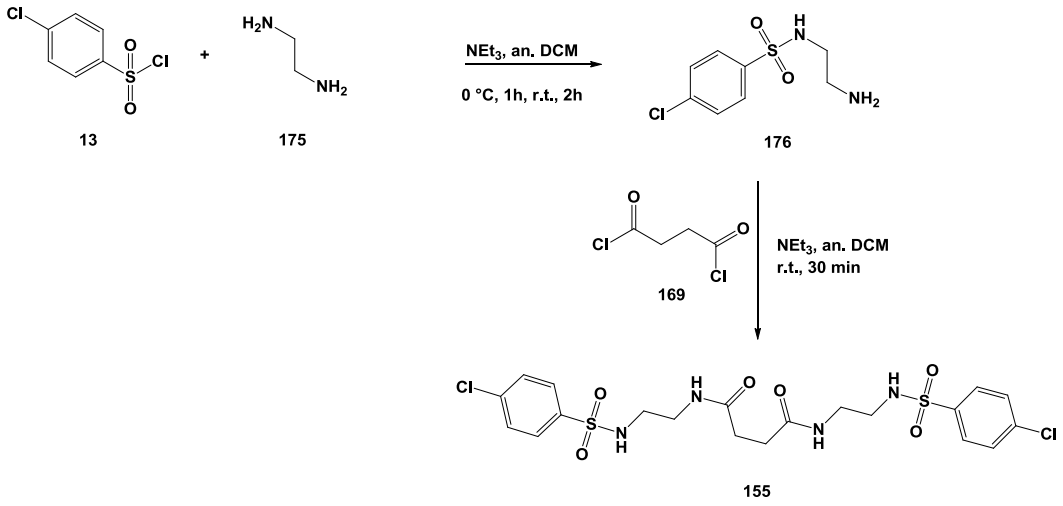


Intermediate	R	Product	Yield %
164	-Cl	**162**	48
165	-Me	**174**	36
166	-H	**174**	64

Scheme 3.4: Final step for the synthesis of compounds **162**, **173-174**

3.2.4 *N,N'*-Bis-(2-(4-chlorophenylsulfonamido)ethyl)-succinamide (155)

Compound **155** was obtained following the previous two-step synthetic strategy, with the only difference being the use of an excess of 1,2-diaminoethane **175** in the first reaction step (scheme 3.5).



13 175 176 169 155

Scheme 3.5: Synthetic pathway for compound **155**

Synthesis of *N*-(2-aminoethyl)-4-chlorobenzenesulfonamide (176)

Intermediate **176** was prepared by reacting 4-chlorobenzenesulfonyl chloride (**13**) with an excess of ethylendiamine **175**, in order to achieve a single nucleophilic displacement on the chloride leaving group.

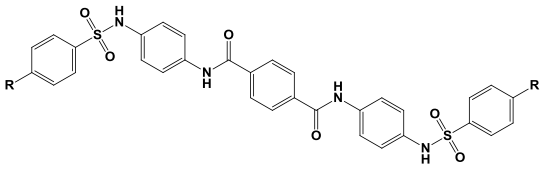
Synthesis of *N*,*N*'-bis-(2-(4-chlorophenylsulfonamido)ethyl)-succinamide (155)

Final compound **155** was obtained with a double nucleophilic displacement of the two leaving groups of succinyl chloride by the free amine group of intermediate **176**.

3.2.5 Biological evaluation

All new derivatives were evaluated for their potential inhibition of HCV replication in the subgenomic replicon assay,[7] as well as for their toxicity in the cytostatic assay. Their biological data are shown in tables 3.5-3.9.

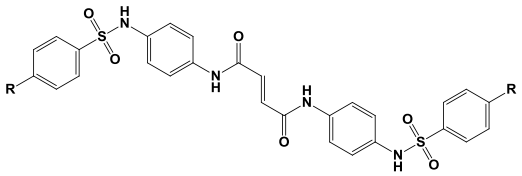
***N,N'*-Bis-(4-arylsulfonylamino-phenyl)-fumaramides**



Compound	R	EC_{50}(μM)	CC_{50}(μM)	EC_{90}(μM)	SI
158	-Cl	140.9	>143.8	>143.8	>1
170	-Me	25.2	>152.7	>152.7	>6

Table 3.5: Activity and cytotoxicity data for compounds **158**, **170**

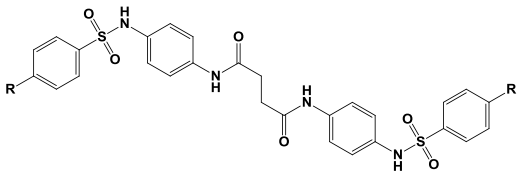
***N,N'*-Bis-(4-arylsulfonylamino-phenyl)-fumaramides**



Compound	R	EC_{50}(μM)	CC_{50}(μM)	EC_{90}(μM)	SI
160	-Cl	>154.9	>154.9	>154.9	-
171	-Me	42	>82.7	-	>2
172	-H	>173.4	>173.4	>173.4	-

Table 3.6: Activity and cytotoxicity data for *N,N'*-bis-(4-arylsulfonylamino-phenyl)-fumaramides

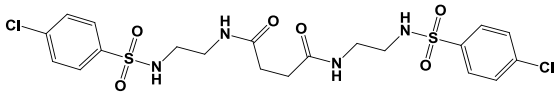
N,N'-Bis-(4-(arylsulfonamido)phenyl)-succinamides



Compound	R	EC_{50}(μM)	CC_{50}(μM)	EC_{90}(μM)	SI
162	-Cl	10.8	>154.4	140.7	>14.3
173	-Me	52.4	>164.8	>164.8	>3.1
174	-H	31.6	172.8	-	5.5

Table 3.7: Activity and cytotoxicity data for *N*-(3-(4-(2-(arylsulfonamido)ethyl)piperazin-1-yl)propyl)arylsulfonamides

N,N'-Bis-(2-(4-chlorophenylsulfonamido)ethyl)-succinamide



Compound	EC_{50}(μM)	CC_{50}(μM)	EC_{90}(μM)	SI
155	>181.3	>181.3	-	-

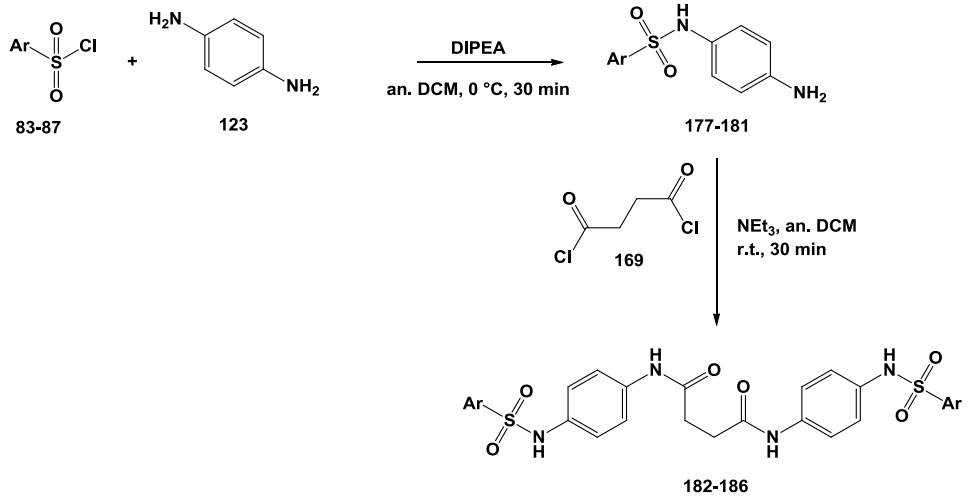
Table 3.8: Activity and cytotoxicity data for compound **155**

Among the new scaffolds designed to optimise compound **12**, compound **162** is associated with a positive antiviral effect against HCV replication, with an EC_{50} value that indicates retention of activity compared to compound **12**. The activity pattern of its analogues **173** and **174** seems to indicate the same effect for a *para*-hydrophobic substituent in the sulfonamide aromatic ring previously seen for the first structural analogues of compound **12**, where antiviral activity was positively correlated with an increased hydrophobic group in this position.

In order to better investigate the antiviral potential of compound **162**, its scaffold was chosen to be further modified.

3.2.6 Synthesis of a second series of *N,N'*-bis-(4-(arylsulfonamido)phenyl)-succinamides

The first modification designed for compound **162** was the substitution of the aromatic sulfonamide groups, in order to explore different hydrophobic groups in the *para* position and increase their occupational volume inserting a condensed aromatic ring.
The synthetic pathway followed for this new series of compounds was the same previously carried out for **162**: starting from the appropriate sulfonyl chloride (**83-87**), *N*-(4-amino-phenyl)-arylsulfonamides **177-181** were obtained in the first reaction step, and then reacted in excess with succinyl chloride (**169**) to give final products **182-186**.

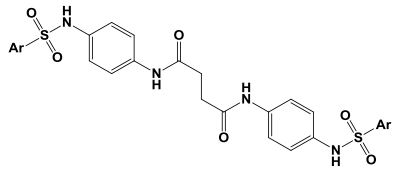


Intermediate	Ar	Yield %	Product	Yield %
177	4-tert-butylphenyl	82	**182**	39
178	4-trifluoromethylphenyl	93	**183**	42
179	4-biphenyl	69	**184**	44
180	1-naphthyl	84	**185**	53
181	8-quinoline	53	**186**	35

Scheme 3.6: Synthetic pathway for compounds **182-186**

3.2.7 Biological evaluation of compounds 182-186

The five new *N*,*N*'-bis-(4-(arylsulfonamido)phenyl)-succinamide derivatives were evaluated for their antiviral potential and cytotoxicity in the subgenomic replicon assay (table 3.9).[7]



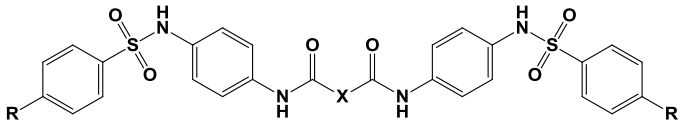
Compound	Ar	EC_{50}(μM)	CC_{50}(μM)	EC_{90}(μM)	SI
182	4-tert-butylphenyl	14.6	>144.7	34.1	>10.7
183	4-trifluoromethylphenyl	86.5	>139.9	>139.9	>1.6
184	4-biphenyl	12.2	>136.8	55	>11.2
185	1-naphthalene	32.9	>147.3	>147.3	>4.5
186	8-quinoline	>146.9	>146.9	>146.9	-

Table 2.20: Biological data for compounds **132-136**

As can be inferred from the data above, the most successful modification corresponds to compound **182**, where the presence of a *tert*-butyl substituent in the *para* position of the phenylsulfonamide groups is associated with activity retention and to an improved EC_{90} value. A similar trend is found for compound **184**, where the presence of a biphenylsulfonamide group is still associated with activity retention. The other three modifications attempted are related to loss of activity, thus confirming the importance of a *para*-hydrophobic substituent in the molecule, but suggesting a negative influence of a bicyclic or heterocyclic aromatic ring.

3.3 Conclusions

In silico comparative analyses of compound **12** against the structures of known symmetrical inhibitors of the HCV NS3 helicase led to the design of four new structural scaffolds, derived to maximise the functional overlapping shared between compound **12** and some of the known active molecules.



The newly designed compounds show the presence of the two equal phenyl-sulfonamide groups of compound **12**, while the linker was modified to achieve a better structural superimposition with the known symmetrical inhibitors. For each new structural family a small series of new derivatives was synthesised and sent for biological evaluation in the HCV replicon assay. One of these families showed antiviral activity in the range of the low micromolar concentration, and was therefore chosen for further development. Data found so far suggest that antiviral activity is associated with the presence of two equal phenylendiamine rings in the linker, connected by a central succinamide group. As had been found for compound **12**, a hydrophobic substituent in the *para* position of the two terminal phenyl groups is required for activity retention.

Due to the positive results initially obtained, a second series of new compounds was designed to explore different aromatic rings on the terminal part of the structure. Two new succinamide analogues, compound **182** and **184**, with a 4-*tert*-butylphenyl and a 4-biphenyl substituent on the two sulfonamide groups, respectively, were found to inhibit HCV replication with EC_{50} values in the range of the low μM. As had happened for the previous series of derivatives of compound **12**, a significant improvement in terms of antiviral activity was not achieved with the modifications attempted so far. With the aim to find more potent inhibitors of the HCV replication with different structural scaffolds, a new structure-based virtual screening was planned and carried out.

3.4 References

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Chapter 4

Thienopyrimidines

4.1 Structure-based virtual screening on the enzyme open conformation

The structure of the enzyme in the open-conformation, high-affinity complex with the single-stranded oligonucleotide substrate was considered for a structure-based evaluation of potential inhibitors of RNA binding. The 3KQH crystal structure (resolution 2.4 Å) was used for all analyses performed.[1]

In the high-affinity complex of the enzyme, domain 2 is shifted outwards from domain 1, and the substrate is located in a wide binding cleft within the interface of the three domains. In order to select a restricted region within this large RNA-binding space, the five structures corresponding to the three main steps of DNA unwinding (PDB IDs 3KQH, 3KQK, 3KQL, 3KQN, 3KQU) were compared to one another:[1] the aim was to identify a relatively fixed region, in which protein residues conserve their relative positions more than the other ones surrounding. A subsite with similar characteristic could represent a good target to inhibit substrate binding.

The sequences of the five structures were aligned and the residues defining the known RNA binding pocket were selected and evaluated with the RMSD function available in MOE 2010.10.[2] By measuring the Root Mean Square Deviation of the relative positions among different protein conformations, it was possible to identify the positional shift of each residue from its starting position, defined as the one found in the 3KQH crystal structure. Following this analysis, significant positional changes were observed for most selected residues, with the main deviations found in domain 2.

Due to the overall elevate of RMSD values, it was decided to focus attention on residues with RMSD values below 3 Å with respect to all the proteins (in blue in figure 4.1), and to consider as fixed regions including residues with RMSD values below 2 Å (in pink in figure 4.1).

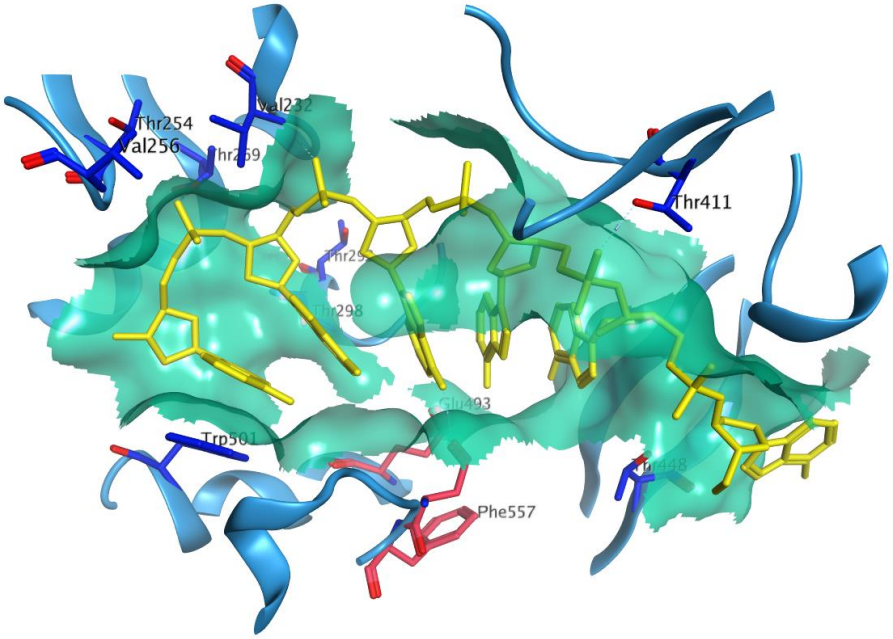


Figure 4.1: Residues with low RMSD values (<3 Å in blue, < 2Å in pink) shown within the 3KQH RNA binding cleft

The residues with the lowest RMSD were identified in Glu493, Asn556 and Phe557, located in the middle of the three domains: this region, and in particular a possible interaction with Glu493, that is essential for enzymatic activity, or the close Asp296, was chosen as the target for the identification of potential inhibitors.

A new pharmacophoric model was built to pre-screen the SPECS database;[3] based on the mutagenesis studies reported for HCV NS3 helicase,[4] the residues essential for enzymatic activity located within the known RNA binding pocket were identified: Thr269, Arg393, Thr411, Glu493 and Trp501 (figure 4.2).

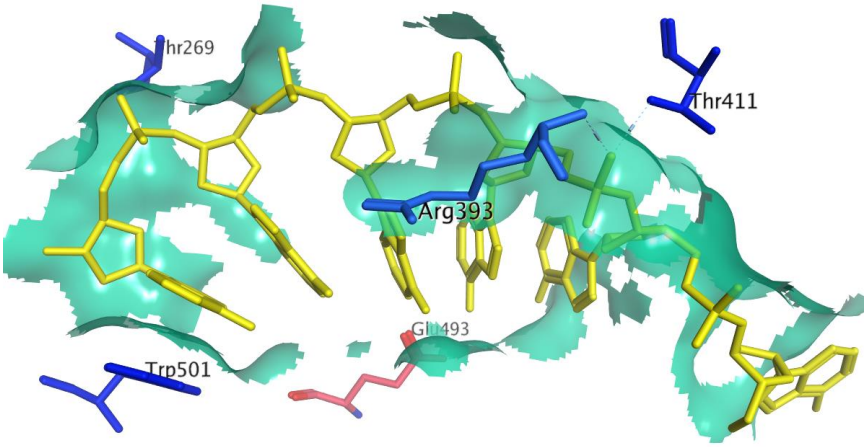


Figure 4.2: Essential residues for helicase activity within the DNA binding cleft

These residues were considered for the pharmacophoric filter, along with the occupation of the region in the middle of the three domains, included in the query as a polar interaction with either Glu493 (F2:Don in figure 4.3) or Asn556 (F3:Don), none of which was set as essential. Other three features were added to target Trp501 (F1:Aro|Hyd), Arg393 (F5:Acc|Ani), both required as essential, and Thr411 (F4:Ani|Acc).

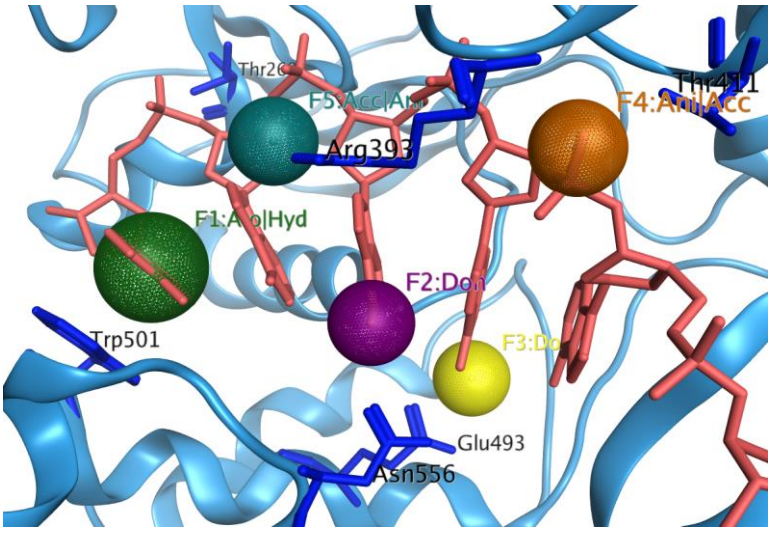


Figure 4.3: Pharmacophoric model based on the 3KQH crystal structure

Once added the exclusion volumes corresponding to the protein surface, a partial match of four was required, and approximately 3000 matching hits were selected for the

docking phase.

In the first virtual screening on the 3KQN structure (Chapter 2.1), the Glide docking program had shown the best potential in locating the greatest number of compounds within the RNA binding cleft, while with Plants and FlexX many structures had to be discarded due to their placement in different subsites. For this reason, it was decided to use the only Glide docking algorithm, run in the standard precision SP mode.[5] The output poses were re-scored with the Glide extra precision scoring function XP, that has been proven to increase effective binding prediction potential after SP docking,[6] Plants ChemPLP and FlexX scoring functions,[7-8] which were combined in a consensus scoring procedure (Chapter 2.1). The 25% best performing structures were visually inspected to identify which ones could best interact with the selected residues and fit the target defined by Glu493 (figure 4.4).

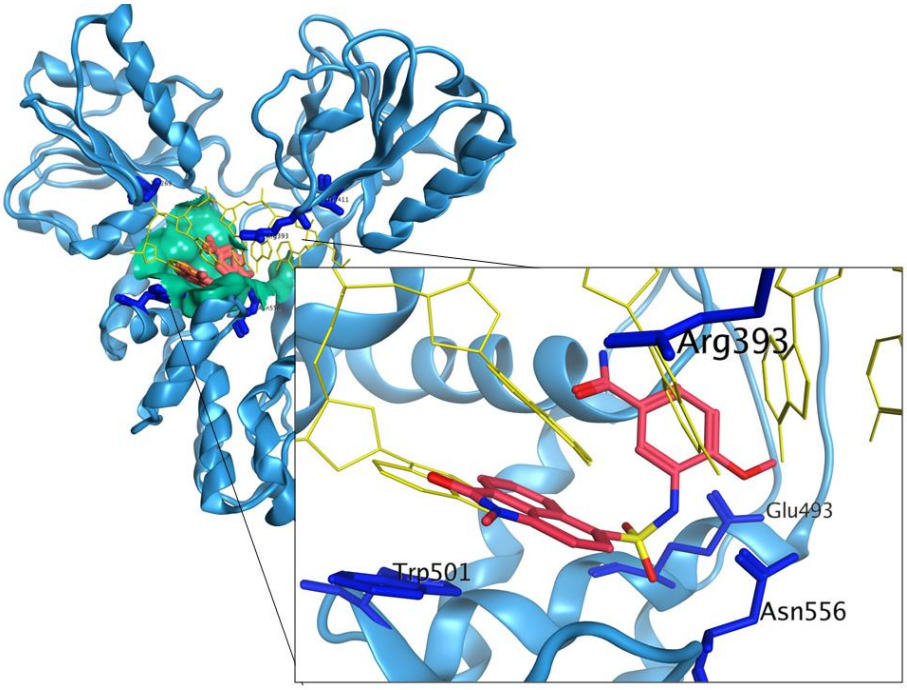


Figure 4.4: Ideal binding mode considered within the 3KQH target site

Twenty-one new compounds were purchased from the SPECS company and tested in the HCV replicon assay (Appendix I). One of them, compound **187** (figure 4.5), showed antiviral activity against HCV replication, with an EC_{50} value lower than 1μM.

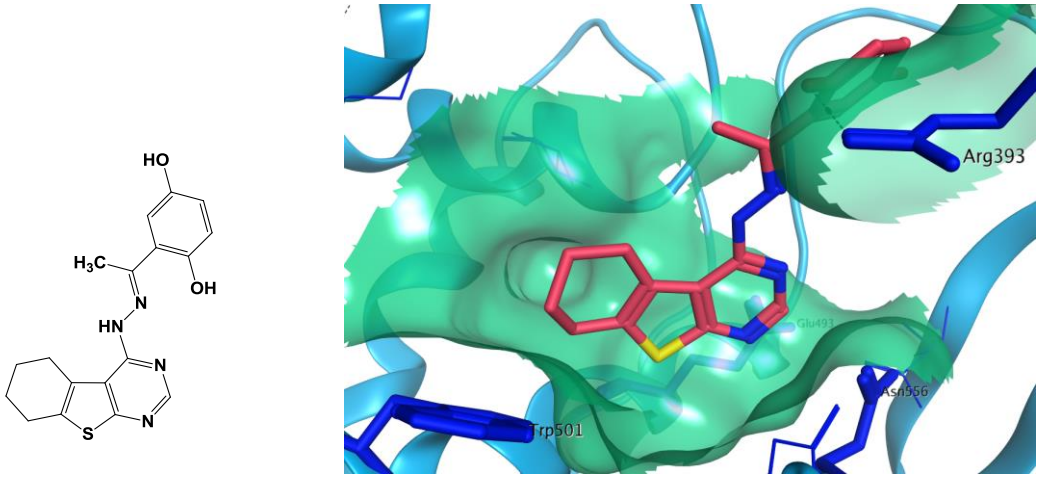


Figure 4.5: Structure and predicted binding mode for compound **187**

As shown in figure 4.5, the predicted binding mode found for compound **187** suggests a good spatial occupation of the target site, with the cyclohexyl portion of the molecule in close proximity to Trp501, the pyrimidine ring filling the space defined by Glu493 and Asn556, and the opportunity of hydrogen-bond formation between the 2'-hydroxyphenyl group and Arg393 lateral chain.

Given its potential to inhibit HCV replication, compound **187** was chosen for further investigation.

Moreover, a structural parallelism could be observed between compound **187** and previously published helicase inhibitors QU663 and compound **7** (figure 4.6).[9]

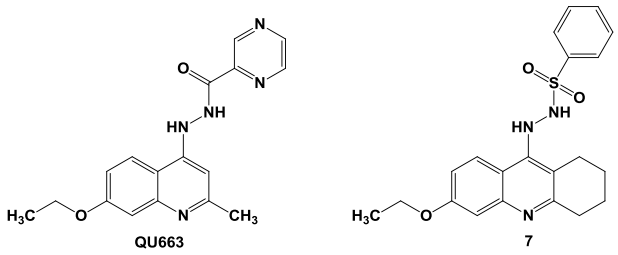


Figure 4.6: Chemical structure of QU663 and compound **7**

Occupational volume and predicted binding mode for compound **187** were found to be similar to the ones of the known inhibitors, further confirming the potential of this hit molecule as a selective NS3 helicase inhibitor.

4.2 Synthesis of tetrahydrobenzo[*b*]thienopyrimidines

Compound **187** presents a central thieno-pyrimidinic nucleus, which is condensed with a tetrahydrobenzene system in position 4 and 5 of the thiophene ring, and functionalised with a 2,5-dihydroxyphenyl-ethylidene moiety via a hydrazone linker in position 4 of pyrimidine (figure 4.7).

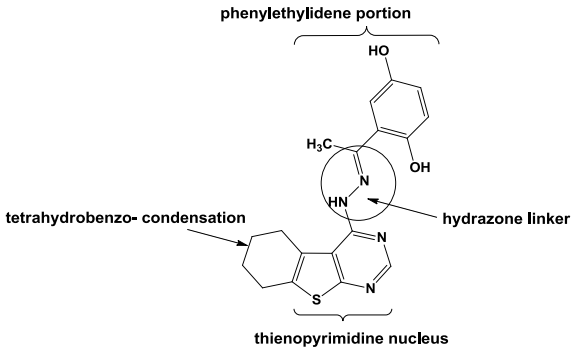
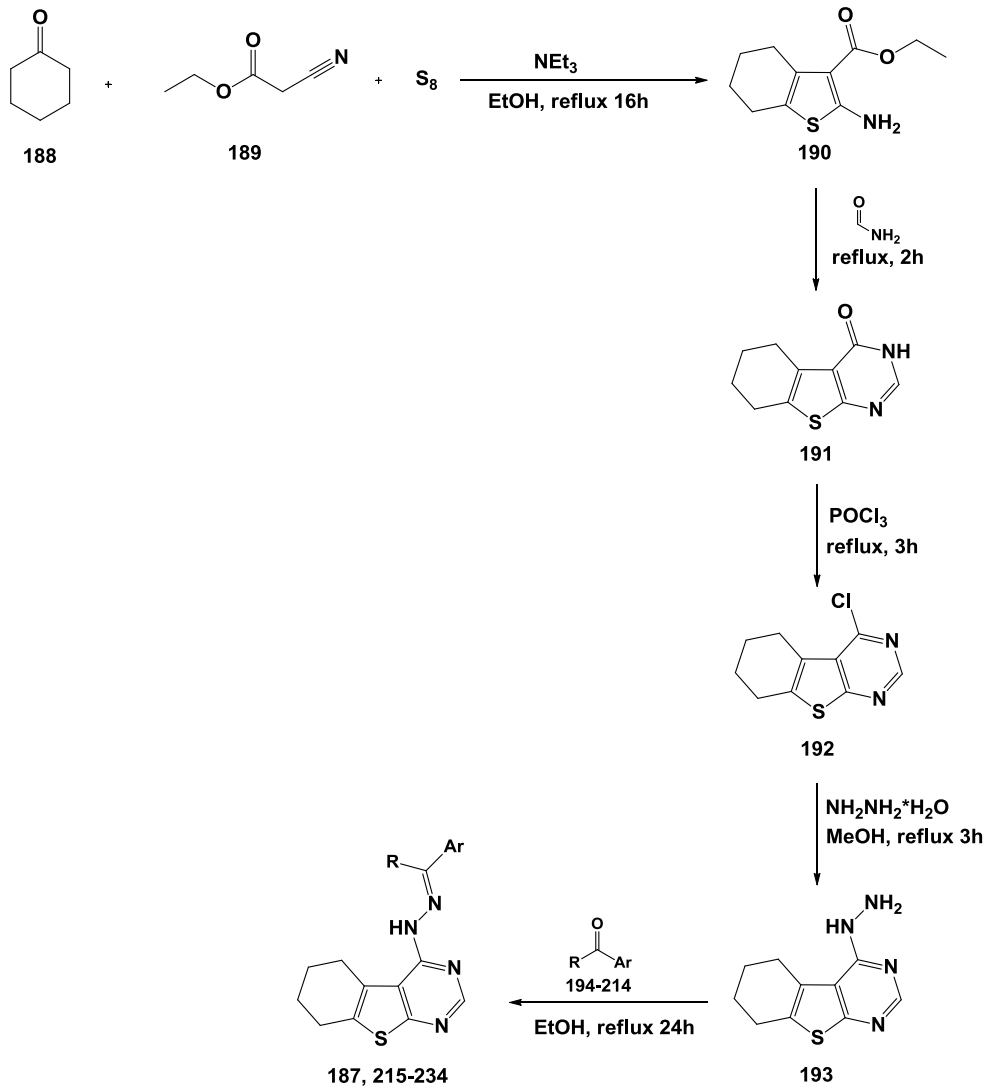


Figure 4.7: Structural features of compound **187**

Compound **187** was resynthesised, in order to confirm the activity values found for the batch bought from the SPECS company, and a first series of new derivatives was prepared to explore different aromatic substituents on the phenylethylidene portion.

4.2.1 (5,6,7,8-Tetrahydro-benzo [4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazones (187, 215-234)

Following reported procedures for the preparation of tetrahydrobenzothieno-pyrimidines, the synthetic pathway shown in scheme 4.1 was planned to resynthesise compound **187** and prepare a first series of new analogues.[10-11]



Scheme 4.1: Synthetic pathway for the synthesis of compounds **187**, **214-234**

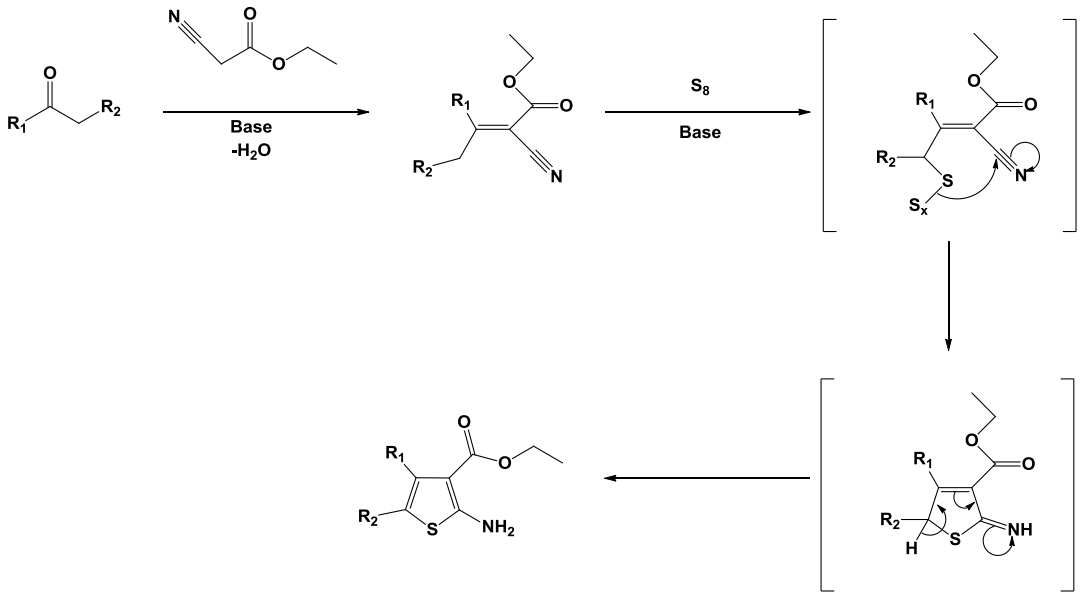
The planned strategy started with the preparation of 2-amino-4,5,6,7-tetrahydrobenzo[*b*] thienophene-3-carboxylic acid ethyl ester (**190**) through a Gewald reaction, followed by treatment of **190** with an excess of formamide to obtain cyclic pyrimidinone **191**, that was subsequently chlorinated using phosphorus oxychloride to give compound **192**. Aromatic nucleophilic displacement of the chloride leaving group with aqueous hydrazine was carried out to form aromatic hydrazine derivative **193**, which was finally used to obtain a Schiff base with ketones **194-203** and aldehydes **204-214**, giving desired compound **187** and its analogues **215-234**.

In order to obtain a preliminary SAR for the scaffold of compound **187**, it was decided to react final intermediate **193** with readily available, differently substituted benzaldehydes and acetophenones, changing only the last step in the reaction scheme to prepare several new derivatives.

The first four common steps of the synthetic route needed to be optimised, due to a significant loss in yield subsequent to the scale up of the starting materials, to allow the use of fourth intermediate **193** for the formation of multiple final products.

Synthesis of ethyl 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thienophene-3-carboxylate (190)[12-13]

A widely used method to prepare poly-substituted 2-aminothiopenes is the Gewald reaction, which involves the condensation of a ketone or aldehyde with an α-cyanoester in the presence of elemental sulphur and a base. The postulated mechanism for this reaction is shown in scheme 4.2.[14-16]



Scheme 4.2: Postulated mechanism for the formation of 2-amino substituted thiophenes

The first step for the formation of the thiophene ring is a Knoevenagel condensation between the ketone and the α-cyanoester, to produce a stable intermediate to which elemental sulphur is added by a mechanism which is still unclear. The intermediate formed is postulated to undergo cyclisation and tautomerisation to give the final poly-substituted 2-aminothiophene.

Compound **190** was obtained by heating cyclohexanone, ethyl cyanoacetate and sulphur at reflux in absolute ethanol, after adding triethylamine.

By changing the reaction time from 16 to 24 h and the purification procedure from flash column chromatography to recrystallisation from $EtOH/H_2O$, it was possible to improve the yield of this step from 70 to 85%.

Synthesis of 5,6,7,8-tetrahydro-3*H*-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-one (191)

Thienopyrimidinones and their derivatives can be obtained by reaction between 2-amino-3-ethoxycarbonylthiophenes and amides, through the formation of intermediate amidines, that undergo an intramolecular cyclisation to give thienopyrimidinones.[17]

Along with compound **190**, formamide was used in excess, as reagent and solvent. The yield was improved from 50 to 93% by refluxing the mixture for 8 h instead of 2 h, and purifying the product by recrystallisation from EtOH/H_2O.

Synthesis of 4-chloro-5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidine (192)

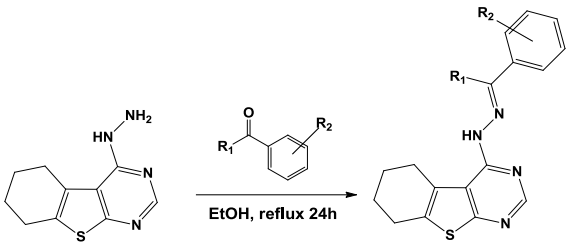
Following reported procedures, pyrimidone derivative **191** was chlorinated by refluxing with an excess of the chlorinating agent, phosphoryl chloride, to afford the formation of the thienopyrimidine system in compound **192**.[12] The yield of this third reaction step was optimised from 20 to 79% by refluxing the mixture for 8 h instead of 3 h, and changing the purification method from recrystallisation from MeOH to flash column chromatography.

Synthesis of (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (193)

Hydrazine derivative **193** was prepared through a nucleophilic aromatic substitution of the chloride leaving group on the pyrimidine system, by reacting compound **192** with an excess of hydrazine monohydrate, and refluxing the mixture in methanol. Desired product was obtained with an improved yield, from 70 to 89%, by refluxing the reaction for 8 h instead of 3 h and changing the recrystallisation system from MeOH to EtOH/H_2O.

Synthesis of (5,6,7,8-tetrahydro-benzo [4,5]thieno[2,3 *d*]pyrimidin-4-*yl*)-hydrazones (187, 215-234)

A first series of twenty new derivatives was readily prepared, along with compound **187**, through the formation of a Schiff base between compound **193** and differently substituted acetophenones (**194-203**) and benzaldehydes (**204-214**), by refluxing the two starting materials in ethanol for 24 h.

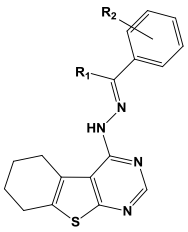


Aldehyde/ketone	Product	R_1	R_2	Yield %
194	**187**	Me	2,5-OH	66
195	**215**	Me	H	62
196	**216**	Me	4-OMe	28
197	**217**	Me	3,4,5-OMe	74
198	**218**	Me	3-F, 4-OMe	75
199	**219**	Me	3,4-O-CH_2-O	27
200	**220**	Me	4-NO_2	76
201	**221**	Me	4-C_6H_{11}	76
202	**222**	Me	2-OH	98
203	**223**	Me	3-OH	99
204	**224**	H	H	46
205	**225**	H	4-OMe	75
206	**226**	H	2-OMe	75
207	**227**	H	3-OMe	57
208	**228**	H	3,4,5-OMe	91
209	**229**	H	3,4-OEt	77
210	**230**	H	4-OH	34
211	**231**	H	4-Br	36
212	**232**	H	3-Br	65
213	**233**	H	4-tBu	61
214	**234**	H	4-iPr	38

Scheme 4.3: Synthesis of final compounds **187**, **215-234**

4.2.2 Biological evaluation of compounds 187, 215-234

Before proceeding with further modifications on the structure of compound **187**, the first series of tetrahydrobenzo[*b*]thienopyrimidines prepared was evaluated for potential antiviral activity in the HCV replicon assay,[18] as well as for associated cytotoxicity, in order to obtain a rational criterion for further studies.



Compound	R_1	R_2	EC_{50}(μM)	CC_{50}(μM)	EC_{90}(μM)	SI
193	-	-	23	243	45.1	10.6
187	Me	2,5-OH	<1	>282	135	>282
215	Me	H	33.6	53.4	>310	1.6
216	Me	4-OMe	17.5	86	>284	4.9
217	Me	3,4,5-OMe	17	43.5	>242	2.6
218	Me	3-F, 4-OMe	>270	5.9	>270	-
219	Me	3,4-O-CH_2-O	28.5	116	-	4.1
220	Me	4-NO_2	60.8	192	-	3.2
221	Me	4-C_6H_{11}	61.2	235	>309	3.8
222	Me	2-OH	2.28	154	4.36	67
223	Me	3-OH	403	>443	>443	>1.1
224	H	H	23.8	41.9	-	1.8
225	H	4-OMe	26.3	40.4	>295	1.5
226	H	2-OMe	6.8	45.6	-	6.7
227	H	3-OMe	21.3	32.9	-	1.5
228	H	3,4,5-OMe	121	204	-	1.7
229	H	3,4-OEt	>252	>252	>252	-
230	H	4-OH	24.8	40.6	-	1.6
231	H	4-Br	15.4	54.7	30	3.6
232	H	3-Br	8.04	60.2	19.6	7.5
233	H	4-tBu	7.83	>274	>274	>35.1
234	H	4-iPr	11.3	178	-	15.8

Table 4.1: Biological data for compounds **187**, **215-234**

Considering biological results, position and nature of the two hydroxy substituents on the phenylethylidene system seem to play an important role for antiviral potential: all attempted modifications in compounds **215-234** were associated with loss of activity, except for compound **222**, where activity improved for the EC_{90} value.

Most of the antiviral potential seems to be associated with the hydroxy substitution in position 2: compound **223**, with the sole 3'-hydroxy group, shows a significant loss of activity in comparison to compound **187**.

As for the phenylidene derivatives **224-234**, at a first evaluation the absence of the phenylethylidene methyl group seems associated with an increased toxicity against cells, showing most of these derivatives low values of CC_{50} and SI. Activity seems to be partially retained only in compound **233**, where the hydrophobic substituent in *para* position is associated with a reduced cytotoxicity while a relatively low EC_{50} value is preserved.

In order to further explore antiviral potential of compounds **187** and **222**, three new series of derivatives were designed. The first new modification was to study the role of the two hydroxy groups and their possible replacement; the second had the purpose of modifying the hydrazone linker, known to be poorly stable in aqueous systems, with a sulfonyl hydrazide and a hydrazide function, respectively, thus increasing stability in water, while maintaining the same length of the original functional group.

4.2.3 Design of a second series of 5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*] pyrimidin-4-*yl*)-hydrazones

Aiming to understand the role of the two hydroxy groups, in particular in position 2', a second series of *N*-(1-aryl-ethylidene)-*N*'-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazines was designed, to change the position of the hydroxy groups from 2 and 5 to 4 in the phenylethylidene ring, and to replace them with methoxy and halogen groups (chlorine and fluorine).

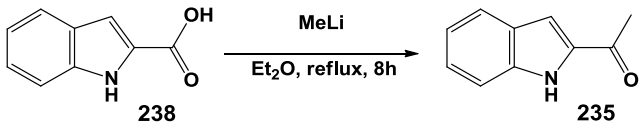
Moreover, in order to mimic polarity of the original substituents and opportunity of hydrogen bond formation, it was planned to replace the phenyl ring with heteroaromatic systems, such as pyrazine, 2-pyridine, indole and benzimidazole, by reacting their ethanone derivatives with final intermediate **193**. The insertion of a pyrazine and 2-pyridine system in this part of the molecule was also supported by the structural similarity observed for compound **187** and helicase inhibitors QU663 and compound **7**.[9] Since the presence of a pyrazine or 2-pyridine group had been demonstrated to be important for inhibitory activity of the published compounds, these same modifications were attempted for compound **187**.

Once synthetically obtained non-commercially available 1-(1*H*-indol-2-yl)ethanone (**235**) and 1-(1*H*-benzo[*d*]imidazol-2-*yl*)ethanone (**236**), 14 new derivatives were prepared following the same synthetic route previously used, where only the last reaction step needed to be changed to obtain different final compounds.

In order to understand the function of the phenylethylidene methyl group for antiviral activity, it was decided to synthesise 2,5-dihydroxy derivative without this methyl function, reacting intermediate **193** with 2,5-dihydroxybenzaldehyde (**191**), according to the usual strategy.

Synthesis of 1-(1*H*-indol-2-*yl*)ethanone (235)

The 2-acetylate form of indole was obtained starting from indole-2-carboxylic acid (**238**), converted to the desired product using methyllithium as a nucleophilic methyl source, as shown in scheme 4.4.

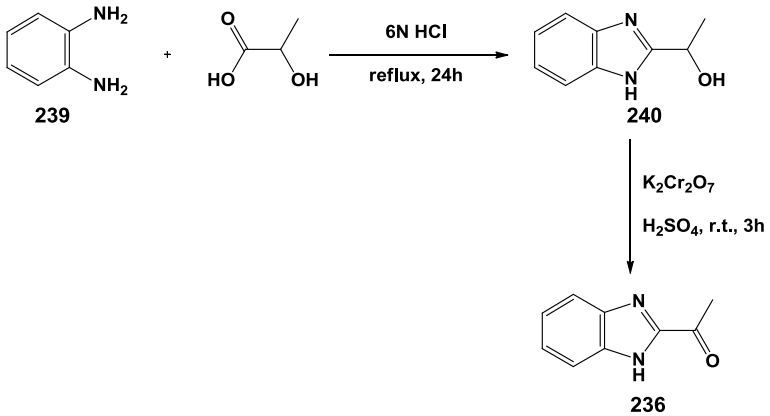


Scheme 4.4: Synthesis of ethanone **235**

Following reported procedures, the reaction was performed at 0°C in anhydrous diethyl ether, in order to control the methylating agent high reactivity towards water.[19]

Synthesis of 1-(1*H*-benzimidazol-2-*yl*)-ethanone (236)

A two-step synthetic approach was followed, as shown in scheme 4.5, to prepare the acetylate form in position 2 of benzimidazole.



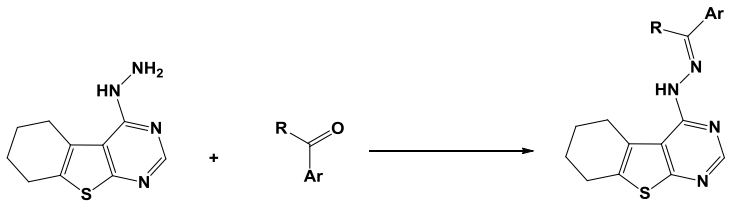
Scheme 4.5: Synthetic strategy for ethanone **236**

Following reported procedures, the 2-substituted benzimidazole nucleus was first obtained through an acid-catalysed ring condensation between *o*-phenylenediamine **239** and D,L-lactic acid, with the formation of a secondary alcohol intermediate, 1-(1*H*-

benzimidazol-2-*yl*)-ethanol (**240**). The secondary alcoholic function of intermediate **240** was subsequently oxidised to ketone using potassium dichromate in diluted sulphuric acid, following an oxidoreduction reaction.[20]

Synthesis of (5,6,7,8-tetrahydro-benzo [4,5]thieno[2,3 *d*]pyrimidin-4-*yl*)-hydrazones (252-265)

Newly designed compounds **252-265** were prepared according to the previously optimised synthetic strategy, by reacting final intermediate **193** in the fifth step with ketones **241**, **242-251**, **235**, **236** and 2,5-dihydroxy-benzaldehyde (**237**).



Aldehyde/ketone	R	Ar	Product	Yield %
241	Me	4-hydroxy-phenyl	**252**	66
237	H	2,5-dihydroxyphenyl	**253**	61
242	Me	2,5-dimethoxyphenyl	**254**	41
243	Me	2-methoxyphenyl	**255**	51
244	Me	3-methoxyphenyl	**256**	35
245	Me	2,5-dichlorophenyl	**257**	74
246	Me	2-chlorophenyl	**258**	94
247	Me	4-chlorophenyl	**259**	77
248	Me	2,5-difluorophenyl	**260**	45
249	Me	2-fluorophenyl	**261**	42
250	Me	pyrazine	**262**	55
251	Me	2-pyridine	**263**	81
235	Me	2-indole	**264**	53
236	Me	2-benzimidazole	**265**	42

Table 4.2: Newly synthesised compounds **252-265**

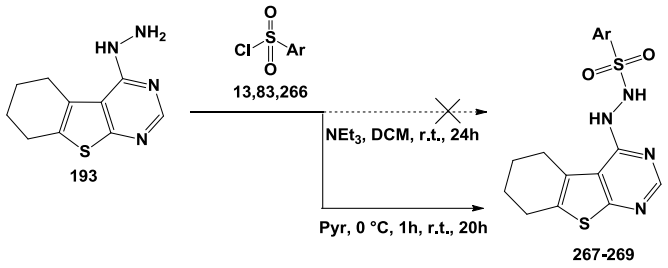
4.2.4 *N*'-(5,6,7,8-Tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-sulfonyl-hydrazides (218-220)

In a first attempt to replace the poorly stable hydrazone linker, it was thought to insert a sulfonyl hydrazide group in the molecule, thus maintaining the same length of the

original structure. Three derivatives with this linker were obtained by reacting final intermediate **193** with sulfonyl chlorides **13**, **83**, **266**.

Due to the impossibility to preserve the original hydroxy groups in the starting arylsulfonyl chlorides, on the basis of the residual activity of compound **233**, a hydrophobic *para* substituent was chosen for aryl sulfonyl hydrazides, in particular 4-*tert*-butyl and 4-chloro, along with a 2-pyridine system, in an attempt to reproduce polarity associated with the 2'-hydroxy group.

The synthetic strategy applied is shown in scheme 4.6.

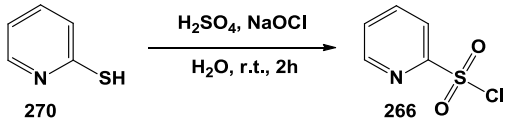


Aryl sulfonyl chloride	Ar	Product	Yield %
83	4-*tert*-butylphenyl	**267**	38
13	4-chlorophenyl	**268**	46
266	2-pyridine	**269**	34

Scheme 4.6: Synthetic strategies for compounds **267-269**

Synthesis of 2-pyridinesulfonyl chloride (266)

2-Pyridinesulfonyl chloride was not commercially available, and it was therefore synthesised, according to reported procedures, by oxidising the thiol group of 2-mercaptopyridine (**270**) to sulfonyl chloride, using NaOCl and sulphuric acid, as shown in scheme 4.7.[21]



Scheme 4.7: Synthesis of 2-pyridine sulfonyl chloride **266**

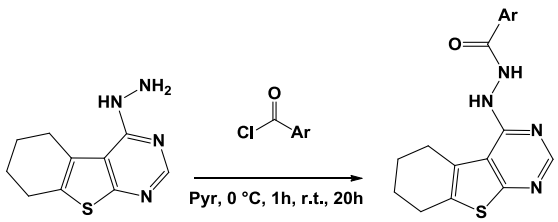
Synthesis of *N*'-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-sulfonyl-hydrazides (267-269)

As reported in scheme 4.7, a first attempt was made to obtain compound **267** by

reacting the two starting materials in anhydrous DCM and adding triethylamine to neutralise the hydrochloric acid formed: in these conditions, a complex mixture of multiple products was obtained, and it was not possible to purify desired compound **267** by chromatographic or recrystallisation techniques. Following reported procedures, the reaction was repeated in anhydrous pyridine, and in this second system desired products **267-279** were finally obtained after flash column chromatography purification.[22]

4.2.5 *N*'-(5,6,7,8-Tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazides (272-273, 278-281)

A second attempt was made to replace the hydrazone bond with a more stable chemical function, while maintaining the length of the linker: a hydrazide group was chosen for a new series of six derivatives. These compounds were obtained according to two different synthetic approaches: compounds **272-273**, carrying a *para* hydrophobic substituent in the phenyl ring, were prepared following the same strategy and reaction conditions used for compounds **267-269**, reacting intermediate **193** with acyl chlorides **271** and **119**, as shown in scheme 4.8.

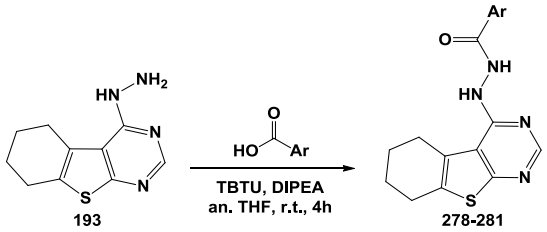


Acyl chloride	Ar	Product	Yield %
271	4-*tert*-butylphenyl	**272**	86
119	4-chlorophenyl	**273**	37

Scheme 4.8: Synthetic strategy for compounds **272-273**

A further small series of four derivatives was obtained through a coupling reaction between intermediate **193** and differently substituted carboxylic acids **274-277**, using TBTU as the coupling agent as done for several previous compounds (Chapter 2). Starting from carboxylic acids instead of chlorides, it was possible to obtain, along with pyrazine and 2-pyridine products **280** and **281**, hydrazides **278** and **279**, where the hydroxy groups of the original active structure are maintained, thus allowing a direct

comparison between the two series of compounds (scheme 4.9).



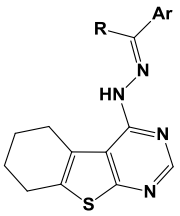
Carboxylic acid	Ar	Product	Yield %
274	2,5-dihydroxyphenyl	**278**	28
275	2-hydroxyphenyl	**279**	35
276	pyrazine	**280**	29
277	2-pyridine	**281**	67

Scheme 4.9: Synthesis of hydrazide compounds **278-281**

4.2.6 Biological evaluation

Newly synthesised thieno-pyrimidines were tested for antiviral activity in the subgenomic replicon and cytostatic assays.[18] Their biological results are shown in tables 4.3-4.5.

(5,6,7,8-Tetrahydro-benzo [4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazones



Compound	R	Ar	EC_{50}(μM)	CC_{50}(μM)	EC_{90}(μM)	SI
252	Me	4-hydroxy-phenyl	>295	>295	>295	-
253	H	2,5-dihydroxyphenyl	0.84	18.2	3.1	21.7
254	Me	2,5-dimethoxyphenyl	36.7	63.6	-	1.7
255	Me	2-methoxyphenyl	8.1	48.9	18.8	6
256	Me	3-methoxyphenyl	16	30	-	1.9
257	Me	2,5-dichlorophenyl	9.4	>256	55.1	>27.2
258	Me	2-chlorophenyl	10.9	70	34.7	6.4
259	Me	4-chlorophenyl	18.6	187	96	10.1
260	Me	2,5-difluorophenyl	30.9	60.3	33.5	2
261	Me	2-fluorophenyl	22.3	39.3	-	1.8
262	Me	pyrazine	0.355	21.1	0.775	59.4
263	Me	2-pyridine	1.04	8.06	3.34	7.8
264	Me	2-indole	17.8	26.3	>277	1.5
265	Me	2-benzimidazole	2.97	50.1	7.94	16.9

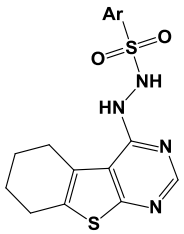
Table 4.3: Antiviral and cytotoxicity data for compounds **252-265**

Biological results for compounds **252-265** further confirm the importance of a hydroxy substituent in position 2' and 5' of the original scaffold: their replacement with methoxy and halogen groups is associated with activity reduction. The same negative effect is observed with a 4'-hydroxy substituent in the aromatic ring, while the absence of the acetophenylic methyl group in compound **253** leads to activity retention, but is associated with increased toxicity against cells, with a CC_{50} value much lower than its

analogue **187**.

A successful replacement of the original hydroxy functions is achieved with the introduction of heteroaromatic rings in the ethylidene portion of the molecule: the presence of pyrazine (**262**), 2-pyridine (**263**) and 2-benzimidazole (**265**) is tolerated, with compound **262** showing the best activity profile found so far. This conclusion cannot be extended to compound **264**, where the 2-indole ring appears to be associated with loss of activity.

N'-(5,6,7,8-Tetrahydro-benzo [4,5]thieno [2,3-*d*]pyrimidin-4-*yl*)-sulfonylhydrazides

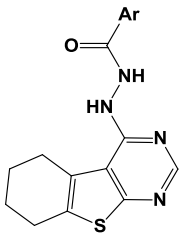


Compound	Ar	EC_{50}(μM)	CC_{50}(μM)	EC_{90}(μM)	SI
267	4-*tert*-butylphenyl	42.8	116	-	2.7
268	4-chlorophenyl	57.6	253	-	4.4
269	2-pyridine	43.9	145	83.2	3.3

Table 4.4: Antiviral and cytotoxicity data for compounds **267-269**

The first attempt to replace the hydrazone bond with a sulfonylhydrazide does not seem to be successful: for all three compounds prepared, this modification is associated with loss of activity.

N'-(5,6,7,8-Tetrahydro-benzo [4,5]thieno [2,3-*d*]pyrimidin-4-*yl*)-hydrazides



Compound	Ar	EC_{50}(μM)	CC_{50}(μM)	EC_{90}(μM)	SI
272	4-*tert*-butylphenyl	13.9	>263	-	>18.9
273	4-chlorophenyl	9.2	12.9	-	1.4
278	2,5-dihydroxyphenyl	0.51	99.9	0.939	195.9
279	2-hydroxyphenyl	0.301	66.4	0.549	221
280	pyrazine	1.12	7.76	2.55	6.9
281	2-pyridine	1.11	8.34	0.819	7.5

Table 4.5: Antiviral and cytotoxicity data for compounds **272-273**, **278-281**

Greater success is associated with the replacement of the hydrazone group with a hydrazide: except for compounds **272** and **273**, all remaining derivatives of this series show EC_{50} values equal to or below 1 μM, with good SI values for two of them, compounds **278** and **279**. Moreover, while a successful replacement of the hydrazone linker was achieved, the importance of the original hydroxyl groups is further confirmed (compounds **278** and **279**), along with their potential replacement with heteroaromatic moieties such as pyrazine (**280**) and 2-pyridine (**281**), as shown for the hydrazone scaffold (compounds **262** and **263**). Compound **279** in particular shows the lowest EC_{50} value found so far, and represents an encouraging starting point for further studies.

4.2.7 Design and synthesis of heteroaromatic-hydrazones (290-294) and hydrazides (302-309)

Due to the successful replacement of the 2',5'-dihydroxyphenyl group in compound **187** with heteroaromatic rings, it was decided to expand the phenylethylidene series: inserting 2-quinoline, to see how the expansion of occupational volume on this level might affect antiviral activity, 3-pyridine, to determine whether the presence of a nitrogen in position 2 is essential for activity retention with the same trend observed for 2'-hydroxy group, 2-pyrrole, 2-furan and 2-thiophene, in order to explore the effect of a

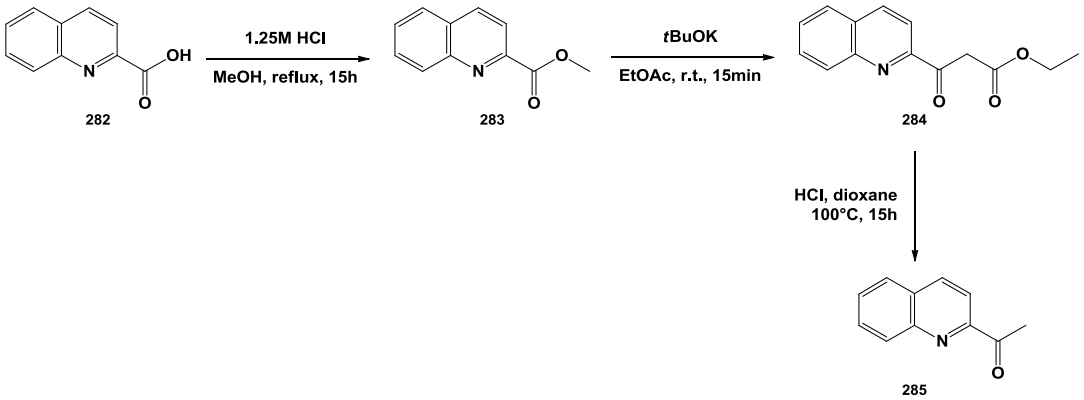
smaller heteroaromatic ring in this portion of the structure.

Moreover, due to the improved activity profile associated with the hydrazide linker, it was planned to further explore this series of compounds. In order to assess whether the activity pattern follows the one observed for hydrazone compounds, it was decided to first replace the aromatic hydrazide substituent with the 3-hydroxyphenyl and the 3-pyridine groups, and then to carry out the same heteroaromatic modifications of the hydrazone series, corresponding to the insertion of 2-indole, 2-benzimidazole, 2-quinoline, 2-pyrrole, 2-furan and 2-thiophene substituents on the hydrazide group.

Once prepared non-commercially available 2-acetylquinoline **285**, five new hydrazone-derivatives were synthesised following the usual route, changing only the last reaction step to obtain final products **283-286**.

Synthesis of 2-acetylquinoline (285)

A three-step synthetic approach was followed to prepare acetylate quinolone in position 2, as shown in scheme 4.10.

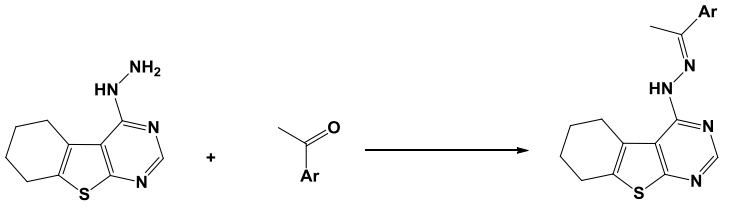


Scheme 4.5: Synthetic strategy for 2-acetylquinoline (**282**)

Starting from quinaldic acid (**282**), following reported procedures, it was decided to first prepare methyl ester **283**, in order to perform a Claisen condensation with ethyl acetate and obtain β-ketoester **284**. The β-ketoester was subsequently treated with hydrochloric acid while heating to give ester hydrolysis first, and then decarboxylation of the free acid formed the desired ketone **285**.[23]

Synthesis of (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazones (290-294)

Five new hydrazone compounds **290-294** were prepared following the synthetic route previously optimised, by reacting intermediate **193** with ketones **285-289**.

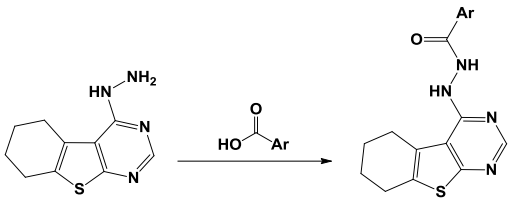


Aldehyde/ketone	Ar	Product	Yield %
285	2-quinoline	**290**	71
286	3-pyridine	**291**	61
287	2-pyrrole	**292**	62
288	2-furan	**293**	36
289	2-thiophene	**294**	44

Table 4.6: Synthesis of compounds **290-294**

Synthesis of *N*'-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazides (302-309)

Eight new hydrazide products were synthesised by a coupling reaction between aryl carboxylic acids **282**, **295-301** and intermediate **193**, following the same procedure used for compounds **274-277** (table 4.7).

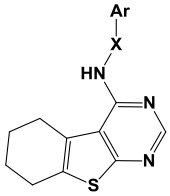


Carboxylic acid	Ar	Product	Yield %
295	3-hydroxyphenyl	**302**	29
296	3-pyridine	**303**	23
297	2-indole	**304**	36
298	2-benzoimidazole	**305**	26
282	2-quinoline	**306**	68
299	2-pyrrole	**307**	65
300	2-furan	**308**	35
301	2-thiophene	**309**	15

Table 4.7: Synthesis of compounds **302-309**

4.2.8 Biological evaluation

Newly synthesised heteroaromatic hydrazones and hydrazides were evaluated for inhibition of HCV replication in the HCV replicon and cytostatic assay.[18] Biological results are shown in table 4.8.



Compound	Ar	Linker X	EC_{50}(μM)	CC_{50}(μM)	EC_{90}(μM)	SI
290	2-quinoline	hydrazone	<0.697	7.23	t.b.d.	t.b.d.
291	3-pyridine	hydrazone	79.4	>309	>103	3.9
292	2-pyrrole	hydrazone	4.8	17	12.8	
293	2-furan	hydrazone	14.9	61.5	-	4.1
294	2-thiophene	hydrazone	22.3	49.8	-	2.2
302	3-hydroxyphenyl	hydrazide	210	90.9	>220	>1.4
303	3-pyridine	hydrazide	128	>307	>307	2.4
304	2-indole	hydrazide	14.8	64.7	-	4.4
305	2-benzimidazole	hydrazide	0.193	2.92	0.679	15.1
306	2-quinoline	hydrazide	23.9	59	-	2.4
307	2-pyrrole	hydrazide	147	>319	-	>2.2
308	2-furan	hydrazide	199	230	-	1.2
309	2-thiophene	hydrazide	31.1	36.7	-	1.2

Table 4.8: Antiviral and cytotoxicity data for compounds **272-273**, **278-281**

Biological data for hydrazone compounds **290-294** suggest, for the presence of a heteroaromatic nitrogen in the phenylethylidene substituent, the same trend in antiviral activity found for hydroxy groups: loss of activity associated with 3-pyridine derivative **291** indicates the same essential role for a nitrogen in position 2 (present in pyrazine and 2-pyridine analogues **262** and **263**), already noticed for the 2'-hydroxyphenyl group. Furthermore, replacement of 6-membered heteroaromatic rings with 5-membered rings is associated with loss of activity.

The same considerations can be extended to the hydrazide series of compounds: among this family of structures, the presence of a 2-hydroxyphenyl group is essential for

activity retention. As observed in the hydrazone derivatives, replacement of the 2-pyridine group with 3-pyridine (compound **303**) is associated with loss of activity, suggesting the same effect for a heteroaromatic ring with a nitrogen in position 2 and a 2-hydrophenyl substituent. Also for this series of compounds, 5-member heteroaromatic groups in the hydrazide part of the molecule are associated with loss of activity, while 2-benzimidazole is correlated to activity retention, but an increased toxicity against the cell can be noticed for this modification. Finally, a 2-indole or 2-quinoline group in the hydrazide scaffold are associated with a decreased antiviral potential.

In conclusion, the general trend in activity profile appears to be the same for the two families of structures, hydrazones and hydrazides: this evidence could suggest the same mechanism of action for the two structural scaffolds, and represents a further confirmation of the successful replacement of the hydrazone linker with a hydrazide function.

4.2.9 Design and synthesis of *N*-aryl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amines (310-312)

Conformational search on compound 222

In an attempt to understand the role of the 2'-hydroxy group in compound **187** and **222**, a conformational search was performed on the structure of **222**.

Using the Conformational Search tool in MOE2010.10,[2] the lowest energy conformation for compound **222** was found to show an internal hydrogen bond between the 2'-hydroxy group and the nitrogen of the hydrazone linker (figure 4.8).

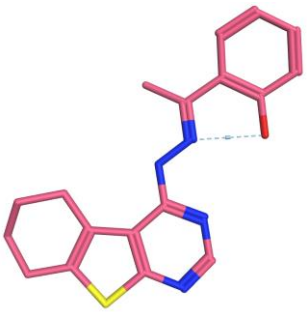


Figure 4.8: Lowest energy conformation found for compound **222**

In order to freeze this interaction and explore a new linker modification, it was planned to replace the hydrazone bond with an aromatic amino group, to be functionalised with

heteroaromatic bicyclic rings, such as benzimidazole (**312**) and quinoline (**311**), along with naphthalene ring (**310**), to be used as potential reference for activity evaluation.

Before carrying out this new modification from a synthetic point of view, MOE Flexible Alignment was used to superimpose the newly designed compounds with the low-energy conformation found for compound **222**, kept rigid during the analysis, to confirm the volume overlapping shared between the modified structures and the parent molecule (figure 4.9).[24]

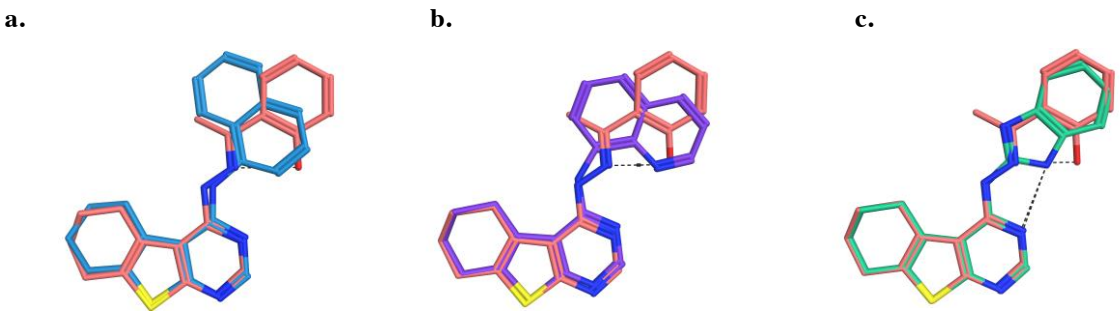


Figure 4.9: Flexible Alignment results of the superimposition between compound **222**, in pink, and a. **310**, in blue; b. **311**, in purple; c. **312**, in green

All alignments shown in figure 4.9 correspond to dU values equal to 0 kcal/mol and large negative S values. The best alignment is found for compound **312** (S= -163): freezing the internal H-bond found in the low energy conformation of compound **222** seems to be most successful with this last modification, where the 2-hydroxyphenylidene function is replaced by a 2-aminobenzimidazole group (**312**, figure 4.9c). With this designed compound, the benzene ring of benzimidazole overlaps completely with the phenyl ring of compound **222**, and the formation of an internal hydrogen bond between imidazole NH group and pyrimidine aromatic nitrogen could make the conformation found for this alignment particularly stable, confirming the good superimposition of benzimidazole ring with the 2-hydoxyphenyl group of the parent molecule.

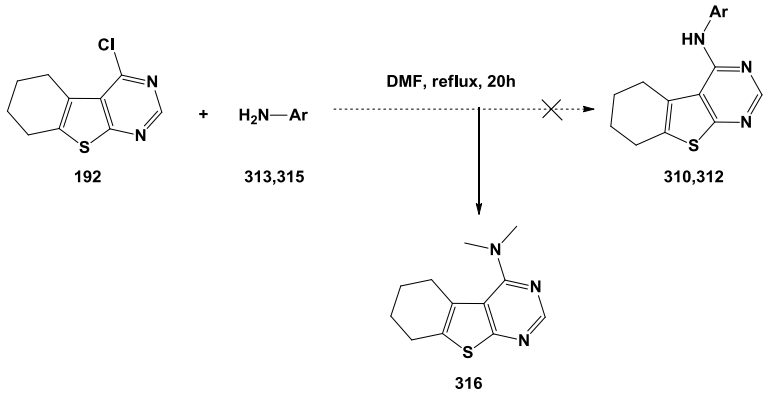
Alignment results show that in the case of compounds **310** (S= -121) and **311** (S=-128) spatial superimposition of the condensed bicyclic aromatic rings and the phenylethylidene group of **222** is not as good as the one found for compound **312**: 1-naphthyl and 8-quinoline groups, respectively, only partially overlap the volume occupied by the 2-hydroxyphenyl hydrazine function. Nevertheless, all three modifications were chosen for synthetic development, in order to use their biological

results as reference for the evaluation of compound **312**.

Synthesis of *N*-aryl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amines (310-312)

In order to replace the hydrazone linker with an amino function, it was planned to react the third intermediate of the usual synthetic pathway, 4-chloro-5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidine (**192**), with commercially available bicyclic aromatic amines.

In a first attempt, chloride **192** was treated with an excess of 1-naphthylamine (**313**) and heated under reflux in anhydrous DMF for 20 h. Under these conditions, the only new species formed and isolated after column chromatography purification was undesired product **316**, as shown in scheme 4.6. In an attempt to see if the formation of the side product was due to a low reactivity of the amine used, the reaction was tried in the same conditions using an excess of 2-aminobenzimidazole (**315**), but also in this case the only new species isolated after column chromatography was compound **316**.

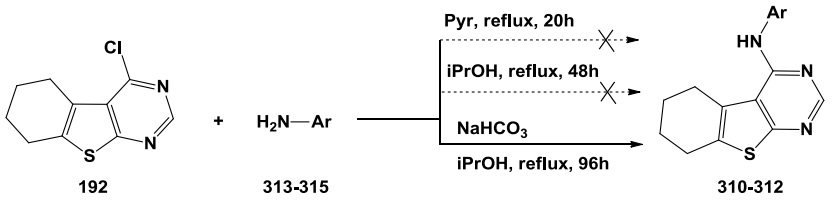


Scheme 4.6: First attempt for the synthesis of compounds **310-312**

To avoid the interference of DMF with the reaction, the solvent was changed, and a second attempt was made refluxing chloride **192** with an excess of 2-aminobenzoimidazole (**315**) in pyridine for 20 h. In this second case, the formation of a mixture of different new species was observed by T.L.C., but all attempts to isolate desired compound **312** by column chromatography or recrystallization were unsuccessful (scheme 4.7.).

The reaction mixture was then refluxed in isopropanol, but only the two starting materials **192** and **315** were recovered after 72 h, with no new species observed by monitoring the reaction via T.L.C.

A successful method was finally identified by adding to the isopropanol solvent $NaHCO_3$ as base: as shown in scheme 4.7, the three desired products **310-312** were obtained according to this procedure, in low yield.

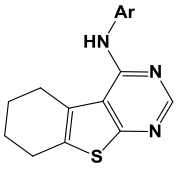


Arylamine	Ar	Product	Yield %
313	1-naphthyl	**310**	21
314	8-quinoline	**311**	14
315	2-benzimidazole	**312**	12

Scheme 4.7: Synthesis of compounds **310-312**

4.2.10 Biological evaluation

Compounds **310-312** were evaluated for their antiviral potential in the HCV replicon assay.



Compound	Ar	EC_{50}(μM)	CC_{50}(μM)	EC_{90}(μM)	SI
310	1-naphthyl	32.4	281	>33.5	8.7
311	8-quinoline	186	>301	>100	>1.6
312	2-benzimidazole	55.4	231	>104	4.2

Table 4.9: Antiviral and cytotoxicity data for compounds **310-312**

Biological results indicate that the last modification, attempted to replace the hydrazone linker and freeze the internal hydrogen bond found for compound **187**, is associated with a significant loss of activity.

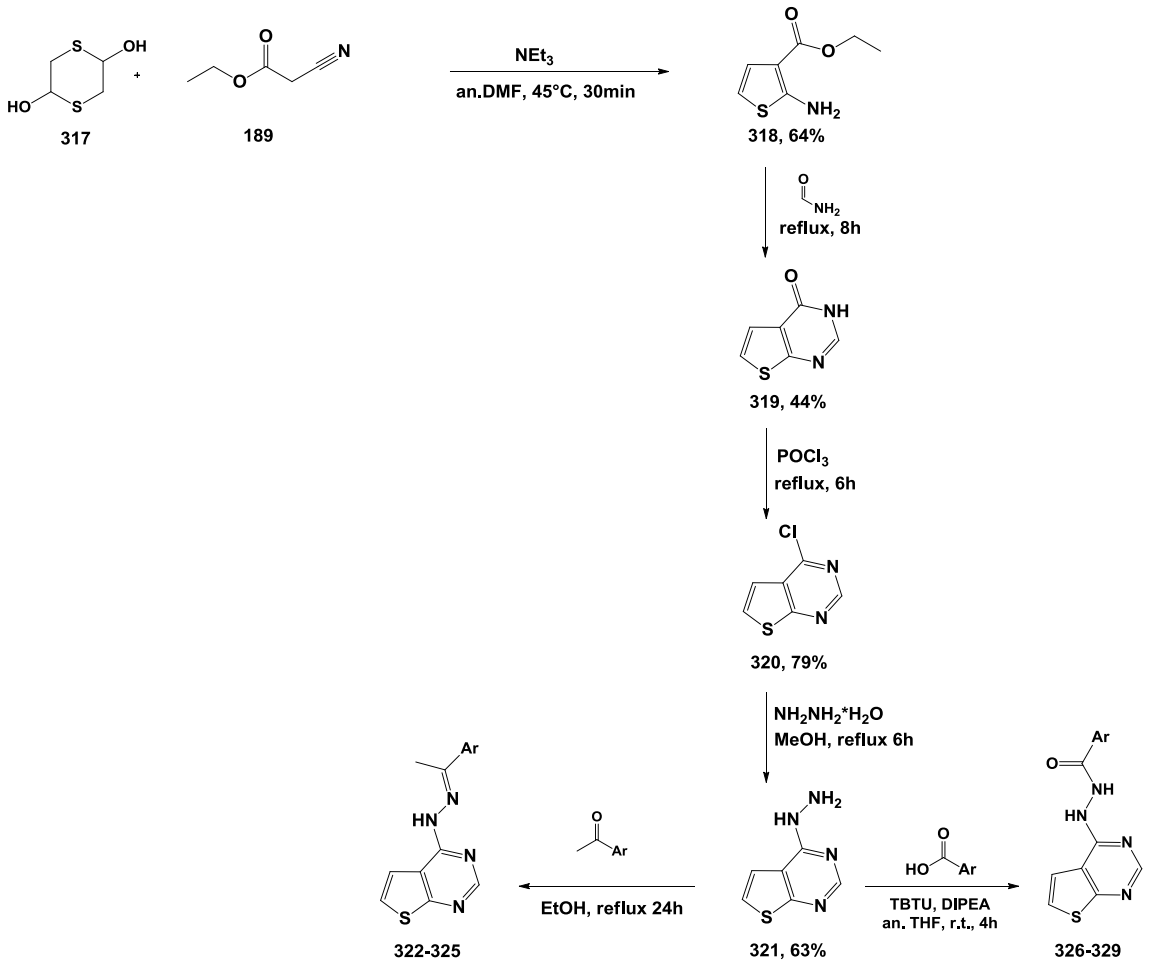
4.3 Synthesis of thieno[2,3-*d*]pyrimidines

Due to the wide exploration of the phenylethylidene aromatic portion and the hydrazone linker done so far, it was decided to start and investigate the role of the tetrahydrobenzo substituent on the thiophene ring.

Several series of new derivatives were designed to remove or replace this part of the molecule, while maintaining the central thienopyrimidine nucleus, either the hydrazone or hydrazide linker, and the most successful modifications on the level of the original 2',5'-dihydroxyphenyl ring.

In particular, five new families of structures were chosen, inserting an unsubstituted thieno-pyrimidine system, a cyclopentane ring condensed to the thiophene ring, an oxidised benzene substituent, two methyl groups, and finally an unsymmetrical substitution with one methyl and one ethyl group.

In order to insert an unsubstituted thienopyrimidine system in the original scaffold, the same five-step synthetic pathway previously validated was used, as shown in scheme 4.8.



Scheme 4.8: Synthetic pathway applied for compounds **322-329**

The only step that changed corresponds to the preparation of ethyl 2-aminothienophene-3-carboxylate (**318**), which takes place through a different version of the Gewald reaction, in which 1,4-dithiane-2,5-diol **317** is condensed with ethyl cyanoacetate.[22] The four remaining steps followed the strategy and conditions already applied for previous tetrahydrobenzo[*b*]thienopyrimidines.

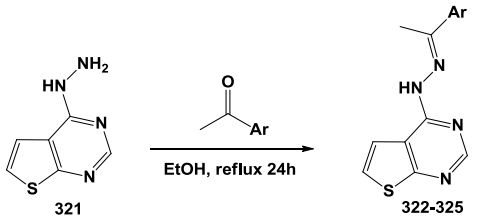
Synthesis of ethyl 2-aminothienophene-3-carboxylate (318)[25]

An improved version of the Gewald reaction uses dimeric forms of an α-sulfanylcarbonyl compound, that is condensed with an α-activated acetonitrile in the presence of a base.

Desired condensation to give 2-aminothiophene **318** was obtained by adding triethylamine to a mixture of ethyl cyanoacetate (**189**) and 1,4-dithiane-2,5-diol (**317**), in anhydrous DMF, and heating at 45 °C for 30 min. The ester intermediate **318** was isolated after flash column chromatography in 64% yield.

4.3.1 *N*-(1-Aryl-ethylidene)-*N*'-(thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazines (322-325)

Four new derivatives were prepared through the formation of a Schiff base between hydrazine intermediate **321** and ketones **194**, **202**, **203** and **250**, by refluxing the two starting materials in ethanol for 24 h.



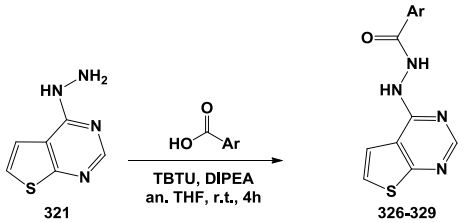
321 322-325

Ketone	Ar	Product	Yield %
194	2,5-dihydroxyphenyl	**322**	81
202	2-hydroxyphenyl	**323**	61
203	3-hydroxyphenyl	**324**	37
250	pyrazine	**325**	96

Scheme 4.9: Synthesis of compounds **322-325**

4.3.2 *N*'-(Thieno[2,3-*d*]pyrimidin-4-*yl*)arylcarbohydrazides (326-329)

A small series of four compounds was obtained with a hydrazide linker in the new thiophene scaffold by reacting intermediate **321** with carboxylic acids **274-277**, according to the coupling procedure previously applied for amide bond formation.



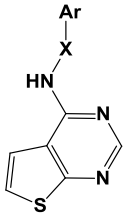
321 326-329

Carboxylic acid	Ar	Product	Yield %
274	2,5-dihydroxyphenyl	**326**	15
275	2-hydroxyphenyl	**327**	19
276	pyrazine	**328**	31
277	2-pyridine	**329**	30

Scheme 4.10: Synthesis of compounds **326-327**

4.3.3 Biological evaluation

Newly synthesised thienopyrimidine derivatives were tested for inhibition of viral replication in the HCV replicon assay:[18] data are shown for both families, hydrazones and hydrazides, in table 4.10.



Compound	Ar	Linker X	EC_{50}(μM)	CC_{50}(μM)	EC_{90}(μM)	SI
322	2,5-dihydroxyphenyl	hydrazone	2.99	226	4.55	75.6
323	2-hydroxyphenyl	hydrazone	4.75	77.2	>10.6	16.3
324	3-hydroxyphenyl	hydrazone	52	78.1	-	1.1
325	pyrazine	hydrazone	4.39	11.9	4.19	2.7
326	2,5-dihydroxyphenyl	hydrazide	2.75	>165	9.4	>60
327	2-hydroxyphenyl	hydrazide	12.9	>349	>38.8	>27
328	pyrazine	hydrazide	0.85	233	2.28	274.1
329	2-pyridine	hydrazide	0.31	138	0.67	445

Table 4.10: Antiviral and cytotoxicity data for compounds **322-329**

Biological data for hydrazone compounds **323-325** suggest loss of activity and an increased toxic effect against cells associated with the removal of the tetrahydrobenzene substituent. Along with lower CC_{50} and SI values in comparison to their original analogues, compounds **323-325** also show higher EC_{50} values, indicating a cytotoxic rather than antiviral effect associated with this modification. Compound **322** represents an exception to this trend: data found so far suggested activity retention for this derivative.

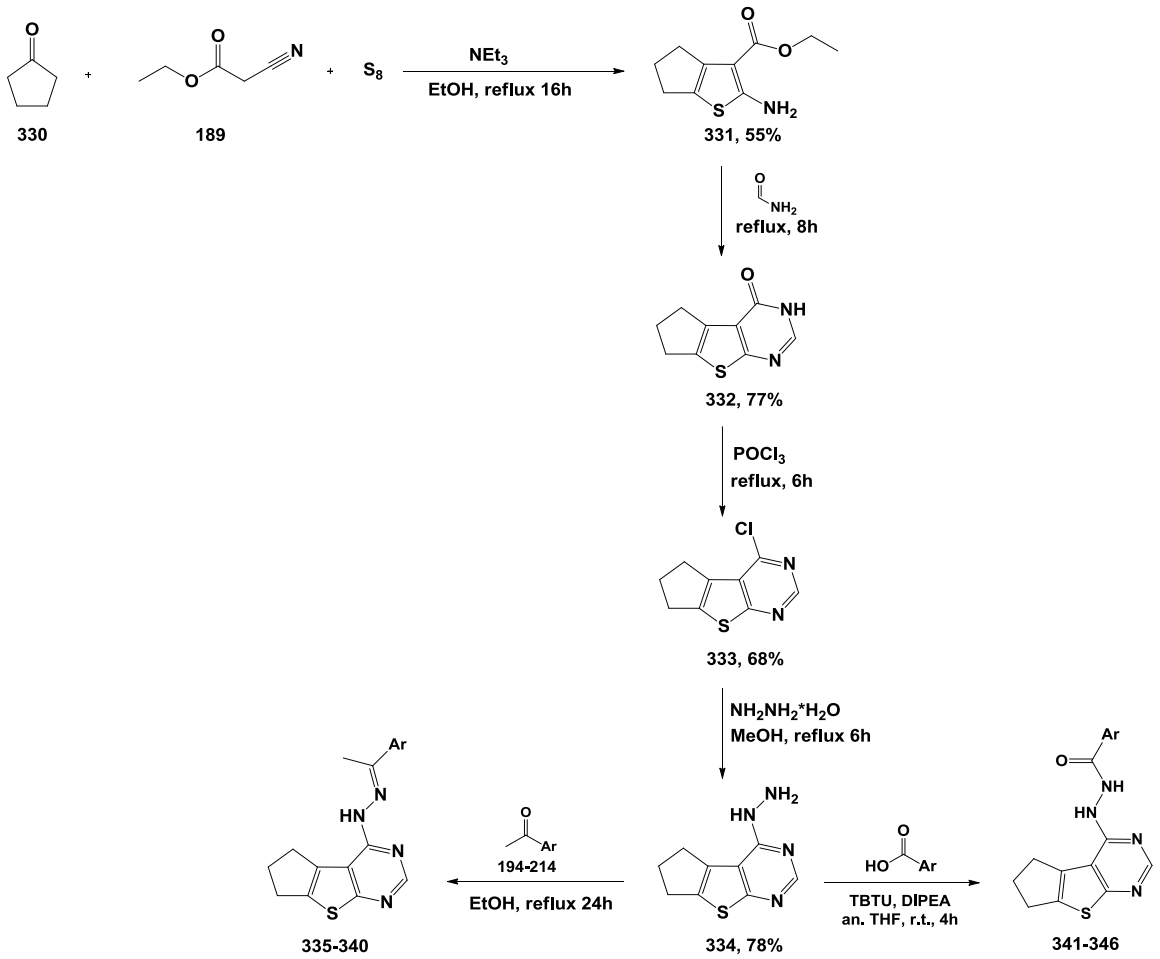
An inconsistent pattern is also found for hydrazide derivatives **326-329**: for hydroxy analogues **326** and **327** antiviral potential seems to be reduced in comparison to original tetrahydrobenzothienyl compounds **278** and **279**, while for pyrazine and 2-pyridine derivatives **328** and **329** this last modification seems to be successful both for activity retention and for a decrease in cytotoxic effect, since their CC_{50} and SI values are higher than the ones found for compounds **280** and **281**.

In conclusion, removal of the substituent on the thiophene portion does not have a consistent effect between the two families of compounds, nor does it show the same trend in activity and toxicity profiles. For hydrazone compounds **323-325**, it is associated with loss of activity and increased cytotoxicity, while among the hydrazide family, it has a different effect on hydroxyphenyl compounds **326-327**, where activity potential is reduced, and heteroaromatic products **328-329**, for which both activity and cytotoxicity profiles are improved.

4.4 Synthesis of cyclopentane[*b*]thienopyrimidines

With the aim to replace the tetrahydrobenzene ring in compound **187** with a condensed cyclopentane system, the previous synthetic pathway was followed, with the only difference being the use, in the first synthetic step corresponding to the Gewald condensation of 2-aminothiophene nucleus, of cyclopentanone (**330**) instead of cyclohexanone, as shown in scheme 4.11. All reagents and conditions for the preparation of the four common intermediates and the two series of final products, hydrazones and hydrazides, were the same previously discussed for tetrahydrobenzo-thienopyrimidine compounds.

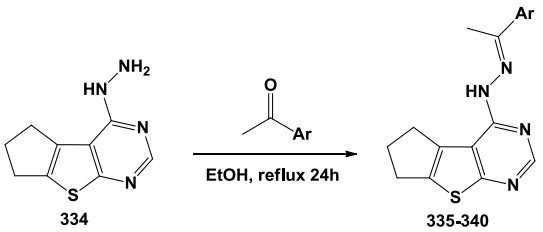
The rationale that guided the selection of ketones and acids to react with the fourth intermediate **334** in the fifth reaction step, for the formation of final products, was the biological evaluation of the previous series of compounds: for both hydrazone and hydrazide series, the aim was to choose the most significant modifications on the phenylethylidene or hydrazide portion of the molecule, to better understand the effect of the new modification on the antiviral potential associated with these structures.



Scheme 4.11: Synthetic pathway applied for compounds **335-346**

4.4.1 *N*-(2,3-Dihydro-1*H*-8-thia-5,7-diaza-cyclopenta[*a*]inden-4-*yl*)-*N*'-(1-phenyl-ethylidene)-hydrazines (335-340)

Six new derivatives were prepared according to the usual procedure for Schiff base formation, by reacting intermediate **334** with ketones **194**, **202**, **203**, **250**, **251** and **298**, under reflux conditions in EtOH for 24h.

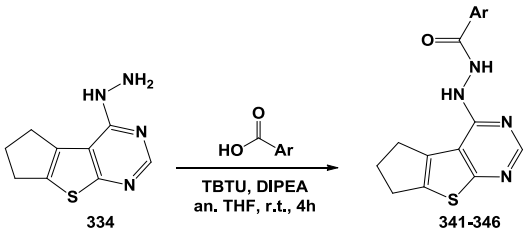


Ketone	Ar	Product	Yield %
194	2,5-dihydroxyphenyl	**335**	74
202	2-hydroxyphenyl	**336**	71
203	3-hydroxyphenyl	**337**	64
250	pyrazine	**338**	42
251	2-pyridine	**339**	36
298	2-benzoimidazole	**340**	58

Scheme 4.12: Synthesis of compounds **335-340**

4.4.2 *N*'-(6,7-Dihydro-5*H*-cyclopenta[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)arylcarbo hydrazides (341-346)

A second series of six new compounds was obtained with a coupling reaction between intermediate **334** and carboxylic acids **274-277**, **297** and **298**, using TBTU as coupling agent as previously done for all the previous compounds with this functional group.

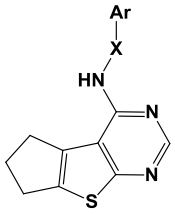


Carboxylic acid	Ar	Product	Yield %
274	2,5-dihydroxyphenyl	**341**	26
275	2-hydroxyphenyl	**342**	30
276	pyrazine	**343**	15
277	2-pyridine	**344**	30
297	2-indole	**345**	38
298	2-benzimidazole	**346**	47

Scheme 4.12: Synthesis of compounds **341-346**

4.4.3 Biological evaluation

Biological results in the HCV replicon assay for compounds **335-346** are shown in table 4.11.[18]



Compound	Ar	Linker X	EC_{50}(μM)	CC_{50}(μM)	EC_{90}(μM)	SI
335	2,5-dihydroxyphenyl	hydrazone	17.8	>367	25.7	>20.6
336	2-hydroxyphenyl	hydrazone	1.72	141	4.07	81.9
337	3-hydroxyphenyl	hydrazone	88	>385	>385	>4.4
338	pyrazine	hydrazone	0.688	1.12	<0.839	1.6
339	2-pyridine	hydrazone	1.18	14	1.44	11.9
340	2-benzimidazole	hydrazone	0.802	6.68	<2.24	8.3
341	2,5-dihydroxyphenyl	hydrazide	2.13	198	3.67	92.9
342	2-hydroxyphenyl	hydrazide	0.186	119	0.352	639.8
343	pyrazine	hydrazide	0.0759	3.18	<0.256	41.9
344	2-pyridine	hydrazide	0.745	6.61	1.79	8.9
345	2-indole	hydrazide	-	47.8	-	-
346	2-benzimidazole	hydrazide	0.594	6.9	1.11	11.6

Table 4.11: Antiviral and cytotoxicity data for compounds **335-346**

As had been noticed for unsubstituted thienopyrimidine compounds **322-329**, replacement of the tetrahydrobenzene substituent with a cyclopentyl ring does not have a consistent effect in terms of antiviral activity between the two families of analogues synthesised, hydrazones and hydrazides, and among different compounds belonging to the same structural family. In the case of hydrazone stuctures, with the exception of compound **335**, which is associated with loss of activity in terms of EC_{50} in comparison to compound **187**, activity potential seems to be retained, but the modification is associated with an increased toxic effect against cells in the case of heteroaromatic derivatives **338-340**.

In the case of hydrazide structures **341-346**, while compound **345** is associated with a toxic effect only, for all remaining analogues in this group activity potential seems to be

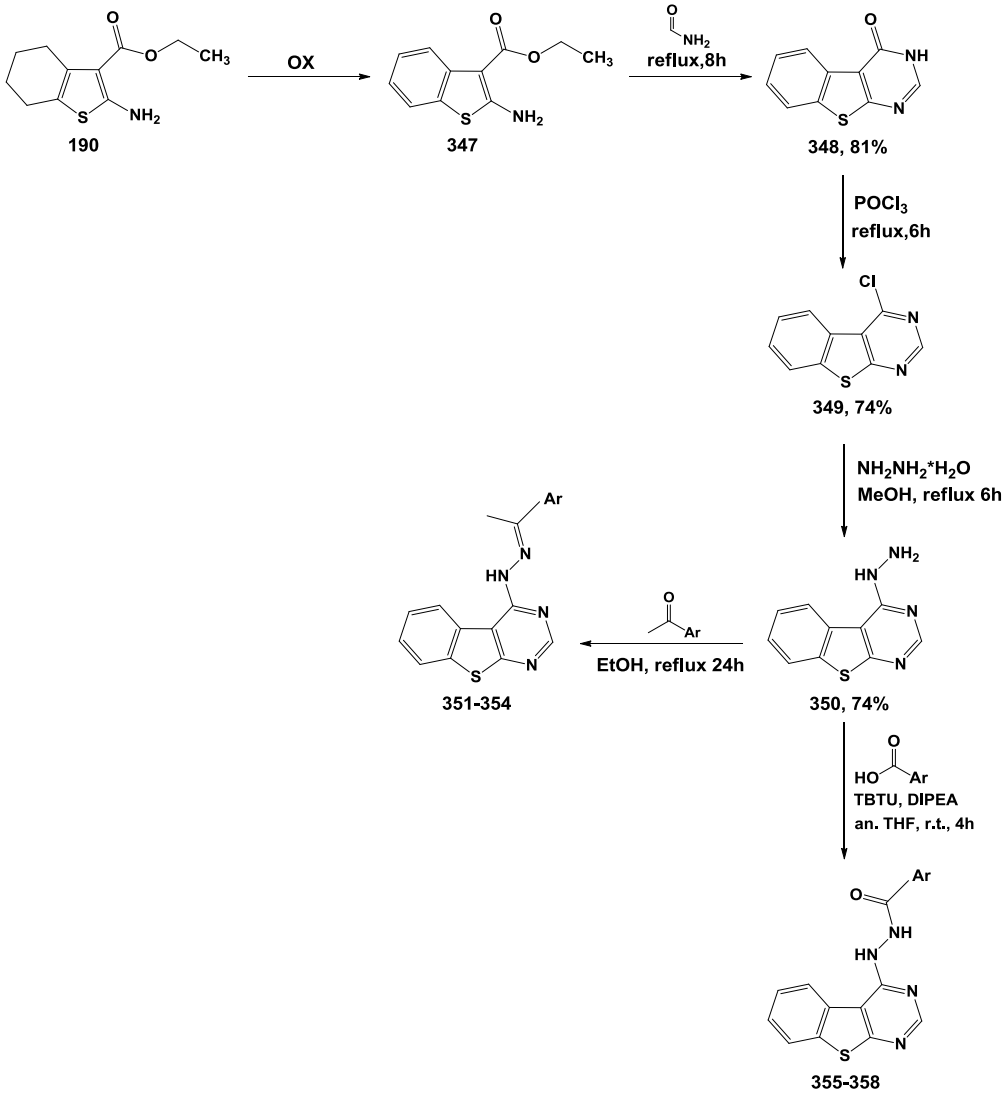
retained, and for two of them, compounds **342** and **343**, an improvement can be noticed both in terms of EC_{50} and SI. Moreover, the EC_{50} associated with compound **343** reaches the lowest value found so far, in the nanomolar range.

Despite the differences found, this last modification is mainly associated with a retained or improved activity profile, suggesting a role in antiviral activity for a cyclic aliphatic substituent on the thiophene ring.

4.5 Synthesis of benzo[*b*]thienopyrimidines

With the purpose to better understand and explore the role of the tetrahydrobenzene substituent on the thiophene, it was planned to aromatise this group and prepare a new series of compounds with a third aromatic ring condensed to the thienopyrimidine system.

In order to obtain final hydrazones and hydrazides with the new tricyclic aromatic nucleus, it was decided to oxidise the cyclohexyl ring in ester intermediate **190**, and then insert aromatised aminoester **347** in the usual synthetic route, as shown in scheme 4.13.



Scheme 4.13: Synthetic approach for compounds **351-358**

Synthesis of ethyl 2-aminobenzo[*b*]thiophene-3-carboxylate (347)

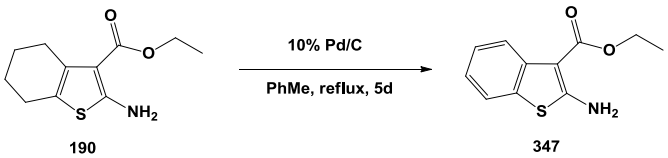
Following reported procedures, a first strategy was carried out to aromatise the tetrahydrobenzene ring in compound **190** as shown in scheme 4.14.[26]

Scheme 4.14: First strategy attempted for the preparation of compound **347**

This first attempt began with the protection of aromatic amine in **190** to cyclopropanecarboxamide in compound **359**, followed by oxidation of the cyclohexyl ring with manganese dioxide in intermediate **360**. The protected amine group was then to be deprotected from the cyclopropylamide group with methyl sulphate, to give tricyclic aromatic ester **347**.

The first step in this pathway, corresponding to the protection of the amine group, was carried out by adding cyclopropane carbonyl chloride to compound **190** in pyridine at 5°C: after 1h the desired product was obtained by acidification of the reaction mixture and precipitation, in 83% yield. The following oxidation step was attempted by adding a large excess of MnO_2 to compound **359** in toluene, and refluxing the mixture for 5 days. After this time, starting material **359** was still the main species present in the reaction mixture, and only 5% product **360** was obtained after flash column chromatography.

Due to the low yield of this second step, it was decided to attempt a different oxidative strategy, without protecting the amino group in compound **190**. The new approach attempted, shown in scheme 4.15, followed the catalytic oxidation of tetrahydrobenzene ring over Pd/C.[27]



Scheme 4.15: Catalytic aromatization of compound **190**

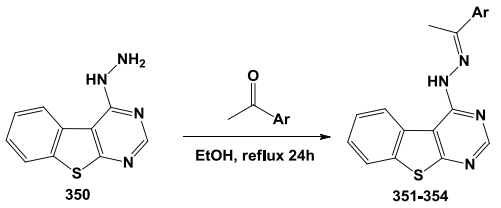
This second strategy was attempted a first time by refluxing compound **190** and 10% Pd/C in toluene for 48 h: most of the starting material was still present, and only 10% of desired product **347** was obtained after chromatographic purification. The reaction was

then repeated by heating at reflux the mixture for 5 days, and in this case 41% of desired product **347** was obtained. Even though the yield was not high and there were four other steps ahead in the synthetic route to obtain final products **351-358**, this second method was preferred for its better yield and the requirement of only one reaction step.

As previously discussed (scheme 4.13), oxidized intermediate **347** was inserted in the usual synthetic route for the preparation of final hydrazones **351-354** and hydrazides **355-358**.

4.5.1 4-(2-(1-Arylethylidene)hydrazinyl)benzo[4,5]thieno[2,3-*d*]pyrimidines (351-354)

Four new hydrazone compounds were obtained by reacting hydrazine intermediate **350** with hydroxyphenyl ketones **194** and **202** and heteroaromatic ketones **250** and **298**.

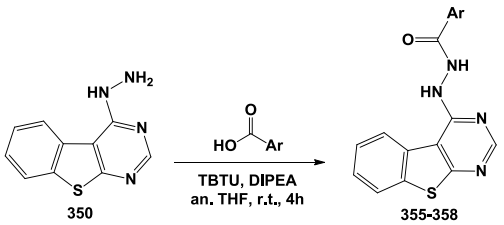


Ketone	Ar	Product	Yield %
194	2,5-dihydroxyphenyl	**351**	73
202	2-hydroxyphenyl	**352**	81
250	pyrazine	**353**	73
298	2-benzimidazole	**354**	62

Scheme 4.16: Synthesis of compounds **351-354**

4.5.2 *N*'-(Benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)arylcarbohydrazides (355-358)

Another small series of four new hydrazide compounds was prepared reacting intermediate **350** and carboxylic acids **274-277**, all characterised by the presence of either hydrophenylfunctions or heteroaromatic rings.

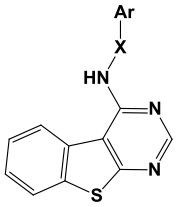


Carboxylic acid	Ar	Product	Yield %
274	2,5-dihydroxyphenyl	**355**	17
275	2-hydroxyphenyl	**356**	14
276	pyrazine	**357**	21
277	2-pyridine	**358**	28

Scheme 4.17: Synthesis of compounds **355-358**

4.5.3 Biological evaluation

Compounds **351-358** were evaluated for their antiviral potential in the HCV replicon assay (table 4.12).[18]



Compound	Ar	Linker X	EC_{50}(μM)	CC_{50}(μM)	EC_{90}(μM)	SI
351	2,5-dihydroxyphenyl	hydrazone	0.343	20.3	0.968	59.2
352	2-hydroxyphenyl	hydrazone	0.0861	12	0.421	139.4
353	pyrazine	hydrazone	0.415	1.99	0.858	6.4
354	2-benzoimidazole	hydrazone	1.1	18.6	3.25	16.9
355	2,5-dihydroxyphenyl	hydrazide	0.715	43.6	2.14	61
356	2-hydroxyphenyl	hydrazide	0.832	108	1.6	129.8
357	pyrazine	hydrazide	0.0783	1.31	0.143	17
358	2-pyridine	hydrazide	0.461	1.54	1.01	3.3

Table 4.12: Antiviral and cytotoxicity data for compounds **351-358**

Aromatisation of the cyclohexyl ring is associated with a retained or improved antiviral potential in comparison to the original structures. In the hydrazone family, improved activity profiles are found for hydroxy derivatives **351** and **352**, with compound **352**

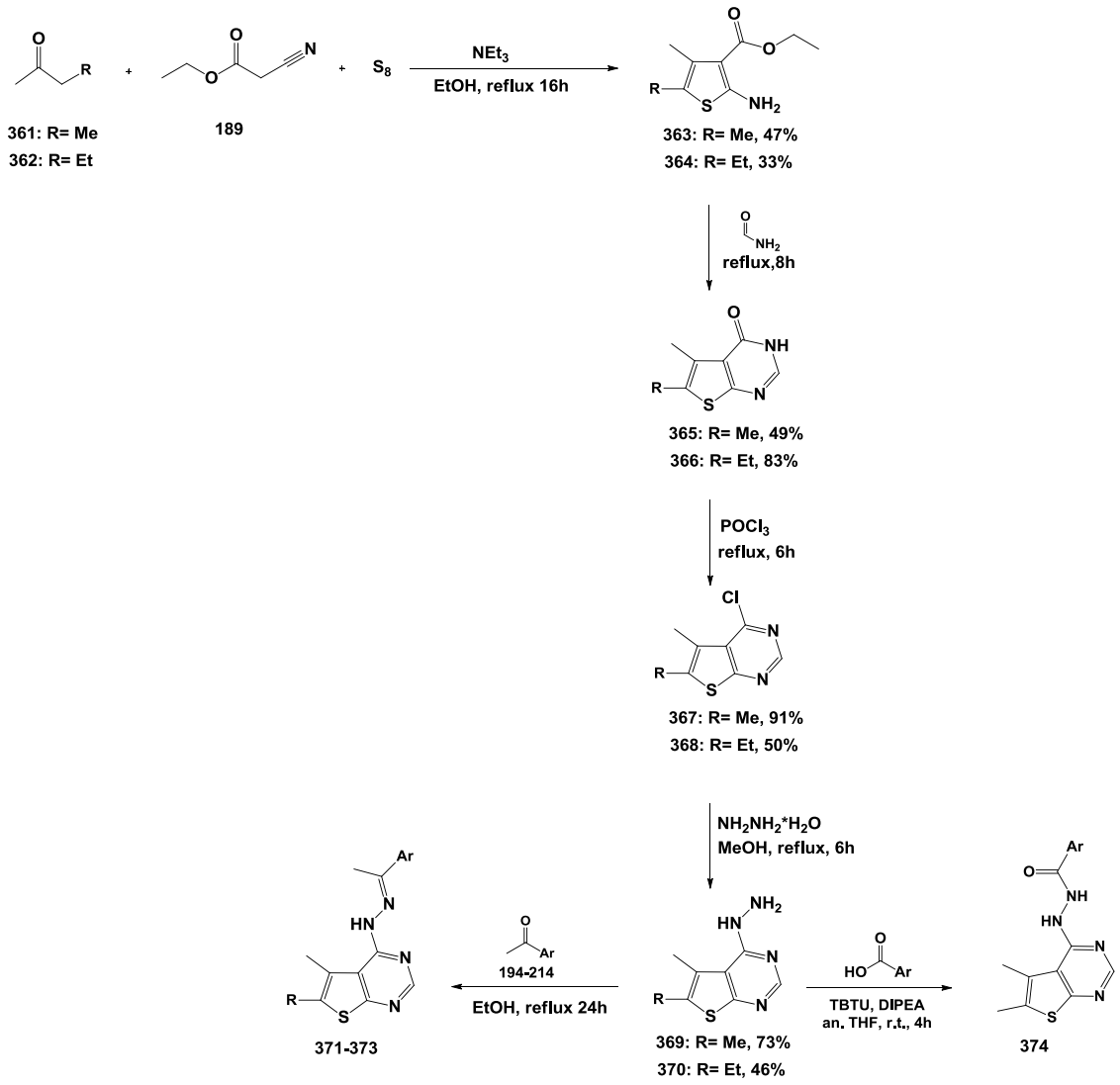
showing an EC_{50} value in the nanomolar range. For heteroaromatic hydrazones **353** and **354**, antiviral profile originally identified for tetrahydrobenzene analogues **262** and **265** appears to be retained. Among the hydrazide family, for hydroxy derivatives **355** and **356** antiviral potential is retained, while heteroaromatic hydrazides **357** and **358**, even if showing low EC_{50} values (in the nanomolar range for compound **357**), are still associated with a toxic effect against cells, as their tetrahydrobenzene analogues **280** and **281**.

In conclusion, this last modification is associated with activity retention, indicating that the rigidity due to a tricyclic system in this part of the molecule is tolerated, and further confirming the importance of the occupational volume, given by an aliphatic or aromatic cyclic substituent, on the the thiophene ring for antiviral activity.

4.6 Synthesis of dimethyl[*b*]thienopyrimidines and 6-ethyl-5-methylthieno[2,3-*d*]pyrimidines

Aiming to further explore the role of the cyclic substituent on the thiophene ring, it was decided to prepare a small series of new derivatives replacing the cyclohexyl group with two methyl groups and a 5-methyl-6-ethyl substituent.

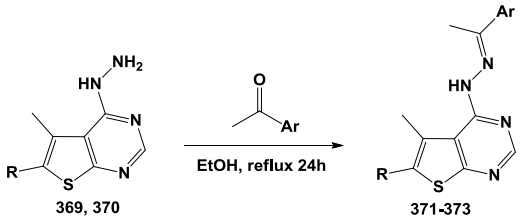
In order to achieve this result, the usual synthetic pathway was followed. Thiophene ring with the desired substituents was obtained according to the Gewald condensation, by reacting ethyl cyanoacetate (**189**) with 2-butanone (**361**) and 2-pentanone (**362**) respectively, as shown in scheme 4.18. The first step in the synthetic route was the only one that needed to be changed.



Scheme 4.18: Synthetic approach for compounds **371-374**

4.6.1 4-(2-(1-Arylethylidene)hydrazinyl)-5,6-dimethylthieno[2,3-*d*]pyrimidines (371-372) and 2-(1-(2-(6-ethyl-5-methylthieno[2,3-*d*]pyrimidin-4-*yl*)hydrazono) ethyl)benzene-1,4-diol (373)

Three new hydrazone compounds were prepared by reacting final intermediates **369** and **370** with hydroxyphenyl ketones **194** and **202**, as shown in scheme 4.19.



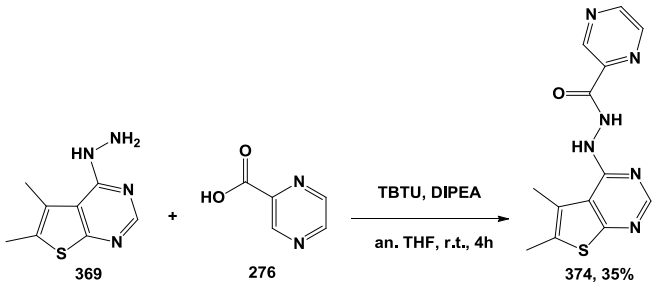
369, 370 371-373

Ketone	Product	R	Ar	Yield %
194	**371**	Me	2,5-dihydroxyphenyl	71
202	**372**	Me	2-hydroxyphenyl	54
194	**373**	Et	2,5-dihydroxyphenyl	53

Scheme 4.19: Synthesis of compounds **371-373**

4.6.2 *N*'-(5,6-Dimethylthieno[2,3-*d*]pyrimidin-4-*yl*)pyrazine-2-carbohydrazide (374)

One new product was obtained by a coupling reaction between hydrazine intermediate **369** and pyrazine carboxylic acid (**276**).

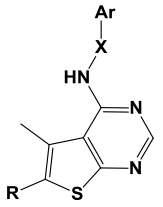


369 276 374, 35%

Scheme 4.20: Synthesis of compound **374**

4.6.3 Biological evaluation

Biological data in the HCV replicon assay for compounds **371-374** are shown in table 4.13.[18]



Compound	R	Ar	Linker X	EC_{50}(μM)	CC_{50}(μM)	EC_{90}(μM)	SI
371	Me	2,5-dihydroxyphenyl	hydrazone	1.3	>305	4.65	>305
372	Me	2-hydroxyphenyl	hydrazone	0.384	93.6	<1.28	243.8
373	Et	2,5-dihydroxyphenyl	hydrazone	0.803	>292	1.62	>363.6
374	Me	pyrazine	hydrazide	0.091	1.58	0.26	17.3

Table 4.13: Antiviral and cytotoxicity data for compounds **371-374**

Hydrazone structures **371-373** are associated with activity retention in comparison to tetrahydrobenzene analogues **187** and **202**: both in the case of 5,6-dimethyl derivative **372** and 5-methyl-6-ethyl compound **373** improved activity and SI values are found.

Biological data for new hydrazide derivative **374** suggest activity retention associated with the new modification: even though it is associated with a lower CC_{50} value compared to original derivative **280**, the EC_{50} found for this structure is in the nanomolar range.

What these data seem to suggest is that replacement of the cyclic substituent on the thiophene system with non-cyclic aliphatic groups is associated with activity retention.

In order to further validate the evidence found so far, it was decided to explore new modifications on the thiophene ring.

New non-cyclic aliphatic modifications were planned: in particular, it was decided to replace one of the 5,6-dimethyl groups with a chlorine, a methoxy and an ethyl ester.

To evaluate the impact of the cyclic substituents, it was decided to increase the size of the aliphatic ring from cyclohexyl to cycloheptyl, and to insert heteroatoms in the cyclohexyl moiety.

Finally, it was planned to explore the role of the pyrimidine aromatic proton, by replacing it with a methyl group.

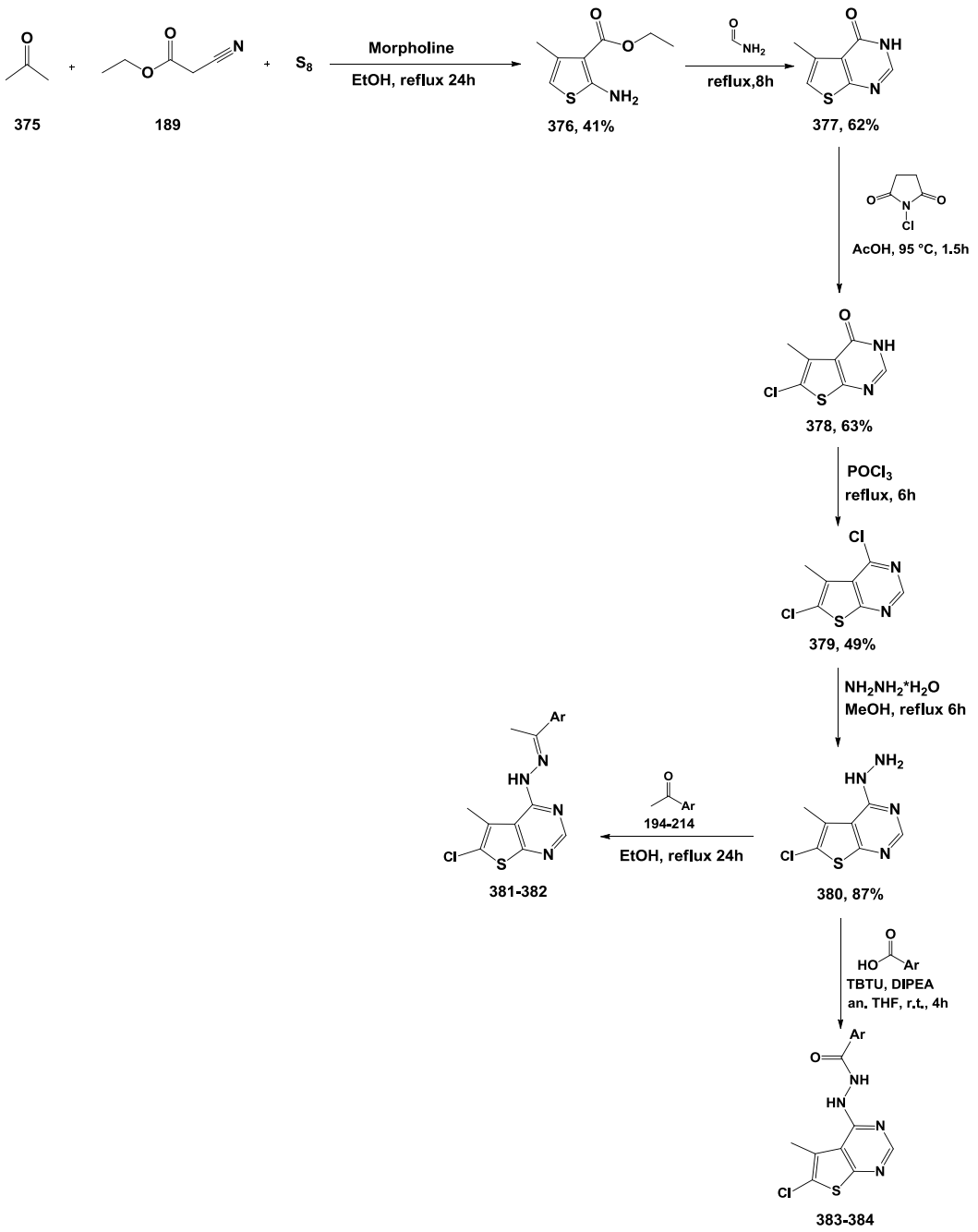
4.7 Synthesis of 6-chloro-5-methyl-thieno[2,3-*d*]pyrimidines

Aiming to further explore the role of the substituent on the thiophene ring, it was decided to prepare a small series of new derivatives by replacing the 6-methyl group with a 6-chloro, 6-methoxy and 6-ethyl carboxylate.

In order to obtain a direct comparison for activity of the new series of compounds, it was decided to prepare one or two hydrazone derivatives, with a 2'-hydroxyphenyl and a pyrazine substituent, and one or two hydrazide derivatives, with the same 2'-hydroxyphenyl and a 2-pyridine group, for each one of the new families.

Insertion of a chlorine in position 6 of the thienopyrimidine ring, according to reported procedures, was planned starting with the preparation of mono-methylated thiophene derivative **376**, by reacting acetone (**375**) and ethyl cyanoacetate (**189**) in the usual conditions for the Gewald condensation.[28] Intermediate aminoester **376** was then condensed with formamide in order to obtain the pyrimidinone ring closure in compound **377**, and position 6 of intermediate **377** was then chlorinated with *N*-chlorosuccinimide.

Once the 6-chloro intermediate **378** was obtained, the usual two remaining steps were followed for the formation of hydrazine intermediate **380**, to be finally functionalised to hydrazones **381-382** and hydrazides **383-384** (scheme 4.21).



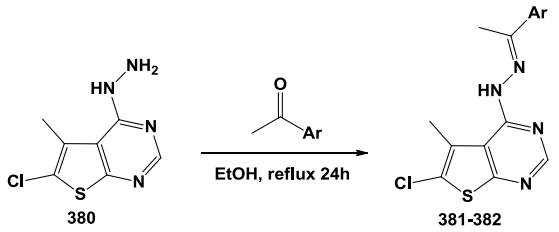
Scheme 4.21: Synthetic route for compounds **381-384**

Synthesis of 6-chloro-5-methylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (379)

As can be seen in scheme 4.21, chlorination of pyrimidinone intermediate **377** was carried out using *N*-chlorosuccinimide as chlorinating agent. The two reagents were heated in glacial acetic acid for 1.5 h under reflux conditions, and the desired chlorinated product **379** was isolated after precipitation from H_2O in 63% yield.

4.7.1 6-Chloro-5-methyl-4-(2-(1-arylethylidene)hydrazinyl)thieno[2,3-*d*] pyrimidines (381-382)

Hydrazine intermediate **380** was reacted with ketones **202** and **250**, in the usual conditions applied for Schiff base formation, to give hydrazone products **381-382**.

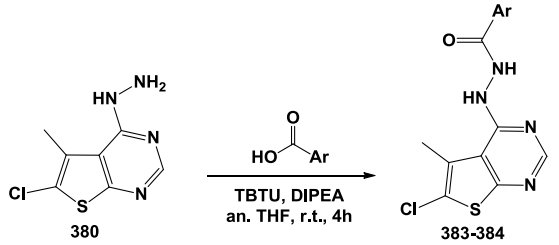


Ketone	Ar	Product	Yield %
202	2-hydroxyphenyl	**381**	77
250	pyrazine	**382**	64

Scheme 4.22: Synthesis of compounds **381-382**

4.7.2 *N*'-(6-Chloro-5-methylthieno[2,3-*d*]pyrimidin-4-*yl*)arylcarbohydrazides (355-358)

A small series of two new hydrazide compounds was obtained with the usual TBTU coupling reaction between intermediate **380** and aryl carboxylic acids **275** and **277**.

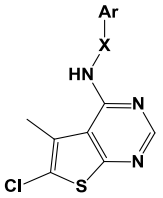


Carboxylic acid	Ar	Product	Yield %
275	2-hydroxyphenyl	**383**	19
277	2-pyridine	**384**	61

Scheme 4.23: Synthesis of compounds **383-384**

4.7.3 Biological evaluation

Newly synthesised compounds **381-384** were evaluated for their antiviral potential in the HCV replicon assay (table 4.14).[18]



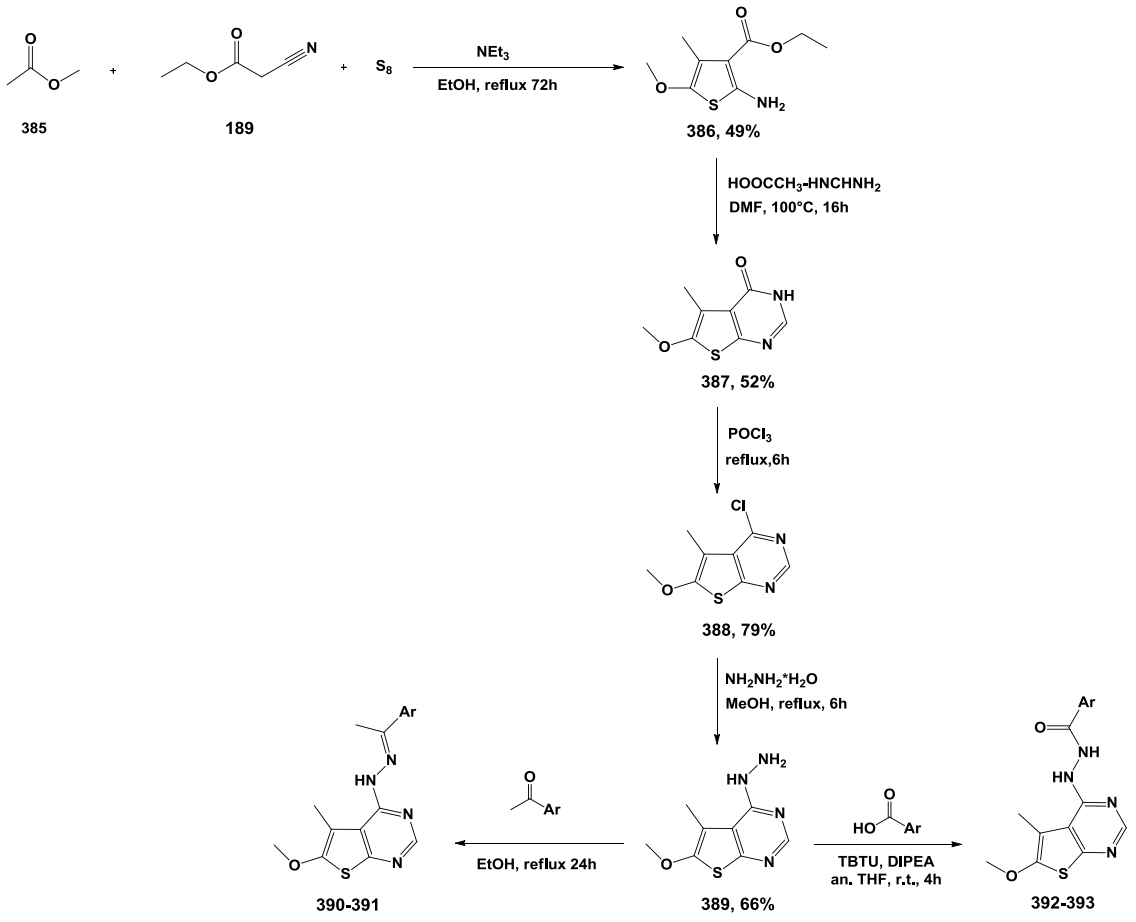
Compound	Ar	Linker X	EC_{50}(μM)	CC_{50}(μM)	EC_{90}(μM)	SI
352	2-hydroxyphenyl	hydrazone	0.17	>9.01	t.b.d.	>56.1
353	pyrazine	hydrazone	0.058	3.13	0.289	53.9
356	2-hydroxyphenyl	hydrazide	0.159	>8.96	t.b.d.	>56.1
358	2-pyridine	hydrazide	0.088	>9.38	0.286	>106

Table 4.14: Antiviral and cytotoxicity data for compounds **381-382**

The presence of a chloride moiety on the thiophene ring is associated with activity retention for the four new compounds prepared. However, the presence of a halogen in this part of the structure could influence the cytotoxicity in a negative fashion, since the CC_{50} values found so far for this new series of compounds, which need to be confirmed with further biological analyses, might be in the low μM range for all of them.

4.8 Synthesis of 6-methoxy-5-methyl-thieno[2,3-*d*]pyrimidines

The 6-ethyl substituent on the thienopyrimidine system was modified to a methoxy group by changing the starting ketone in the first step of the previously validated synthetic pathway: as can be seen in scheme 4.24, methoxy-aminoester intermediate **386** was obtained by Gewald condensation between methoxyacetone (**385**) and ethyl cyanoacetate (**189**). In this case, the first reaction step had to be repeated for a longer time than usual: after refluxing the reaction mixture for 24 h as was done for all previous series of compounds, desired product **386** was obtained in a low 30% yield after chromatographic purification. The reaction was repeated and left under reflux conditions for 72 h: in this case desired intermediate **386** was obtained in an improved yield of 49%, which was considered acceptable to perform the next reaction step.



Scheme 4.24: Synthetic route for compounds **390-393**

Synthesis of 6-methoxy-5-methylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (387)

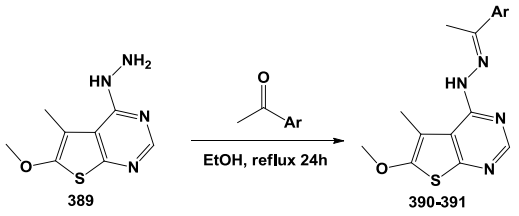
Condensation of the thienopyrimidinone ring had been previously carried out by

refluxing aminoester intermediates in formamide, which has a high boiling point of 220°C and is difficult to be removed by chromatographic techniques. All previous thienopyrimidinone products of the condensation step had been precipitated out from the reaction mixture by addition of water.

Due to the fact that intermediate aminoester **386** was isolated after column chromatography as an oil, and given the low reactivity of the starting materials in the first step of the synthetic pathway, it was thought to change the reaction system for this step, in order to use milder conditions and allow a potential recovery of the starting material, compound **386**. For these reasons it was decided to avoid formamide as reagent and solvent, and pyrimidinone ring condensation was attempted using formamidine acetate salt in anhydrous DMF, heating the mixture at 100 °C for 16 h. After removal of the solvent under vacuum, desired thienopyrimidinone product **388** was precipitated from water and obtained pure in a 52% yield.

4.8.1 6-Methoxy-5-methyl-4-(2-(1-arylethylidene)hydrazinyl)thieno[2,3-*d*] pyrimidines (390-391)

Two new hydrazone products were prepared for this series of compounds, by reacting final intermediate **389** with a small excess of ketones **202** and **250** (scheme 4.25).



389 390-391

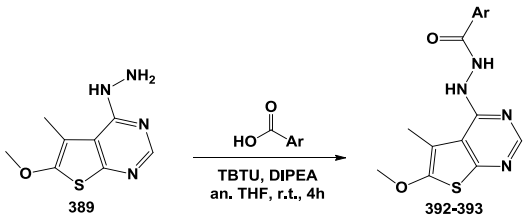
Ketone	Ar	Product	Yield %
202	2-hydroxyphenyl	**390**	50
250	pyrazine	**391**	43

Scheme 4.25: Synthesis of compounds **390-391**

4.8.2 *N*'-(6-Methoxy-5-methylthieno[2,3-*d*]pyrimidin-4-*yl*)arylhydrazides (392-393)

Two new hydrazide derivatives were obtained according to the coupling procedure previously carried out for all hydrazide analogues prepared, reacting compound **389**

with carboxylic acids **275** and **277**.

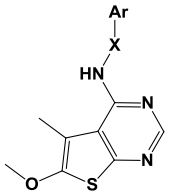


Carboxylic acid	Ar	Product	Yield %
275	2-hydroxyphenyl	**392**	19
277	2-pyridine	**393**	61

Scheme 4.26: Synthesis of compounds **392-393**

4.8.3 Biological evaluation

Biological data (HCV replicon assay) for newly synthesised compounds **390-393** are shown in table 4.15.[18]



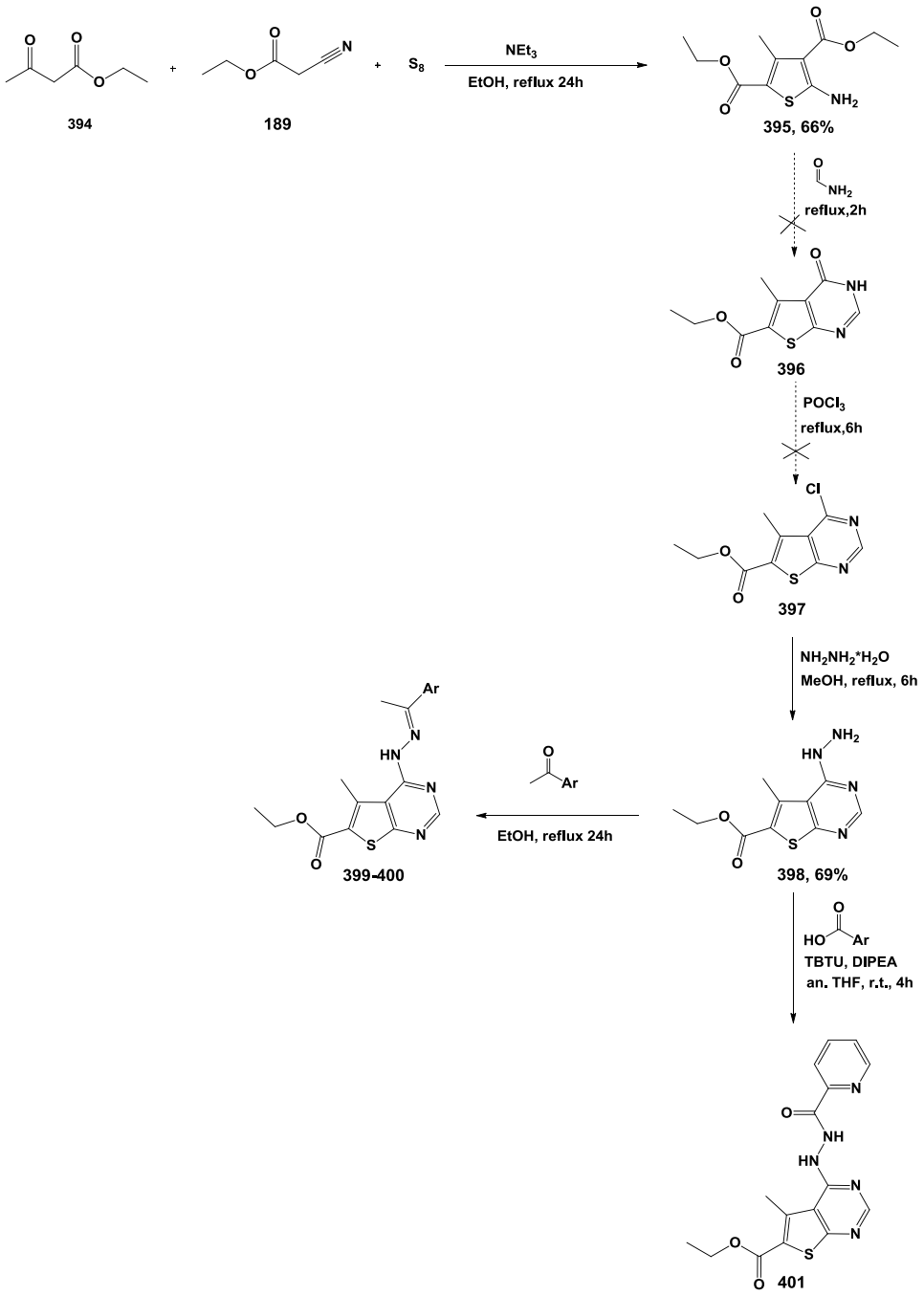
Compound	Ar	Linker X	EC_{50}(μM)	CC_{50}(μM)	EC_{90}(μM)	SI
390	2-hydroxyphenyl	hydrazone	<0.264	>305	t.b.d.	>1155
391	pyrazine	hydrazone	<0.267	0.337	<0.267	>1.22
392	2-hydroxyphenyl	hydrazide	<0.263	30.5	<0.788	>138
393	2-pyridine	hydrazide	<0.275	16.5	<0.275	>60

Table 4.15: Antiviral and cytotoxicity data for compounds **390-393**

The presence of a methoxy substituent on the thiophene ring is generally associated with a retained antiviral potential among the four new compounds tested. However, this modification is also related to an inconsistent effect as for the cytotoxicity: the CC_{50} and SI values found for compound **391** indicate a toxic effect induced by this last modification in the case of the pyrazine-hydrazone substrate. This trend cannot be seen in the other three derivatives prepared. Further studies are ongoing to establish EC_{50} and EC_{90} values for the new compounds and to confirm the trend found so far.

4.9 Synthesis of ethyl-5-methyl-thieno[2,3-*d*]pyrimidine-6-carboxylates

With the purpose to replace the ethyl group in position 6 of thienopyrimidine system with an ethyl ester function, the usual five-step synthetic route was attempted, as shown in scheme 4.27, by changing the ketone in the first step and starting from Gewald condensation between ethyl acetoacetate (**394**) and ethyl cyanoacetate (**189**).



Scheme 4.27: Strategy attempted for the synthesis of compounds **399-401**

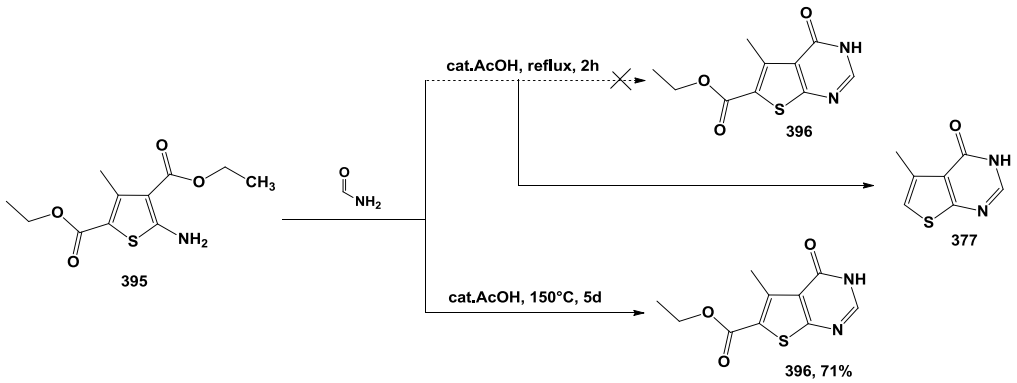
The first step was successful with the usual condition applied for the Gewald condensation, and desired aminoester **395** was obtained after chromatographic purification in 66% yield.

The following two steps however, corresponding to pyrimidinone ring condensation and chlorination of pyrimidinone intermediate with aromatisation of pyrimidine ring, did not work in the usual conditions optimised with the previous series of compounds, and needed to be attempted in different systems.

Synthesis of ethyl 5-methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-6-carboxylate (396)

In the case of diester **395**, condensation with formamide to give pyrimidinone compound **396** did not work in the usual reflux conditions. As can be seen in scheme 4.27, a first attempt was made by refluxing intermediate **395** in formamide for 2 h. In this case, starting material **395** was completely consumed, but the formation of a complex mixture of products was observed, and it was not possible to purify desired product **396** nor to confirm its formation either by NMR or MS experiments.

Following reported procedures, it was decided to add a catalytic amount of acetic acid, and the reaction was repeated under reflux in formamide.[29]



Scheme 4.28: Second and third attempts for the synthesis of compound **396**

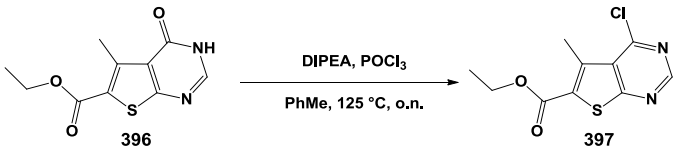
As shown in scheme 4.28, catalytic AcOH was added to compound **395** in formamide, and the reaction mixture was heated under reflux for 2h. In this case, the formation of a thienopyrimidinone product was observed, and it was isolated and purified by recrystallization. However, NMR experiments suggest that the new pyrimidinone species corresponds to decarboxylation product **377**: this was attributed to the combination of the presence of an acid catalyst and the strong heating conditions.

Before trying with a different condensation system, such as formamidine acetate in DMF, the reaction was repeated at a lower temperature, 150°C. In this third attempt, the conversion of starting material **395** into a new species was monitored by T.L.C., and after 5 days of heating, desired product **396** was finally obtained in 71% yield after recystallisation from EtOH/H_2O.

Ethyl 4-chloro-5-methylthieno[2,3-*d*]pyrimidine-6-carboxylate (397)

A first attempt was made to chlorinate and aromatise intermediate **396** to obtain chloro-pyrimidine **397** in the usual reaction system. With this procedure, the formation of a mixture of different products was noticed, and it was not possible to purify nor confirm the presence of desired compound **397**.

According to literature, it was decided to use $POCl_3$ only as reagent, changing the solvent to toluene, and to add a base to neutralise phosphoric acid formed (scheme 4.29).[30]



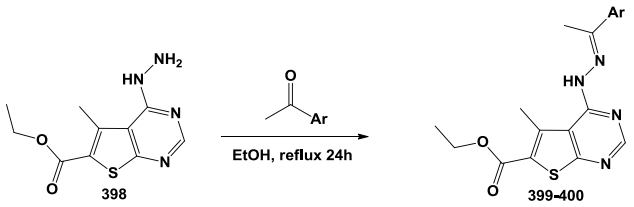
Scheme 4.29: Modified conditions to obtain compound **397**

Following this new procedure, desired product **397** was obtained in 79% yield after flash column chromaphic purification.

The remaining steps in the synthetic route were successful in the usual reaction conditions.

4.9.1 Ethyl 5-methyl-4-(2-(1-phenylethylidene)hydrazinyl)thieno[2,3-*d*]pyrimidine-6-carboxylates (399-400)

Two hydrazone final products were obtained by reacting hydrazine intermediate **398** with ketones **202** and **250** (scheme 4.30).

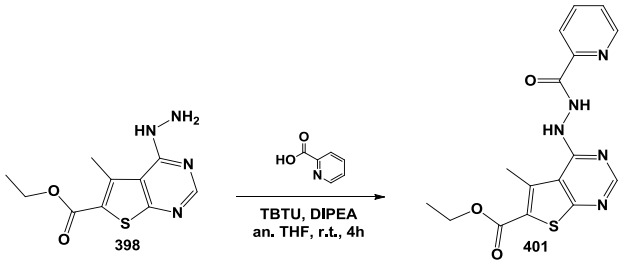


Ketone	Ar	Product	Yield %
202	2-hydroxyphenyl	**399**	29
250	pyrazine	**400**	45

Scheme 4.30: Synthesis of compounds **399-400**

4.9.2 Ethyl 5-methyl-4-(2-picolinoylhydrazinyl)thieno[2,3-*d*]pyrimidine-6-carboxylate (401)

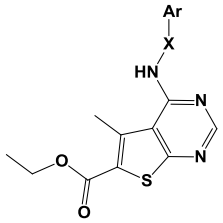
One new hydrazide compound was prepared by a coupling reaction between intermediate **398** and picolinic acid (**277**) (scheme 4.31).



Scheme 4.31: Synthesis of compound **401**

4.9.3 Biological evaluation

Ethyl ester compounds **399-401** were evaluated for their antiviral potential in the HCV replicon assay.[18] Biological data are shown in table 4.16.



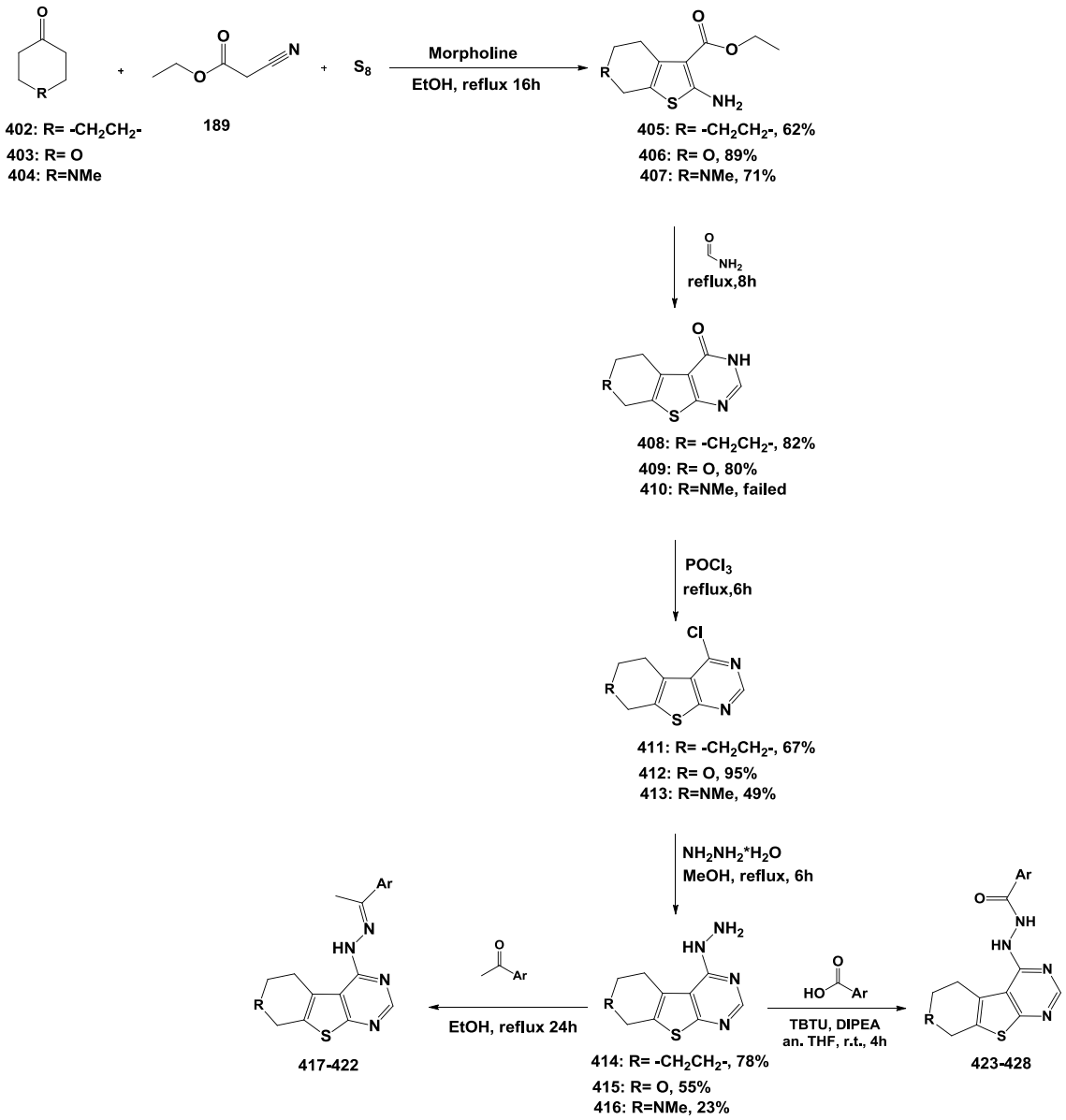
Product	Ar	Linker X	EC_{50}(μM)	CC_{50}(μM)	EC_{90}(μM)	SI
399	2-hydroxyphenyl	hydrazone	<0.234	187	<0.234	>799
400	pyrazine	hydrazone	<0.244	0.281	<0.244	>1.16
401	2-pyridine	hydrazide	<0.243	19.8	<0.243	>81.5

Table 4.16: Antiviral and cytotoxicity data for compounds **399-401**

The presence of an ethyl ester substituent on the thiophene ring is in general associated with a retained antiviral effect, confirming the potential of structural expansion in this part of the molecule. Only in the case of compound **400**, this last modification is also associated to an increased cytotoxic effect: further studies are ongoing to confirm this trend and establish the EC_{50} value for the three new compounds prepared.

4.10 Synthesis of 6,7,8,9-tetrahydro-3*H*-cyclohepta[4,5]thieno[2,3-*d*]pyrimidines, 5,6-dihydro-3*H*-pyrano[4',3':4,5]thieno [2,3-*d*]pyrimidines and 7-methyl-5,6,7,8-tetrahydropyrido [4',3':4,5]thieno[2,3-*d*]pyrimidines

With the purpose to further modify the cyclic substituent on the thienopyrimidine ring, it was decided to expand the original cyclohexyl group to a cycloheptyl one, and to insert heteroatoms on the aliphatic six-member system. This result was achieved by using different cyclic ketones, cycloheptanone (**402**), tetrahydro-4*H*-pyran-4-one (**403**) and *N*-methyl-4-piperidone (**404**), respectively, in the first step of the previously optimised synthetic route (scheme 4.32).



Scheme 4.32: Strategy for the synthesis of compounds **417-428**

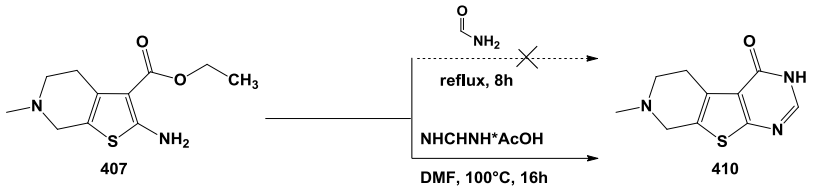
As shown above, the usual synthetic route fitted well the first two families of structures, with a cycloheptyl and a dihydropyran substituent attached to the thiophene ring. For the *N*-methyl-tetrahydropiridine series of compounds, the second step in the synthetic route needed to be changed from the usual conditions.

Synthesis of 7-methyl-5,6,7,8-tetrahydropyrido[4',3':4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (410)

In the *N*-methyl-tetrahydropyridine series of compounds, condensation of the pyrimidinone nucleus was not successful with the usual reaction conditions: in a first attempt, aminoester intermediate **407** was refluxed with an excess of formamide for 8 h,

but even if formation of desired product **410** was confirmed by MS experiments, it was not possible to isolate it pure with recrystallisation or column chromatography techniques, due to its interaction with formamide, used in large excess.

As shown in scheme 4.33, the reaction was repeated using DMF as solvent and formamidine acetate as reagent to obtain pyrimidinone ring condensation: also in this second attempt, it was not possible to completely purify desired product **410**, due to its interaction with DMF. Most DMF was removed under high vacuum and the crude oily residue was used for the next step without further purification.



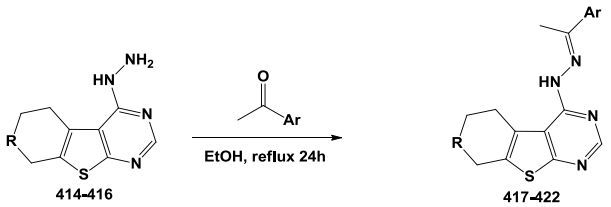
Scheme 4.33: Attempts for the synthesis of compound **410**

4-Chloro-7-methyl-5,6,7,8-tetrahydropyrido[4',3':4,5]thieno[2,3-*d*]pyrimidine (413)

As discussed above, it was decided to use crude pyrimidinone intermediate **410** for the chlorination step without purification. Usual reaction conditions for this step require the addition of an excess of phosphorus oxychloride, reagent and solvent, and the mixture is refluxed for 6 h. In this case, the formation of a thick precipitate was noticed immediately after addition of $POCl_3$, and monitoring of the reaction by T.L.C. did not indicate any conversion of the starting material into the product after 4 h. An insoluble salt on the methyl-tetrahydropyridine amine group might have been formed in the acid reaction environment. The reaction mixture was cooled to 0°C, a small excess of NEt_3 was then added to neutralise the potential salt, and finally the mixture was heated under reflux for 6 h. The solid precipitate solubilised immediately after addition of the base, while complete conversion of the starting material was confirmed by T.L.C. after 6 h. Chloro-pyrimidine intermediate **413** was isolated in 49% yield over two steps after flash column chromatography purification.

4.10.1 4-(2-(1-Phenylethylidene)hydrazinyl)[4,5]thieno[2,3-*d*]pyrimidines (417-422)

Hydrazine intermediates **414-416** were reacted with ketones **202** and **250** to obtain six new hydrazone products, as shown in scheme 4.34.

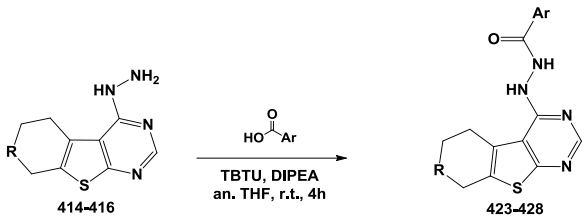


Ketone	Product	R	Ar	Yield %
202	**417**	CH_2CH_2	2-hydroxyphenyl	39
250	**418**	CH_2CH_2	pyrazine	43
202	**419**	O	2-hydroxyphenyl	94
250	**420**	O	pyrazine	86
202	**421**	NCH_3	2-hydroxyphenyl	23
250	**422**	NCH_3	pyrazine	53

Scheme 4.34: Synthesis of compounds **417-422**

4.10.2 [4,5]Thieno[2,3-*d*]pyrimidin-4-*yl*) arylhydrazides (423-428)

Six new hydrazide products were obtained with a coupling reaction between intermediates **414-416** and aryl carboxylic acids **275** and **277**.

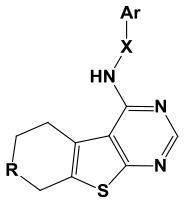


Ketone	Product	R	Ar	Yield %
275	**423**	CH_2CH_2	2-hydroxyphenyl	38
277	**424**	CH_2CH_2	2-pyridine	30
275	**425**	O	2-hydroxyphenyl	23
277	**426**	O	2-pyridine	83
275	**427**	NCH_3	2-hydroxyphenyl	30
277	**428**	NCH_3	2-pyridine	41

Scheme 4.35: Synthesis of compounds **423-428**

4.10.3 Biological evaluation

Newly synthesised compounds **417-428** were evaluated in the HCV replicon assay.[18]



Compound	R	Ar	Linker X	EC_{50}(μM)	CC_{50}(μM)	EC_{90}(μM)	SI
417	CH_2CH_2	2-hydroxyphenyl	hydrazone	0.268	51.5	0.757	192
418	CH_2CH_2	pyrazine	hydrazone	0.195	3.83	0.368	19.6
419	O	2-hydroxyphenyl	hydrazone	0.512	>294	1.59	574
420	O	pyrazine	hydrazone	0.528	4.42	1.22	8.4
421	NCH_3	2-hydroxyphenyl	hydrazone	0.834	30	1.79	36
422	NCH_3	pyrazine	hydrazone	<0.767	>295	<0.767	>384
423	CH_2CH_2	2-hydroxyphenyl	hydrazide	0.451	144	<1.65	319
424	CH_2CH_2	2-pyridine	hydrazide	0.272	16.6	1.74	61
425	O	2-hydroxyphenyl	hydrazide	0.887	202	1.65	227.7
426	O	2-pyridine	hydrazide	1.19	62.3	2.05	52.4
427	NCH_3	2-hydroxyphenyl	hydrazide	<0.733	>281	<0.733	>383
428	NCH_3	2-pyridine	hydrazide	1.45	99.7	2.47	68.8

Table 4.17: Antiviral and cytotoxicity data for compounds **417-428**

As can be deduced from table 4.17, the new modifications on the level of the thiophene substituent are associated with activity retention, both for hydrazone and hydrazide series of compounds. Data obtained so far suggest that the enlargement of the cyclohexyl ring does not affect antiviral potential, and the same indication can be obtained also in the case of the insertion of a heteroatom in the original six-membered aliphatic ring. The presence of an N-methyl group in the ring is as well associated with activity retention.

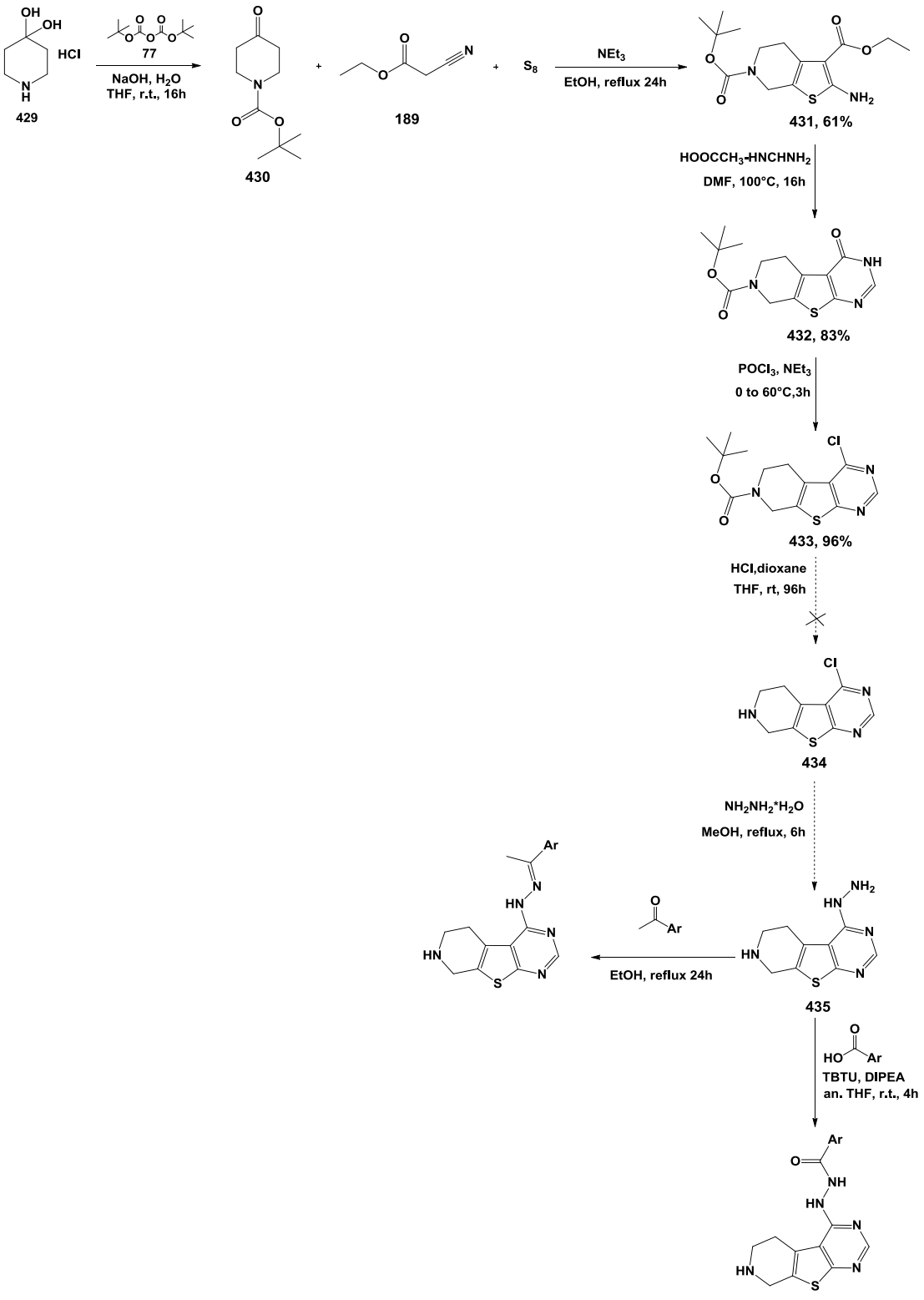
In conclusion, modifications carried out so far suggest that an expanded or heteroatomic aliphatic ring on the thiophene is tolerated and does not interfere with antiviral activity: while the presence of a substituent seems to be required for activity retention, data found so far indicate the potential for expansion of the occupational volume in this part of the molecule.

4.11 Synthesis of 5,6,7,8-tetrahydropyrido[4',3':4,5]thieno [2,3-*d*]pyrimidines

Aiming to evaluate the effect of heteroatoms in the aliphatic cyclohexyl substituent on the thiophene system, along with the preparation of previously discussed dihydropyran and *N*-methyl-tetrahydropyridine compounds, a tetrahydropyridine ring was inserted in this part of the structure.

In order to avoid potential interference of a free amine group in the first three steps of the usual synthetic pathway, 4-piperidone hydrochloride (**429**) was protected with BOC protecting group. Protected ketone *tert*-butyl 4-oxopiperidine-1-carboxylate (**430**) was then inserted in the usual synthetic route, and used to form thieno-aminoester intermediate **431** according to Gewald condensation, followed by pyrimidinone ring closure in compound **432**, and chlorination with aromatisation of pyrimidine system in compound **433**. As can be seen in scheme 4.34, the plan at this point was to deprotect the amino group of compound **433** into free amine **434**, which was then to be reacted with hydrazine to give final intermediate **435**.

The deprotection step in this first strategy attempted was not straightforward.



Scheme 4.34: First synthetic strategy attempted for tetrahydropyridine compounds

Synthesis of *tert*-butyl 4-chloro-5,6-dihydropyrido[4',3':4,5]thieno[2,3-*d*]pyrimidine-7(8*H*)-carboxylate (433)

Following reported procedures, the protected pyridmidinone system was aromatised into pyrimidine **433** and chlorinated using phosphorus oxichloride in the presence of

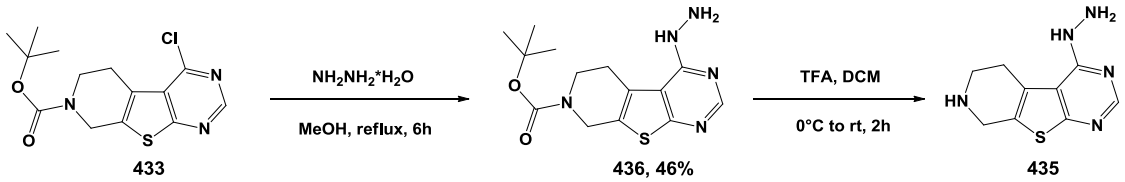
triethylamine.[31] The reaction mixture was stirred under milder heating than the usual reflux conditions, and desired product **433** was isolated in 96% yield after flash column chromatography.

Synthesis of 4-hydrazinyl-5,6,7,8-tetrahydropyrido[4',3':4,5]thieno[2,3-*d*]pyrimidine (435)

In the first strategy attempted, as shown in scheme 4.34, intermediate **433** was to be deprotected and the remaining steps in the synthetic pathway were to be completed with the free amine group in the tetrahydropyridine ring.

Nevertheless, after removal of the BOC group in acid conditions using a saturated solution of HCl in dioxane, it was not possible to obtain deprotected intermediate **434** as a pure species: all attempts to precipitate it from the reaction mixture failed, and this compound could only be isolated as an impure oily residue after several neutralisations and extractions. The following step of aromatic nucleophilic displacement with hydrazine was performed anyway, and even if the presence of desired hydrazine intermediate **435** as the main species was confirmed by MS, its isolation was not successful, and the oily residue obtained was considered not pure enough to attempt any further reaction.

Attempting to reduce purification difficulties, it was decided to carry out the aromatic nucleophilic displacement to obtain hydrazine species on BOC-protected chloride **433** (scheme 4.35).



433 436, 46% 435

Scheme 4.35: Second deprotecting strategy applied to obtain intermediate **435**

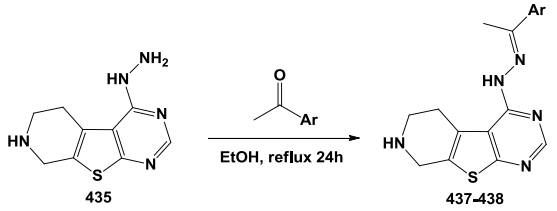
BOC-protected hydrazine intermediate **436** was successfully obtained in the usual reaction system, with 46% yield after recrystallization from EtOH/H_2O.

Deprotection of compound **436** was then performed using trifluoroacetic acid in DCM: it was not possible to obtain the free-amine product **435** as a pure compound, due to its oily nature. MS experiments confirmed however its presence as the main species in the crude residue; it was therefore decided to use it in the final step for the formation of two hydrazone products. This decision was taken due to the high solubility of crude oily

compound **435** in EtOH and to the fact that all hydrazone products prepared so far were isolated by precipitation from the reaction mixture and subsequent recrystallisation. Also in this case, Schiff base products could be isolated in the same manner, thus removing the impurities present in the starting material.

4.11.1 4-(2-(1-Phenylethylidene)hydrazinyl)-5,6,7,8-tetrahydropyrido[4',3':4,5] thieno [2,3-*d*]pyrimidines (437-438)

Two new hydrazone compounds were finally obtained for this series, by reacting final intermediate **435** with ketones **202** and **250** (scheme 4.36). Both of them precipitated as yellow solids from the reaction mixture, and were recrystallised from EtOH.

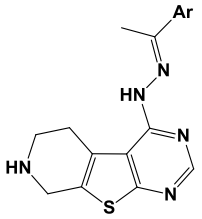


Ketone	Ar	Product	Yield %
202	2-hydroxyphenyl	**437**	23
250	pyrazine	**438**	10

Scheme 4.36: Synthesis of compounds **437-438**

4.11.2 Biological evaluation

Biological data in the HCV replicon assay for compounds **437-438** are shown in table 4.16.[18]



Compound	Ar	EC_{50}(μM)	CC_{50}(μM)	EC_{90}(μM)	SI
437	2-hydroxyphenyl	0.061	>2.95	t.b.d.	>47.7
438	pyrazine	0.038	7.71	t.b.d.	201

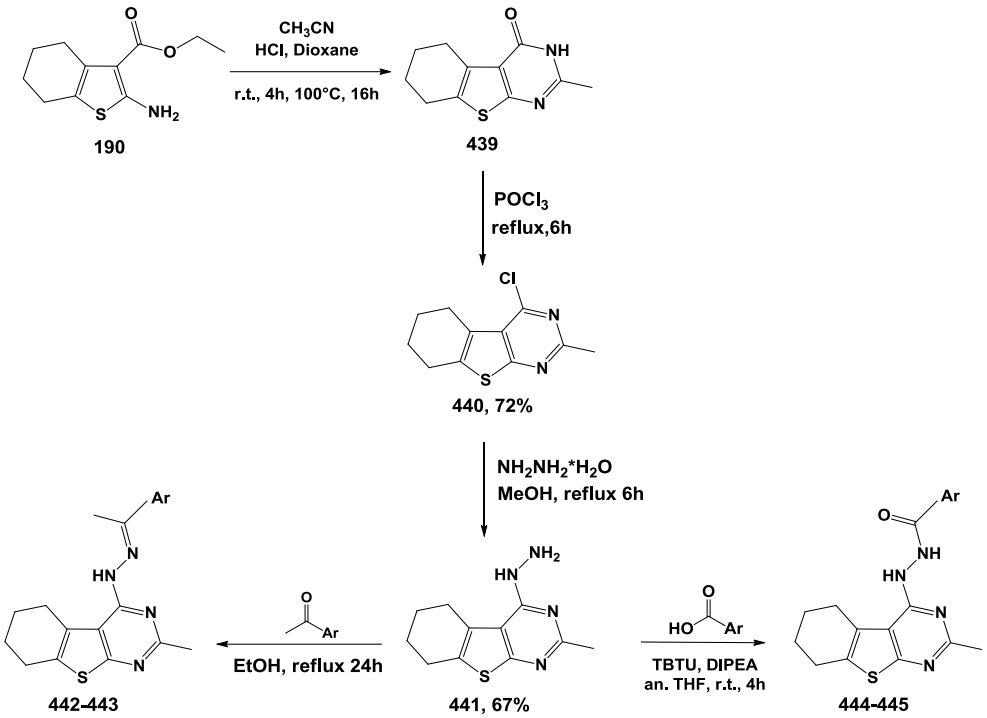
Table 4.16: Antiviral and cytotoxicity data for compounds **437-438**

The presence of a tetrahydropiridine substituent on the thiophene ring is associated with a retained activity profile in the hydrazone family of structures: the two new derivatives prepared, compounds **437** and **438**, show EC_{50} values in the nanomolar range.

4.12 Synthesis of 2-methyl-5,6,7,8-tetrahydro[1]benzothieno[2,3-*d*]pyrimidines

With the purpose to explore the role of the aromatic proton in the pyrimidine ring in the original tetrahydrobenzothiophene nucleus, its replacement with a methyl group was designed and carried out.

As shown in scheme 4.37, this result was achieved by condensing aminoester intermediate **190** with acetonitrile instead of formamide, in order to obtain pyrimidinone intermediate **439** with a methyl group replacing the aromatic proton. Compound **439** was then inserted in the previously optimised synthetic route, to give functionalised hydrazine intermediate **441**, which was reacted to give both hydrazone and hydrazide products, as had been done for the past series of derivatives.



Scheme 4.37: Synthetic approach for compounds **442-445**

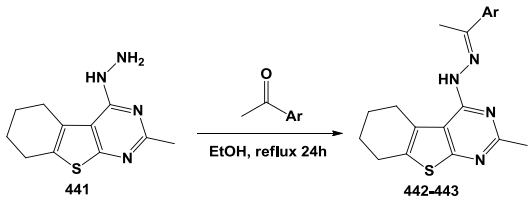
Synthesis of 2-methyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (439)

According to optimised reported procedures, the methyl-pyrimidinone ring in compound **439** was condensed reacting aminoester **190** with acetonitrile in a saturated HCl solution in dioxane, under pressure in a sealed tube.[32] Aminoester **190** forms a thick precipitate in the reaction system; the mixture was therefore sonicated in an

ultrasonic bath for 4 h, in order to solubilise the starting material, and then heated at 100°C for 16 h. Desired pyrimidinone intermediate **439** was then precipitated off by addition of water, filtered, dried and obtained in high purity with 40% yield.

4.12.1 2-Methyl-4-(2-(1-arylethylidene)hydrazinyl)-5,6,7,8-tetrahydrobenzo[4,5] thieno[2,3-*d*]pyrimidines (442, 443)

Two hydrazone final products were prepared for this family of compounds by reacting hydrazine intermediate **441** with ketones **202** and **250**.

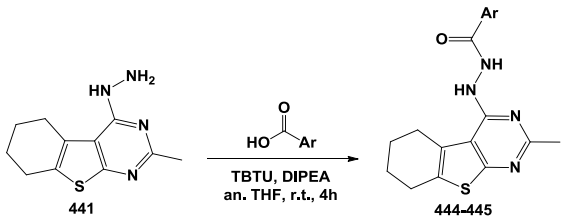


Ketone	Ar	Product	Yield %
202	2-hydroxyphenyl	**442**	86
250	pyrazine	**443**	59

Scheme 4.38: Synthesis of compounds **442** and **443**

4.12.2 *N*'-(2-Methyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)aryl hydrazides (444, 445)

A small series of two new hydrazide compounds was synthesised reacting intermediate **441** and carboxylic acids **275** and **277**, following as usual a TBTU-promoted coupling reaction.

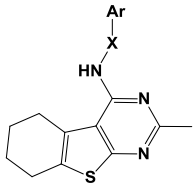


Carboxylic acid	Ar	Product	Yield %
275	2-hydroxyphenyl	**444**	36
277	2-pyridine	**445**	38

Scheme 4.39: Synthesis of compounds **444** and **445**

4.12.3 Biological evaluation

Compounds **442-445** were tested for their antiviral potential in the HCV replicon assay (table 4.12).[18]



Compound	Ar	Linker X	EC_{50}(μM)	CC_{50}(μM)	EC_{90}(μM)	SI
442	2-hydroxyphenyl	hydrazone	>284	>284	>284	-
443	pyrazine	hydrazone	0.069	>3	0.24	>42.7
444	2-hydroxyphenyl	hydrazide	1.26	25	3.07	19.8
445	2-pyridine	hydrazide	0.077	>2.95	0.32	>38.1

Table 4.17: Antiviral and cytotoxicity data for compounds **442-445**

Methylation of the pyrimidine ring is associated with an inconsistent effect among the series of compounds tested: while in general this modification induces activity retention, with EC_{50} values in the nanomolar range for compounds **443** and **445**, antiviral potential is completely lost in compound **442**, carrying a 2'-hydorxyphenyl group in the original hydrazone structure. Further biological studies are ongoing to confirm the heterogeneous activity profile found for this last modification.

4.13 Synthesis of 6-ethylthieno[2,3-*d*]pyrimidines

A series of recently published structures were considered interesting for a potential comparison with thienopyrimidine compounds synthesised and evaluated so far: compound **446** (figure 4.8) was discovered as the most potent derivative of a series of inhibitors of cyclin-dependent kinase CDK4, with IC_{50} values of 0.75 μg/mL for the inhibition of CDK4 activity, 1.610 μg/mL for antiproliferative activity in human colon carcinoma HCT116 cell lines, and 0.556 μg/mL antiproliferative activity in human lung carcinoma PC6 cell lines.[33]

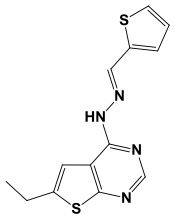


Figure 4.8: Structure of compound **446**

Cyclin-dependent kinases CDKs are a family of kinases involved in the regulation of growth, proliferation and apoptosis of eukaryotic cells;[34] they coordinate the progress of cells through the phases of the cell cycle and phosphorylate the retinoblastoma tumour suppressor protein Rb. When hyperphosphorylated, Rb triggers the release of E2F proteins, transcription factors which cause activation of gene expression, leading cells to enter the S phase.[35] CDK activities are down-regulated in normal cells by tumour suppressor p16 and other kinase inhibitors.[36] In many tumours, mutations, deletions and silencing of p16 or Rb gene are found, and deregulation of Rb pathway and CDK4 seems to play an important role in cancer progression.[37]

Inhibition of CDK4 expression has been demonstrated to cause hypophosphorylation of Rb, with a subsequent accumulation of cells in G1 phase,[38] while CDK4 knockdown in mammary tumour cells inhibits tumour formation.[39] CDK4 inhibitors represent therefore a potential anticancer strategy.

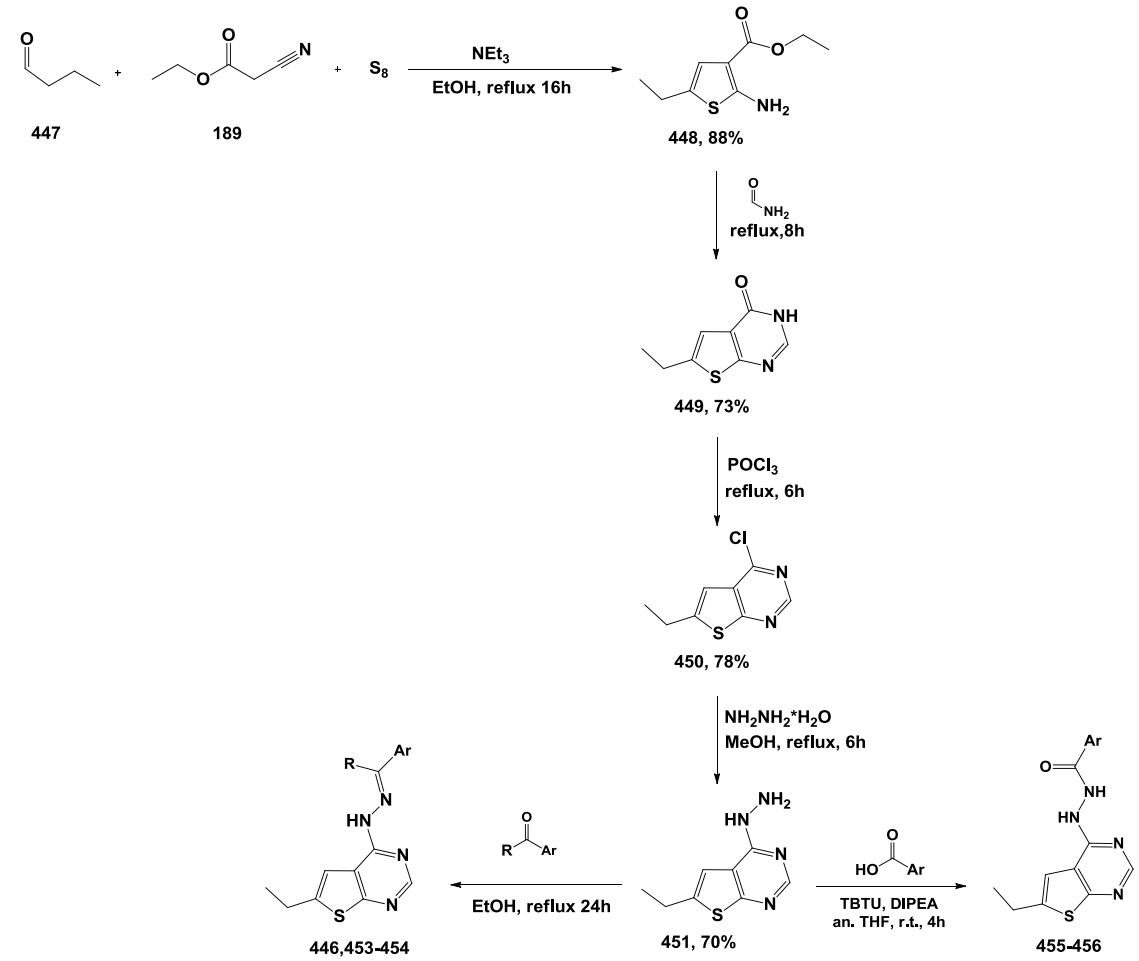
Evidence has been found suggesting that up-regulation of CDK4 and its regulatory subunit cyclin D1 are activated in cirrhosis, a precancerous condition that leads to the development of HCC, hepatocellular carcinoma, and this up-regulation is related to differentiation and progression of HCC.[40] Moreover, HCV core protein has been recognised to be strongly involved in HCC pathogenesis: it interacts with cellular

proteins and transduction pathways which regulate transcription of proto-oncogenes, and has been proven to be directly implicated in transformation and immortalisation of cells.[41] In this scenario, one of the regulatory systems led to up-regulation is cyclin D1-CDK4,[42] while inactivation of p16 gene is frequently associated with HCC tumours.[43]

Due to the fact that the HCV replicon assay is performed on a human hepatoma cell line, the opportunity of an interaction with an overexpressed cellular factor, such as CDK4, was taken into consideration for thienopyrimidine structures evaluated so far, due to the structural similarities they share with compound **446**. Their potential inhibition of kinases is going to be evaluated in future studies.

Nevertheless, as a proof of concept, it was decided to re-synthesise compound **446** and have it tested against HCV replication in the replicon assay. Moreover, its 6-ethylthienopyrimidine scaffold was used to prepare four new compounds, two hydrazone and two hydrazide derivatives, with the most successful hydroxyphenyl and heteroaromatic substitutions found for the previous series of structures discussed in this study. Finally, a sixth new product was designed to insert the thiophen-2-ylmethylene substituent of compound **446** in the tetrahydrobenzothienopyrimidine scaffold of compound **187**.

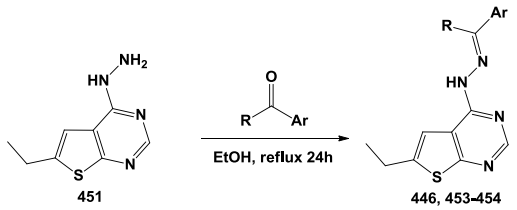
As can be seen in scheme 4.40, starting from butyraldehyde **447** in the first step of Gewald condensation, the usual five-step synthetic approach was followed to obtain compound **446** and final products **452-456**.



Scheme 4.40: Synthetic approach for compounds **442-445**

4.13.1 6-Ethyl-4-(2-(1-arylethylidene)hydrazinyl)thieno[2,3-*d*]pyrimidines (446, 453-454)

Along with compound **446**, two hydrazone final products were obtained for this group of compounds, through Schiff base formation between hydrazine intermediate **451** and carbonyl compounds **452**, **202** and **250**.

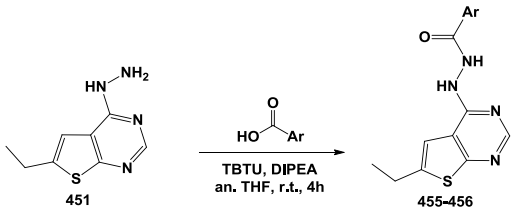


Aldehyde/Ketone	R	Ar	Product	Yield %
452	H	2-thiophene	**446**	46
202	Me	2-hydroxyphenyl	**453**	44
250	Me	pyrazine	**454**	71

Scheme 4.41: Synthesis of compounds **446**, **453-454**

4.13.2 *N*'-(6-Ethylthieno[2,3-*d*]pyrimidin-4-*yl*)-2-arylcarbohydrazides (455-456)

Two hydrazide compounds were prepared by reacting intermediate **451** with carboxylic acids **275** and **277**.

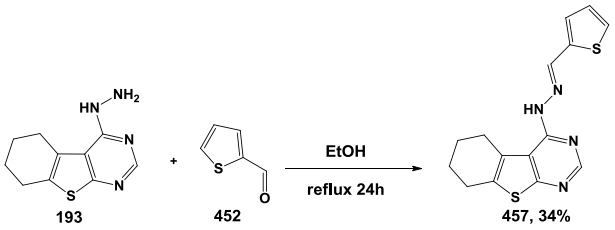


Carboxylic acid	Ar	Product	Yield %
275	2-hydroxyphenyl	**455**	28
277	2-pyridine	**456**	26

Scheme 4.42: Synthesis of compounds **444-445**

4.13.3 4-(2-(Thiophen-2-*yl*methylene)hydrazinyl)-5,6,7,8-tetrahydrobenzo[4,5] thieno[2,3-*d*]pyrimidine (457)

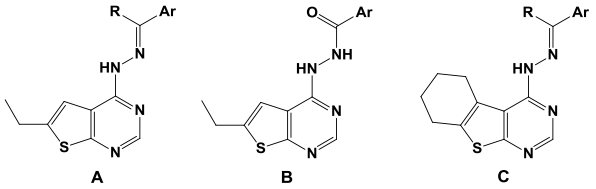
Thiophene-2-carboxaldehyde **452** was reacted with compound **193** to obtain final product **457**, as shown in scheme 4.43.



Scheme 4.43: Synthesis of compound **457**

4.13.4 Biological evaluation

Compounds **446** and **453-457** were evaluated in the HCV replicon assay.[18] Biological results are shown in table 4.18.



Compound	R	Ar	Scaffold	EC_{50}(μM)	CC_{50}(μM)	EC_{90}(μM)	SI
446	H	2-thiophene	A	4.69	22.8	-	4.9
453	Me	2-hydroxyphenyl	A	1.8	24.6	-	13.7
454	Me	pyrazine	A	0.895	4.55	0.662	5.1
455	-	2-hydroxyphenyl	B	1.46	>318	2.97	>217.8
456	-	2-pyridine	B	1.12	41.1	2	36.7
457	H	2-thiophene	C	4.91	40.5	-	8.2

Table 4.18: Antiviral and cytotoxicity data for compounds **446**, **453-547**

Biological data for compound **446** do not suggest significant activity against viral replication: its effect seems to be more correlated to cytotoxicity rather than inhibition of HCV replication, with a profile similar to the ones found for some of the previously tested hydrazone compounds (**215-234**, **252-265**, **290-294**), which were not considered as potential antiviral hits.

The same observation can be extended to compound **457**: as its ethenyl-thiophene analogue **294**, it is not associated with a significant antiviral potential. These data are in line with the trend previously found in the structure-activity relationships observed so far for these compounds.

As for the 6-ethyl substitution on the thienopyrimidine system in hydrazone compounds

453 and **454**, this modification is associated with loss of activity in the case of 2-hydroxyphenyl product **453**, and to an increased cytotoxic effect in the case of pyrazine derivative **454**, in comparison to previously discussed analogues carrying these two ethenylic aromatic groups.

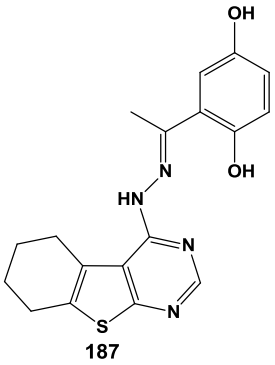
This observation cannot be transferred to hydrazide products **455** and **456**: these compounds show a retained antiviral profile in comparison to previously tested analogues with the same aromatic hydrazide substituents.

What can be concluded is that, as for CDK4 inhibitor **446**, its biological evaluation does not suggest the same antiviral potential found for thienopyrimidine hit structures identified in the course of this study. This assumption needs however to be confirmed with specific enzymatic tests, which may or may not exclude inhibition of CDK4 as the mechanism of action for compound **187** and its analogues.

Regarding instead the 6-ethyl substitution on the thienopyrimidine system, this modification seems to have a different influence between the two series of structures, hydrazones and hydrazides: while in the first case it is associated with loss of antiviral potential, in the second it appears instead to confer retention of antiviral activity. This evidence may indicate that the substituent on the thienopyrimidine system has a greater influence on antiviral activity for hydrazone structures, and its effect may be compensated in the hydrazide series by a stronger interaction with the target given by the hydrazide group.

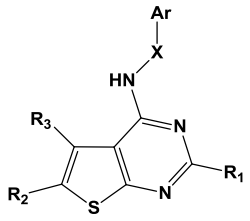
4.14 Conclusions

Following molecular modelling studies, compound **187** was identified as a hit for the inhibition of HCV replication in the HCV replicon assay. Its structure was the starting point for the design and synthesis of 128 new analogues, which were evaluated for their antiviral potential against the HCV replication.



187

According to biological results obtained so far, antiviral activity originally found for compound **187** was confirmed, and several new derivatives showed an improved antiviral effect, with some of them being associated with EC_{50} values in the nanomolar range.



Structure-activity relationships could be identified with the modifications performed on the structural scaffold of compound **187**: the presence of a hydrazone linker in the molecule is essential, but this bond can be successfully replaced with a hydrazide group, which is believed to be more stable in aqueous conditions. Moreover, an ethylidene group on the hydrazone bond is important for the cytotoxicity profile: the absence of the methyl group on this bond is associated with low CC_{50} and SI values.

In the case of both hydrazone and hydrazide structures, the linker has to be functionalised with a six-membered aromatic ring carrying a hydroxyl group in position 2, or a heteroaromatic ring with a nitrogen in position 2: among the different series of

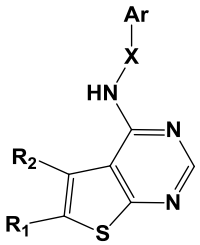
derivatives prepared, the most successful modifications are obtained with a 2-hydroxyphenyl, a 2-pyridine or a pyrazine group in this part of the structure. The presence of a 2-bendimidazole group is tolerated, while all the other modifications attempted were associated with loss of activity.

The presence of an aliphatic or aromatic substituent on the thienopyrimidine nucleus appears to be essential for activity retention, and suggests expansion potential for this part of the structure: when the original tetrahydrobenzene ring is completely removed, an enhanced cytotoxic effect can be observed, while the aromatisation of this group is associated with activity retention. Replacement of this substituent with a smaller cyclopentyl ring or with a bigger cycloheptyl group is associated with a retained antiviral profile, and the same effect is seen with the insertion of heterocyclic functions such as an *N*-methyl-tetrahydropyridine ring, a pyran group or a tetrahydropiridine ring.

The original tetrahydrobenzene group on the thiophene ring can also be replaced with aliphatic groups such as methyl, ethyl, methoxy and ethyl ester moieties: all these modifications are associated with retention of activity. Biological data obtained so far suggest that the occupational volume in this part of the molecule is important for antiviral activity.

The role of the pyrimidine proton has also been explored: its replacement with a methyl group is associated with an inconsistent effect among the structural derivatives prepared, and even if the general trend suggests activity retention, further biological evaluations are required to confirm this assumption.

The most promising modifications found so far are summarised in table 4.19.



Compound	Ar	Linker X	R_1, R_2	EC_{50}(μM)	CC_{50}(μM)	EC_{90}(μM)	SI
343	pyrazine	hydrazide	cyclopentyl	0.0759	3.18	<0.256	41.9
352	2-hydroxyphenyl	hydrazone	benzene	0.0861	12	0.421	139.4
357	pyrazine	hydrazide	benzene	0.0783	1.31	0.143	17
374	pyrazine	hydrazide	dimethyl	0.091	1.58	0.26	17.3
437	2-hydroxyphenyl	hydrazone	tetrahydropyridine	0.061	>2.95	t.b.d.	>47.7
438	pyrazine	hydrazone	tetrahydropyridine	0.038	7.71	t.b.d.	201

Table 4.19: Thienopyrimidine derivatives with EC_{50} values in the nanomolar range.

Future modifications of the original structure could involve the replacement of the central thienopyrimidine nucleus with different heteroaromatic scaffolds, in order to determine the impact of this condensed ring on antiviral activity.

Further biological studies are ongoing in order to determine the actual EC_{50}, EC_{90} and CC_{50} values for some of the compounds prepared, along with the determination of the viral or cellular target of these structures, and potentially their mechanism of action.

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Chapter 5

Pyrrolones

5.1 Ligand-based Virtual Screening

An essential requirement for mutual recognition between small molecules and their protein targets is shape complementarity.[1] Common docking-based virtual screening techniques, even though evaluating shape complementarity between a hit and the binding pocket, usually estimate the ligand binding energy by a scoring procedure that emphasises electrostatics rather than shape.[2] In ligand-based screening methods, for which a high accuracy in finding active molecules has been demonstrated, the shapes of known ligands are compared with unknown ones, while the chemical properties of known molecules can be included in the comparison as pharmacophoric or electrostatic models. These methods based on ligand similarity can evaluate two-dimensional similarities, such as fingerprints,[3] or three-dimensional similarities, focusing on the occupational volume acquired by molecules. While 2D comparison methods tend to find hits with the same functional groups of the query molecule,[4] 3D methods are focused on the occupational volume associated with each molecule, thus allowing the identification of novel lead structures with a higher level of scaffold diversity. In 3D similarity search softwares such as ROCS, pharmacophoric properties or associated electrostatic potential of the query molecule can be implemented to the search, thus enriching the use of complementarity shape with chemical features that might be important for ligand-target recognition.[5]

In order to explore the potential of a shape-comparison method in the search for HCV NS3 helicase inhibitors, this type of approach was applied for a new screening of the SPECS library.

Due to the wide chemical diversity and variability of target subsites associated with known helicase inhibitors, the structures of the dye soluble blue HT, compound **1**, and its chemical derivatives were chosen as a starting point,[6] since compound **1** is the only helicase inhibitor for which a crystal structure in complex with the enzyme is available (PDB ID 1ZJO, figure 5.1).[7]

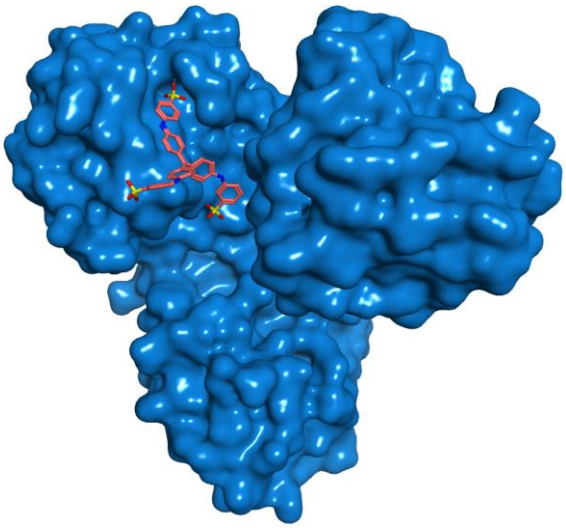


Figure 5.1: 1ZJO crystal structure. Compound **1** in complex with the HCV NS3 helicase

Many of the published HCV NS3 helicase inhibitors have recently been re-tested in both an optimised enzymatic assay and a replicon cell-based assay:[8] among them, one structural derivative of compound **1**, the triphenylmethane analogue **458** (figure 5.2),[6] has been confirmed to inhibit both the enzyme (IC_{50} 17 +/- 7 μM) and the viral replication (~80% inhibited at 10 μM compound) without relevant cytotoxicity (~75% cell viability at 10 μM compound).[8]

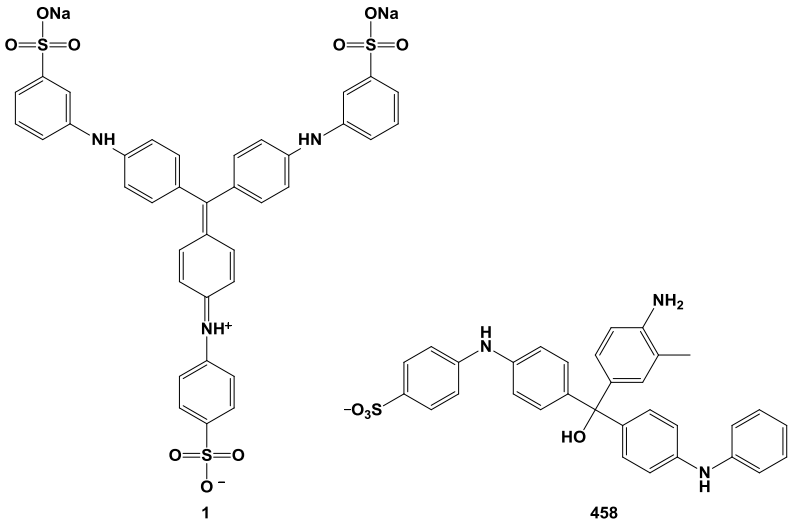


Figure 5.2: Structure of compounds **1** and **458**

Due to its activity profile, the structure of compound **458** was chosen as the query molecule for a shape comparison-based screening of the SPECS library,[9] performed with ROCS 3D similarity search software.[5]

5.1.1 Conformational search for ROCS query edition

In order to evaluate the shape complementarity between target query and screened compounds, the program considers the shape-density overlapping volumes of molecular shape in each superimposition, and the superimposed molecules are scored on the basis of a Tanimoto-like overlapping value of molecular volumes, the higher it is (between 0 and 1), the most similar the two compared volumes are considered.

In such context, the conformational state of the query molecule plays an essential role for hits selection, since all analysed structures will be scored on the basis of their overlapping with the target conformation. Conformation selection for the query structure becomes crucial, along with the evaluation of the different three-dimensional states that the molecules to screen can assume.

As had been previously done for the two pharmacophoric searches of the SPECS library, a conformational search with MOE2010.10 was performed for the creation of a conformational database of the compounds to be screened.[10]

A conformational analysis was performed on the srtucture of compound **458**, in order to identify low-energy three-dimensional states to be used as the query in the shape screening. Multiple low-energy conformations were obtained. For a more accurate selection of the target query, results found for compound **458** were compared to the conformation of compound **1** in the 1ZJO crystal structure: MOE Flexible Alignment tool was used for this purpose.[11] The low-energy conformations found for compound **458** were rigidly aligned to compound **1**, and the one corresponding to the best alignment (dU=0 kcal/mol, S= -220, figure 5.3) was chosen as target query for ROCS screening.

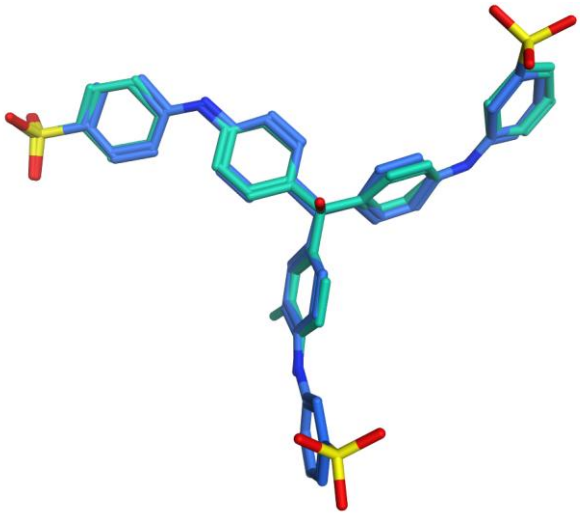


Figure 5.3: Conformational superimposition between compound **1**, coloured in blue, and compound **458**, in cyan.

5.1.2 Shape complementarity search of the SPECS database

The conformational database obtained for SPECS compounds was analysed against the selected conformation of compound **458** with the vROCS 3.1.2 software,[5] considering both shape and electrostatics complementarity with the target query for the evaluation of screened structures.

All screened conformations were ranked on the basis of their similarity score with the given target molecule. In order to choose a small number of hits to purchase and test in the cell-based replicon assay, a selection was made among the screened molecules in order to identify those showing the highest values of similarity index (Combo-shape tanimoto score) and the major number of different conformations matching the query.

On the basis of these parameters, six compounds were finally selected (Appendix I).

One of them, compound **459** (figure 5.4), showed antiviral effect in the replicon assay, with an EC_{50} and EC_{90} value of 38 and 149 μM, respectively, and a CC_{50}>204 μM. Its scaffold was chosen for further development.

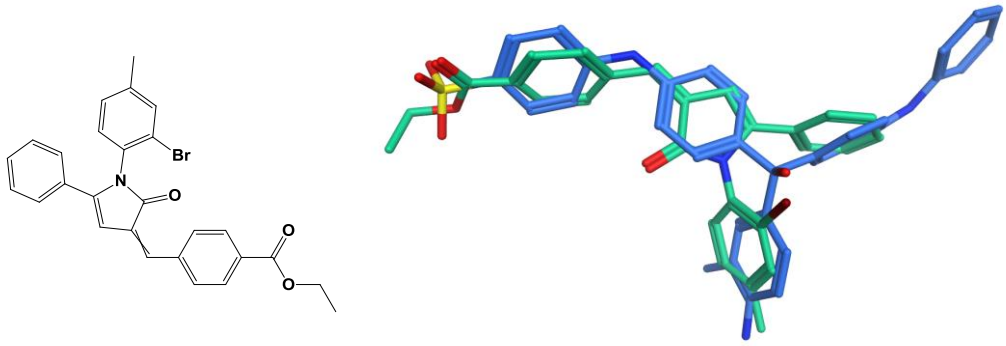


Figure 5.4: chemical structure and best alignment of compound **459** (aligned in green against compound **458**, in blue)

5.2 Synthesis of 1-aryl-3-arylidene-5-phenyl-1*H*-pyrrol-2(3*H*)-ones

The structure of compound **459** shows a central pyrrolone nucleus, substituted in position 1 and 5 with two aromatic rings, and linked in position 2 with a phenylidene system (figure 5.5).

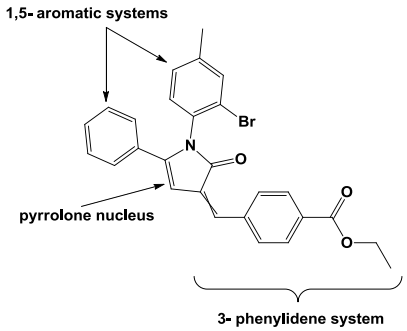
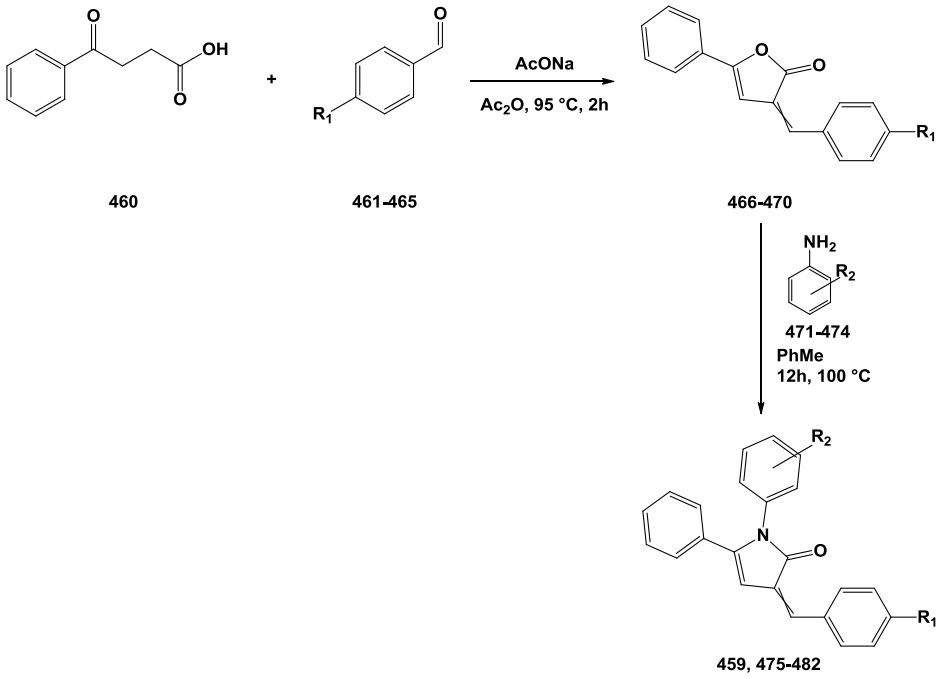


Figure 5.5: Chemical features of compound **459**

In order to confirm the antiviral potential associated with this scaffold, it was decided to synthesise, along with the original structure, a preliminary series of triphenyl-pyrrolone derivatives by only varying the aromatic substituents on the 1-phenyl and 3-phenylidene rings. For the preparation of desired compounds, a two-step synthetic pathway was planned, as shown in scheme 5.1.

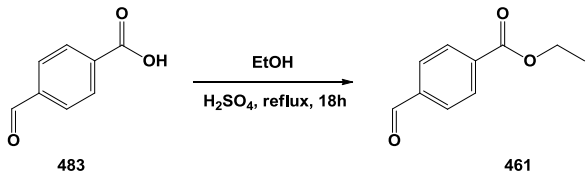


Scheme 5.1: Synthetic pathway planned for compounds **459**, **475-482**

The strategy carried out began with the condensation between 3-benzoyl propanoic acid (**460**) and differently *para* substituted benzaldehydes, with the formation of intermediates **466-470** through furanone ring closure, followed by phenyl-amine displacement of the cyclic ester with the formation of the pyrrolone system in final products **459**, **475-482**.

Synthesis of ethyl 4-formylbenzoate (461)[12]

Due to the presence of a 4-benzoic acid ethyl ester substituent in the structure of compound **459**, benzaldehyde **461** was synthesised by a Fischer esterification of 4-formyl-benzoic acid (**483**), which was refluxed with ethanol in presence of sulphuric acid to give desired ethyl 4-formylbenzoate (**461**), as shown in scheme 5.2.

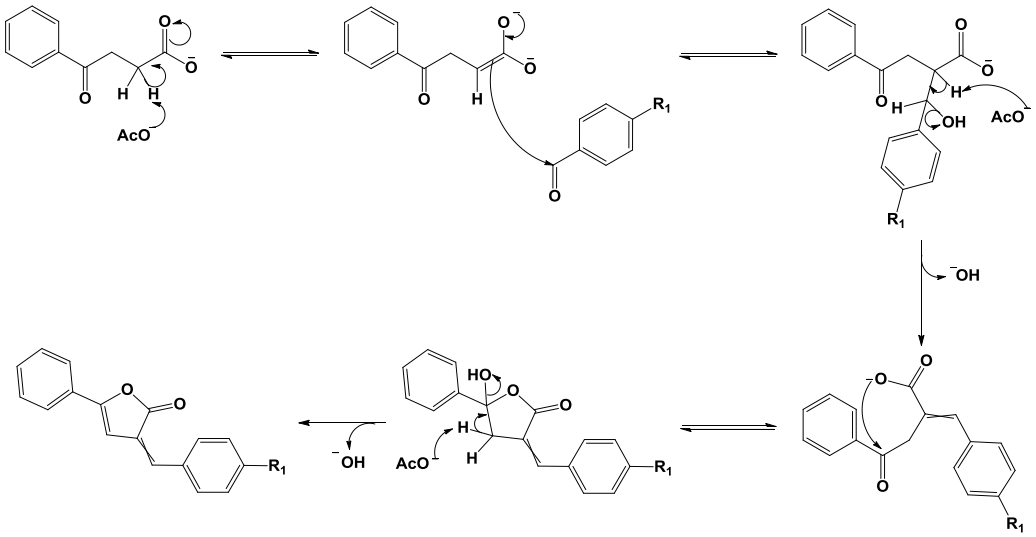


483 461

Scheme 5.2: Synthesis of starting bendaldehyde **461**

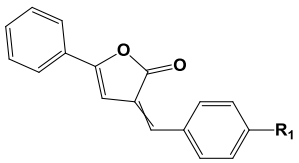
5.2.1 3-Arylidene-5-phenylfuran-2(3*H*)-ones (466-470)

Following reported procedures, furanone intermediates 258-262 were obtained by heating the starting materials at 95 °C in acetic anhydride in presence of sodium acetate. [13] The formation of the central butenolide ring is believed to follow a Perkin condensation between the β-benzoylpropionic acid and aryl aldehyde,[14] which are thought to form an aldol-condensation product that subsequently undergoes internal cyclisation with the formation of furanone ring, as shown in scheme 5.3.



Scheme 5.3: Postulated mechanism for the formation of compounds **466-470**

Following this procedure, five butenolide intermediates were prepared by changing the starting benzaldehyde (table 5.1). For this preliminary series of compounds it was decided to maintain a common unsubstituted phenyl ring in position 5 of the final pyrrolone derivatives, while exploring a few different substitutions in the *para* position of the 3-phenylidene system.



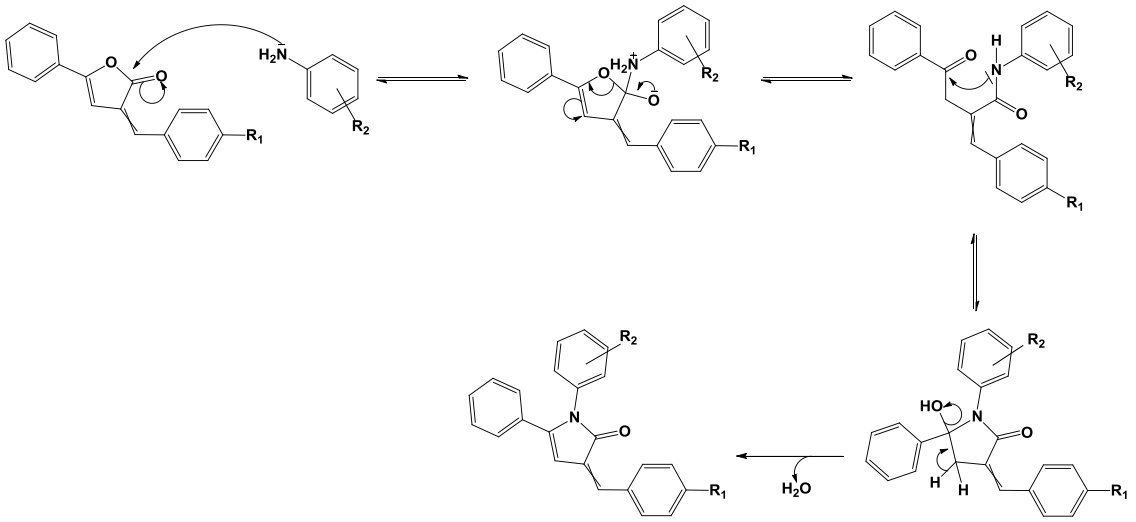
Aldehyde	R_1	Furanone intermediate	Yield %
461	COOEt	**466**	31
462	H	**467**	35
463	OMe	**468**	33
464	Br	**469**	51
465	Ph	**470**	41

Table 5.1: Chemical structure of furanone intermediates **466-470**

5.2.2 1-Aryl-3-arylidene-5-phenyl-1*H*-pyrrol-2(3*H*)-ones (459, 475-482)

According to reported procedures, a first attempt to obtain final compound **459** was made by heating at 100 °C in toluene furanone intermediate **466** with 2-bromo-4-methyl-aniline (**471**). [15-16] Formation of the final pyrrolone product is thought to take

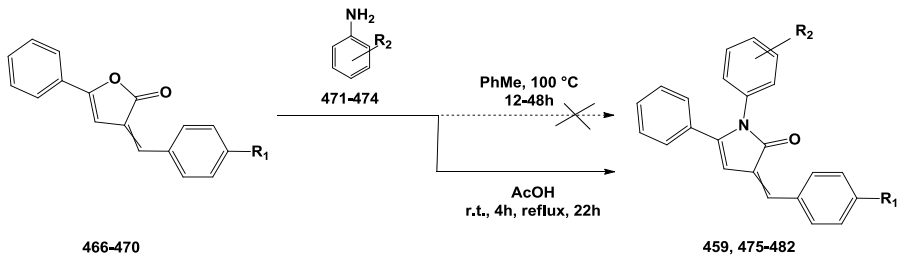
place through an intermediate stage where the furanone ring is opened as a result of the ammonolysis of the starting compound by the aromatic amine. The amide initially formed is then believed to undergo cyclisation to a tautomeric 5-hydroxyl species, followed by dehydration with the formation of pyrrolone ring (scheme 5.4).[16]



Scheme 5.4: Postulated mechanism for the formation of compounds **459**, **475-482**

The reaction was tried with different molar ratios of the reagents and time was increased from 12 to 48 h, but formation of desired product **459** was not observed in this solvent system, while the starting material **461** was recovered after flash column chromatography purification.

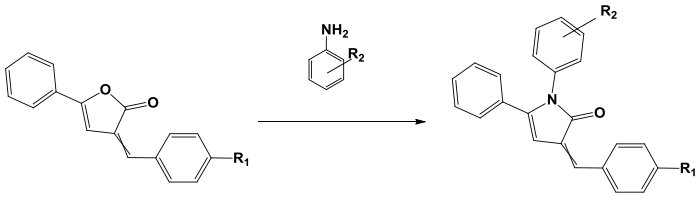
Following reported procedures for the synthesis of phthalimides,[17] in an attempt to enhance the elecrophilic characteristics of the ketone carbonyl carbon of the intermediate amide, and in order to create a dehydrating environment to favour the formation of final pyrrolone products, toluene was replaced with glacial acetic acid, and the mixture was heated under reflux for 22 h, as shown in scheme 5.5.



Scheme 5.5: Attempted conditions for the synthesis of compounds **459**, **475-482**

Eight new derivatives, along with compound **459**, were obtained with the new synthetic

procedure, by combining intermediates **461-465** with differently substituted anilines (**471-474**). Their structures are shown in table 5.2.

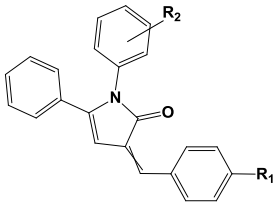


Furanone intermediate	R_1	Aniline	R_2	Product	Yield %
461	COOEt	**471**	2-Br, 4-Me	**459**	44
462	H	**472**	H	**475**	34
461	COOEt	**472**	H	**476**	57
463	OMe	**472**	H	**477**	39
464	Br	**472**	H	**478**	29
465	Ph	**472**	H	**479**	27
461	COOEt	**473**	4-Me	**480**	32
461	COOEt	**474**	2,3-Me	**481**	56
462	H	**471**	2-Br, 4-Me	**482**	50

Table 5.2: Structure of compounds **459**, **475-482**

5.2.3 Biological evaluation

Newly synthesised triphenylpyrrolone structures were tested for antiviral activity in the subgenomic replicon and cytostatic assays.[18] Their biological results are shown in table 5.3.



Product	R_1	R_2	EC_{50}(μM)	CC_{50}(μM)	EC_{90}(μM)	SI
459	COOEt	2-Br, 4-Me	20.2	>205	61.7	>10.1
475	H	H	4.9	29	15.3	5.9
476	COOEt	H	22.8	99.6	73.2	3.2
477	OMe	H	5.31	97.2	21.6	18.3
478	Br	H	3.52	25.7	11.6	7.3
479	Ph	H	17.4	156	61.7	8.9
480	COOEt	4-Me	22.9	>244	82	>10.7
481	COOEt	2,4-Me	43.1	90.8	-	-
482	H	2-Br, 4-Me	3.3	>240	11.3	>73

Table 5.3: Biological results for compounds **459**, **475-482**

Re-synthesised compound **459** confirmed its activity profile, with EC_{50}, EC_{90} and CC_{50} values close to the ones previously found for the batch acquired from SPECS.

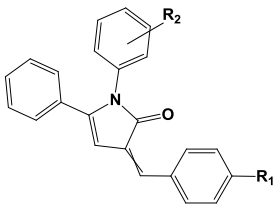
Considering the substitutions attempted so far, the presence of an ethyl ester function in the 3-phenylidene ring does not appear to be essential for activity retention, since compound **482**, where this group is removed, is the most active found for this series of compounds, with EC_{50} and EC_{90} values in the low μM range. A similar profile can be found also for derivatives **475**, **477** and **478**, where the 1-phenyl ring is unsubstituted and the ethyl ester group is replaced respectively with 4-H, 4-Me and 4-Br groups, but these modifications are associated with increased cytotoxicity.

Antiviral potential associated with triphenylpyrrolone compounds was only partially explored with the modifications carried out so far, but activity confirmation obtained for compound **459** and improved antiviral profile found for derivative **482** could be an encouraging starting point for future development.

5.3 Conclusions

In the course of this final part of the study, ligand-based molecular modelling techniques were applied to the analysis of the HCV NS3 helicase enzyme.

A shape-comparison screening of commercially available compounds led to the identification of a third hit molecule, compound **459**, associated with a positive anti-HCV potential in cellular assays. The original structure was successfully re-synthesised, together with a preliminary series of eight new analogues, all of which have been tested in the HCV replicon assay.



The antiviral potential originally found was confirmed for compound **459**, and new structural derivatives were associated with an improved antiviral activity in the low μM range: the most active analogue in this first series of structures is compound **482**, for which an improvement of antiviral potential is observed in terms of both EC_{50} and CC_{50} values. A preliminary SAR evaluation suggests that the antiviral effect is mainly due to the *N*-phenyl substituent: the original 2-bromo-4-methyl-phenyl ring is essential for activity, while with the removal of the ethyl ester function on the phenylethylidene ring of compound **459** is associated with an improved antiviral profile. These results represent a promising starting point for future studies, with which different substituents could be investigated for all the three aromatic rings, along with the replacement of the central pyrrolone nucleus with new scaffolds.

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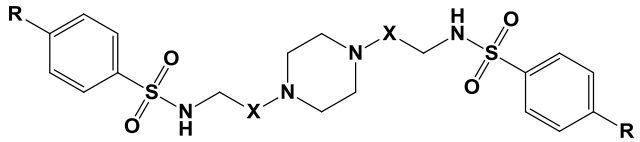
Conclusions

In the course of this study, different molecular modelling techniques were applied to the analysis of the NS3 helicase enzyme with the aim to identify potential inhibitors of HCV replication.

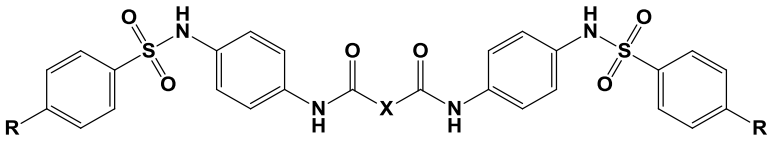
A first virtual screening study on the enzyme closed conformation allowed the identification of compound **12**, which was associated to low-μM antiviral effect in the replicon assay.



12: R= Cl, X= $(CH_2)_2$

Starting from the structure of compound **12**, 61 new derivatives were designed and synthesised to explore the antiviral effect associated to its scaffold, and several compounds were found to inhibit the viral replication at low μM concentration. The presence of two equally substituted phenyl-sulfonamide groups is essential for retention of activity, along with the presence of the central piperazine nucleus and two equal three-carbon aliphatic linkers. A hydrophobic substituent in the *para* position of the terminal aromatic rings is also essential for antiviral activity, along with the preservation of the symmetry of the molecule. A significant improvement in terms of potency could not be achieved with this series of structures, therefore further computer-based analyses were planned and carried out.

A ligand-based optimisation approach on compound **12** guided the synthesis of a first series of nine derivatives belonging to four new structural scaffolds.

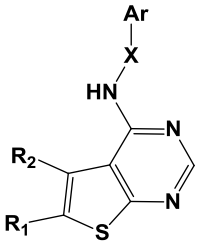


162: R= Cl, X= $(CH_2)_2$

Due to the antiviral potential associated to one of the new structures synthesised, compound **162**, an extended series of five new analogues was synthesised, and two derivatives showed anti-HCV activity in the low μM range. Two equal *para*-phenylendiamine groups are required in the linker for activity retention, along with the presence of a central succinamide group and a *para*-hydrophobic substituent on the two phenyl-sulfonamide groups. Even if activity retention was obtained with different new

compounds, the desired improvement in potency was not achieved for the antiviral effect of this new series of compounds. A second virtual screening study was therefore planned with the aim to find more potent inhibitors of the HCV replication.

The second virtual screening was performed on the crystal structure of the NS3 helicase enzyme in the open conformation, and among the new structures screened and selected for biological evaluation compound **187** was identified as a HCV replication inhibitor with a sub-μM activity: its structure was the starting point for the design and synthesis of 128 derivatives, which were biologically evaluated against HCV replication in the replicon assay.

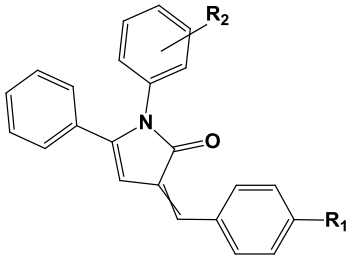


187: R_1, R_2= C_4H_8, X= $NCCH_3$, Ar= 2,5-OH-Ph

Structural modifications aiming to explore the antiviral potential associated with this new scaffold led to the synthesis of several new compounds with an improved antiviral potential, and some of them were associated to EC_{50} values in the nanomolar range. The presence of an aliphatic, aromatic or heterocyclic substituent on the thiophene ring is essential for antiviral activity, along with a hydrazone or hydrazide linker group between the thienopyrimidine ring and a second aromatic group. This second aromatic ring has to be a 2-hydroxyphenyl group or a six-membered heteroaromatic ring with a nitrogen in position 2, such as 2-pyridine or pirazine. Further biological evaluations are ongoing to identify the biological target and the mechanism of action of these promising antiviral agents, while future studies could include the substitution of the central thienopyrimidine ring with different heteroaromatic moieties, further expansion of the occupational volume of the substituent on the thiophene ring, and the replacement of the pyrimidine proton with new functional groups, since the only modification carried out so far on this part of the structure was the insertion of a methyl group.

Finally, a shape-comparison ligand-based screening allowed to the identification of a third hit molecule, compound **459**, associated to antiviral effect in the low μM range. Its structure was successfully re-synthesised, along with a first series of eight new

analogues.



459: R_1= COOEt, R_2= 2-Br, 4-Me
482: R_1= H, R_2= 2-Br, 4-Me

Among the small series of compounds prepared, the antiviral potential originally found was confirmed for compound **459**, while its derivative compound **482** shows improved antiviral activity in the replicon assay. Biological data obtained so far suggest that inhibition of the viral replication is mainly associated with the 2-bromo, 4-methyl substituents on the *N*-phenyl ring, which are essential for activity, while the removal of the ethyl ester function on the phenylethylidene moiety leads to an improved activity profile. The results found so far for this last series of compounds represent a promising starting point for future structural modifications, which could include the exploration of different aromatic modifications on the three phenyl rings, the removal of these rings one at a time, the substitution of the central pyrrolone group with different heterocycles. Even though these findings represent a successful application of computer-aided techniques for the identification of biologically active compounds, specific studies are required to define the mechanism of action of these molecules, and confirm whether or not their molecular target can be identified as the HCV NS3 helicase.

Chapter 6

Experimental

6.1 General information

All chemicals, reagents and solvents were purchased from Aldrich or purified by standard techniques.

Thin Layer Chromatography

Silica gel plates (Merck Kieselgel $60F_{254}$) were used and were developed by the ascending method. After solvent evaporation, compounds were visualised by irradiation with UV light at 254 nm and 366 nm.

Column Chromatography

Glass columns were dry packed in the appropriate eluent under gravity, with Woelm silica (32-63 mm). Samples were applied as a concentrated solution in the same eluent. Fractions containing the product were identified by TLC, combined and the solvent removed *in vacuo*.

NMR Spectroscopy

^{1}H, ^{13}C, NMR spectra were recorded on a Bruker AVANCE 500 spectrometer (500 MHz and 75 MHz respectively) and auto calibrated to the deuterated solvent reference peak. Chemical shifts are given in δ relative to tetramethylsilane (TMS); the coupling constants (J) are given in Hertz. TMS was used as an internal standard (δ = 0 ppm) for ^{1}H NMR and $CDCl_3$ served as an internal standard (δ = 77.0 ppm) for ^{13}C NMR.

6.1.1 Molecular Modelling

All molecular modelling studies were performed on a MAC pro 2.66 GHz Quad-Core Intel Xeon, running Ubuntu.

Molecular Operating Environment (MOE) 2010.10 and Maestro (Schrodinger version 9.0) were used as molecular modelling softwares.

All minimisations were performed with MOE until RMSD gradient of 0.001 Kcal mol^{-1} $Å^{-1}$ with the AMBER99 force field. Partial charges were automatically calculated.

Conformational analyses were performed with MOE 2010.10; conformers with a strain

energy >4 kcal/mol were discarded, and the maximum number of conformations per ligand was set to 500. In every step MOE default settings were applied.

Pharmacophoric filters were created within MOE 2010.10 choosing the PCH (polar-charged-hydrophobic) scheme.

Docking experiments were carried out using LEadIT-FlexX version 2.1.0, Plants version 1.1 and GlideSP module in Maestro with the default options.

Outputs of all docking experiments were refined with Glide SP refine-do not dock tool.

All refined docking results were rescored using Plants ChemPLP, FlexX and Glide SP scoring functions.

Selection of the best docked poses was made by building a consensus scoring function as follows: in each docking study, for each scoring function applied the first quartile value, fquart, was evaluated to determine the threshold limit under which relies the best 25% of the scoring results (negative energy values). The mathematical function Sign was then applied for each scoring value of each docked pose, in order to determine whether it belongs to the best 25% (sign +1) or not (sign -1). Finally, the three sign values, each one corresponding to the performance according to one scoring function, for a given docked pose were summed and only the ones with a sign sum equal to +3 (or +2, meaning that one of the assigned scoring values was equal to the fquart value) were chosen.

Referring to the three scoring functions Glide SP, Plants ChemPLP and FlexX as A, B and C respectively, the *consensus* scoring function equation for a given docked pose X was the following:

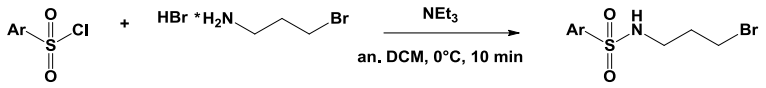
$$\text{Sign sum}(X) = \text{sign}(\text{fquart}_A - X_A) + \text{sign}(\text{fquart}_B - X_B) + \text{sign}(\text{fquart}_c - X_c)$$

Shape-comparison screening was performed with vROCS version 3.1.2. Both shape and colour screen criteria were applied to the query. Output conformations were ranked by the Tanimoto Combo score and the Shape Tanimoto score.

6.2 Synthesis of piperazine structures

6.2.1 General procedures 1-11

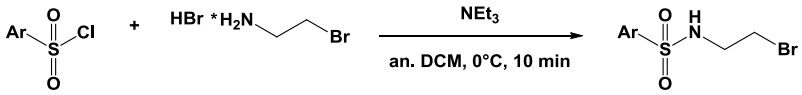
General procedure 1: synthesis of *N*-(3-bromopropyl)arylsulfonamides



Arylsulfonyl chloride (4 mmol) and 3-bromopropylamine hydrobromide (**21**) (1.0 g, 4.6 mmol) were suspended in anhydrous DCM (14 mL) under nitrogen atmosphere. The reaction mixture was cooled to 0°C in an ice-bath and then treated dropwise with triethylamine (1.34 mL, 9.6 mmol) over a period of 10 min.

The reaction mixture was stirred for 10 min under ice-cooling, then diluted with DCM (50 mL) and washed with 2M hydrochloric acid solution (2 x 60 mL) and brine (2 x 60 mL). The organic solvent was evaporated at reduced pressure after drying over $MgSO_4$ to give the pure N-(3-bromopropyl)arylsulfonamides.

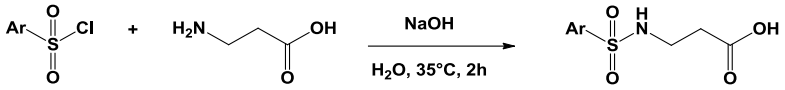
General procedure 2: *N*-(2-bromoethyl)arylsulfonamides



Arylsulfonyl chloride (4.2 mmol), and 2-bromoethylamine hydrobromide (**37**) (1.0 g, 4.8 mmol) were suspended in anhydrous DCM (14 mL) under nitrogen atmosphere. The reaction mixture was cooled to 0°C in an ice-bath and then treated dropwise with triethylamine (1.4 mL, 10 mmol) over a period of 10 min.

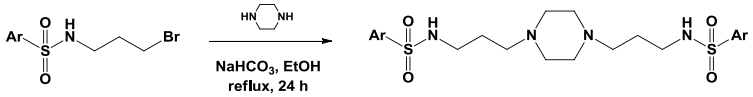
The reaction mixture was stirred for ten min under ice-cooling, then diluted with DCM (50 mL) and washed with 2M hydrochloric acid solution (2 x 60 mL) and brine (2 x 60 mL). The organic solvent was evaporated at reduced pressure after drying over $MgSO_4$ to give pure *N*-(2-bromoethyl)arylsulfonamides.

General procedure 3: synthesis of arylsulfonylamino propionic acids



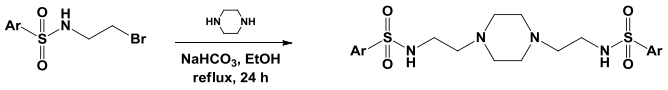
To partially dissolved β-alanine (**54**) (0.6 g, 6.8 mmol) in distilled water (4 mL) was added a solution of aqueous NaOH 2M (3.4 mL), followed by the portionwise addition of arylsulfonyl chloride (9.6 mmol). The reaction mixture was stirred at 35°C and a solution of 1 M aqueous NaOH was added portionwise to maintain a pH of approximately 9. After complete consumption of alkali, stirring was continued at 35°C for an additional 1 h. Unreacted arylsulfonyl chloride was removed by filtration, and the reaction mixture was acidified with 5M aqueous HCl at 0°C to pH 2. The aqueous solution with solid precipitate was stored at 4°C o.n. The crystals formed were collected by filtration, washed with cold water and dried at reduced pressure to afford pure 3-arylsulfonylamino propionic acids.

General procedure 4: synthesis of *N,N'*-(3,3'-(piperazine-1,4-di*yl*)bis(propane-3,1-di*yl*))diarylsulfonamides



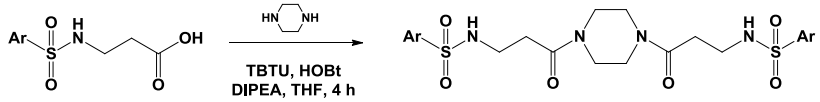
Piperazine (0.050 g, 0.6 mmol) and $NaHCO_3$ (0.109 g, 1.3 mmol) were suspended in absolute ethanol (9 mL). *N*-(3-Bromopropyl)arylsulfonamide (1.3 mmol), was then added portionwise to the suspension and the reaction mixture was stirred under reflux for 24 h. The solvent was evaporated under reduced pressure and the crude residue was purified by flash column chromatography to afford pure *N,N'*-(3,3'-(piperazine-1,4-di*yl*)bis(propane-3,1-di*yl*))diarylsulfonamides.

General procedure 5: synthesis of *N,N'*-(2,2'-(piperazine-1,4-di*yl*)bis(ethane-2,1-di*yl*)) diarylsulfonamides



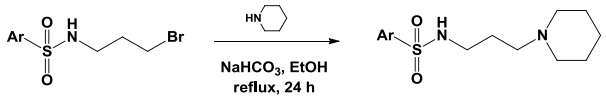
Piperazine (0.050 g, 0.6 mmol) and $NaHCO_3$ (0.109 g, 1.3 mmol) were suspended in absolute ethanol (9 mL). *N*-(2-Bromoethyl)arylsulfonamide (1.3 mmol), was then added portionwise to the suspension and the reaction mixture was stirred under reflux for 24 h. The solvent was evaporated under reduced pressure and the crude residue was purified by flash column chromatography to afford pure *N,N'*-(2,2'-(piperazine-1,4-diyl)bis(ethane-2,1-diyl))diaryl sulfonamides.

General procedure 6: *N,N'*-(3,3'-(piperazine-1,4-di*yl*)bis(3-oxopropane-3,1-di*yl*)) diarylsulfonamides



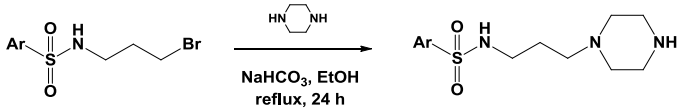
3-Arylsulfonylamino propionic acid (1.3 mmol), TBTU (0.45 g, 1.4 mmol) and HOBt (0.19 g, 1.4 mmol) were suspended in anhydrous THF (9 mL) at r.t. DIPEA (0.7 mL, 4.2 mmol) was then added to the reaction mixture, followed by piperazine (41) (0.05 g, 0.6 mmol). The reaction mixture was left stirring at r.t. for 4 h. The organic solvent was then removed at reduced pressure and the residue was suspended in EtOAc (100 mL). The organic layer was washed with saturated $NaHCO_3$ solution (2 x 70 mL), then with saturated NH_4Cl solution (2 x 70 mL) and finally with brine (70 mL). The organic solvent was removed under vacuum after drying over $MgSO_4$. The crude residue was purified by flash column chromatography to afford pure *N,N'*-(3,3'-(piperazine-1,4-diyl)bis(3-oxopropane-3,1-diyl))-diarylsulfonamides.

General procedure 7: synthesis of *N*-(3-(piperidin-1-*yl*)propyl)arylsulfonamides



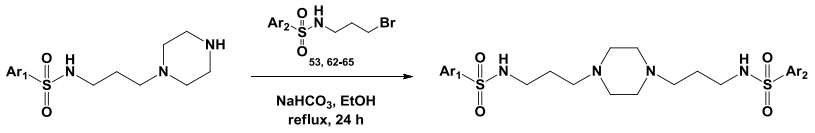
Piperidine (0.1 mL, 1.2 mmol) and $NaHCO_3$ (0.109 g, 1.3 mmol) were suspended in absolute ethanol (9 mL). *N*-(3-Bromopropyl)arylsulfonamide (1.3 mmol), was then added portionwise to the suspension and the reaction mixture was stirred under reflux for 24 h. The solvent was evaporated under reduced pressure and the crude residue was purified by flash column chromatography to afford pure *N*-(3-(piperidin-1-*yl*)propyl)aryl sulfonamides.

General procedure 8: synthesis of *N*-(3-piperazin-1-*yl*-propyl)-arylsulfonamides



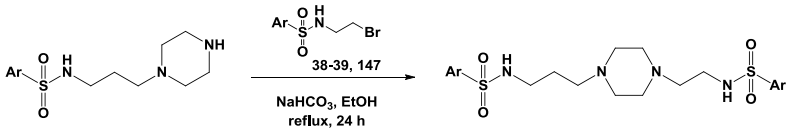
Piperazine (2.00 g, 23.2 mmol) and $NaHCO_3$ (3.25 g, 38.7 mmol) were suspended in absolute ethanol (21 mL). *N*-(3-Bromopropyl)arylsulfonamide (7.7 mmol) was then added portionwise to the suspension and the reaction mixture was stirred under reflux for 24 h. The reaction mixture was then concentrated under reduced pressure. The residue was diluted with EtOAc (30 mL), washed with water (3 x 30 mL) and the organic solvent was removed under vacuum after drying over $MgSO_4$. The crude residue was purified by flash column chromatography to afford pure *N,N'*-(3,3'-(piperazine-1,4-diyl)bis(propane-3,1-diyl))diaryl- sulfonamides.

General procedure 9: synthesis of *N*-{3-[4-(3-arylsulfonylamino-propyl)-piperazin-1-yl]-propyl}-arylsulfonamides



A mixture of *N*-(3-piperazin-1-*yl*-propyl)-arylsulfonamide (1.0 mmol), *N*-(3-bromopropyl)arylsulfonamide (1.5 mmol) and $NaHCO_3$ (0.16 g, 2 mmol) in absolute ethanol (7 mL) was stirred under reflux for 24 h. The solvent was then evaporated under reduced pressure and the crude residue was purified by flash column chromatography to afford pure *N,N'*-(3,3'-(piperazine-1,4-diyl)bis(propane-3,1-diyl))diarylsulfonamides.

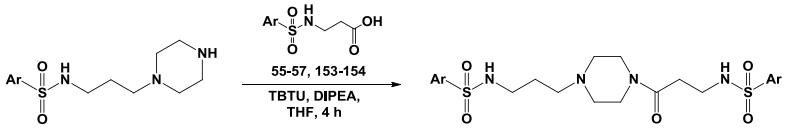
General procedure 10: synthesis of *N*-(3-(4-(2-(arylsulfonamido)ethyl)piperazin-1-*yl*) propyl)arylsulfonamides



A mixture of *N*-(3-piperazin-1-*yl*-propyl)-arylsulfonamide (1.0 mmol), *N*-(2-bromoethyl)arylsulfonamide (1.5 mmol) and $NaHCO_3$ (0.16 g, 2 mmol) in absolute ethanol (7 mL) was stirred under reflux for 24 h.

The solvent was evaporated under reduced pressure and the crude residue was purified by flash column chromatography to afford pure *N*-(3-(4-(2-(arylsulfonamido) ethyl)piperazin-1-*yl*)propyl)arylsulfonamides.

General procedure 11: synthesis of *N*-(3-(4-(3-(arylsulfonamido)propanoyl) piperazin-1-*yl*)-3-oxopropyl)aryl sulfonamides

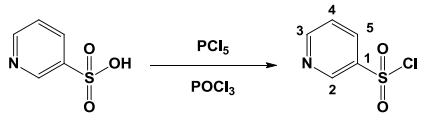


3-Arylsulfonylamino propionic acid (1.1 mmol) and TBTU (0.38 g, 1.2 mmol) were suspended in anhydrous THF (6 mL) at r.t. DIPEA (0.4 mL, 2.4 mmol) was then added to the reaction mixture, followed by *N*-(3-piperazin-1-*yl*-propyl)-arylsulfonamide (1 mmol). The reaction mixture was left stirring at r.t. for 4 h. The organic solvent was then removed at reduced pressure and the residue was diluted with EtOAc (30 mL). The organic layer was washed with saturated $NaHCO_3$ solution (2 x 30 mL) and brine (30 mL). The organic solvent was removed under vacuum after drying over $MgSO_4$. The crude residue was purified by flash column chromatography to afford pure *N*-(3-(4-(3-(arylsulfonamido)propanoyl)piperazin-1-*yl*)propyl) arylsulfonamides.

6.2.2 Arylsulfonyl chlorides (89, 90)

Pyridine-3-sulfonyl chloride (89)[1]

($C_5H_4ClNO_2S$; M.W.= 177.6)



109 **89**

A mixture of pyridine-3-sulfonic acid (**109**) (2 g, 12.6 mmol), phosphorous pentachloride (3.91 g, 18.8 mmol) and phosphorous oxychloride (10 mL) was heated at 120°C o.n. Excess phosphorous oxychloride was then evaporated under reduced pressure and the residue was quenched with ice and partitioned between water (50 mL) and diethyl ether (50 mL). The pH of the aqueous phase was adjusted by addition of solid sodium bicarbonate to pH 8, then the organic layer was separated and washed successively with saturated sodium bicarbonate solution (40 mL), water (40 mL) and brine (40 mL). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure to give pure pyridine-3-sulfonyl chloride (**89**) as a yellow oil.

Yield: 1.45 g (65%)

^{1}H-NMR ($CDCl_3$), δ: 7.62 (m, 1H, H-aromatic), 8.34 (m, 1H, H-aromatic), 8.99 (m, 1H, H-aromatic), 9.28 (s, 1H, H-2).

^{13}C-NMR ($CDCl_3$), δ: 124.23, 134.60 (CH, C-aromatic), 141.15 (C, C-1), 147.62, 155.46 (CH, C-aromatic).

4-Chlorosulfonyl-benzoic acid ethyl ester (90)[2]

($C_9H_9ClO_4S$; M.W.= 248.6)

110 **90**

4-Chlorosulfonylbenzoic acid (**110**) (1 g, 4.5 mmol) was suspended in thionyl chloride (8 mL) and DCM (4 mL) and the reaction was heated at reflux for 2 h. The solvent was

removed *in vacuo* and ice-cold ethanol (4 mL) was added to the residue. The mixture was stirred for 10 min. in an ice-bath before the addition of 7 mL of ice-cold water.

The resulting precipitate was collected by filtration, washed with cold water and dried under vacuum to afford pure 4-chlorosulfonyl-benzoic acid ethyl ester (**90**) as a white solid.

Yield: 0.64 g (57%)

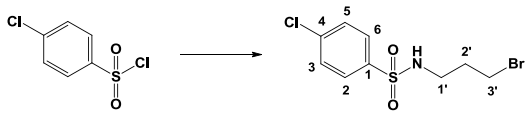
^{1}H-NMR ($CDCl_3$), δ: 1.45 (t, J= 7.1 Hz, 3H, H-3'), 4.47 (q, J= 7.1 Hz, 2H, H-2'), 8.13 (d, J= 8.8 Hz, 2H, H-aromatic), 8.29 (d, J=8.8, 2H, H-aromatic).

^{13}C-NMR ($CDCl_3$), δ: 14.22 (CH_3, C-3'), 62.14 (CH_2, C-2'), 127.02, 130.81 (CH, C-aromatic), 136.45, 147.36 (C, C-1, 4), 164.44 (C, C-1').

6.2.3 *N*-(3-Bromopropyl)arylsulfonamides (22-29, 91-99)

_N_-(3-Bromo-propyl)-4-chloro-benzenesulfonamide (22)[3]

($C_9H_{11}BrClNO_2S$; M.W.= 312.6)



13 **22**

General procedure 1;

Pale yellow wax;

T.L.C. System: DCM-*n*hexane 4:1 v/v, Rf: 0.35.

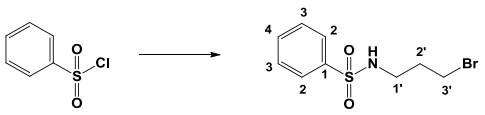
Yield: 1.16 g (94%)

^{1}H-NMR ($CDCl_3$), δ: 2.03-2.09 (m, 2H, H-2'), 3.12-3.18 (m, 2H, H-1'), 3.45 (t, J= 6.2 Hz, 2H, H-3'), 4.86 (t, J= 6.2 Hz, 1H, NH), 7.53 (d, J= 8.6 Hz, 2H, H-aromatic), 7.84 (d, J= 8.6 Hz, 2H, H-aromatic).

^{13}C-NMR ($CDCl_3$), δ: 29.99, 32.26, 41.47 (CH_2, C-2', 1', 3'), 128.54, 129.54 (CH, C-aromatic), 138.29, 139.39 (C, C-1, 4).

_N_-(3-Bromo-propyl)-benzenesulfonamide (23)[4]

($C_9H_{12}BrNO_2S$; M.W.= 278.1)



14 **23**

General procedure 1;

Pale yellow oil;

T.L.C. System: DCM-*n*hexane 4:1 v/v, Rf: 0.31.

Yield: 1.06 g (97%)

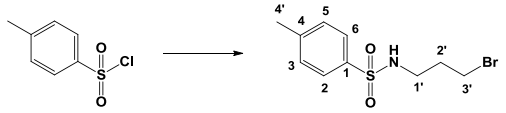
^{1}H-NMR ($CDCl_3$), δ: 2.03-2.07 (m, 2H, H-2'), 3.12-3.16 (m, 2H, H-1'), 3.43 (t, J=6.2, 2H, H-3'), 5.05 (t, J= 6.2, 1H, NH), 7.52-7.58 (m, 2H, H-3), 7.61 (tt, J_1= 7.4, J_2= 1.4, 1H, H-4), 7.88-7.92 (m, 2H, H-2).

^{13}C-NMR ($CDCl_3$), δ: 30.11, 32.34, 41.46 (CH_2, C-2', 1', 3'), 126.94, 129.24, 132.83

(CH, C-3, 4, 2), 139.73(C, C-1).

***N*-(3-Bromo-propyl)-4-methyl-benzenesulfonamide (24)[5]**

($C_{10}H_{14}BrNO_2S$; M.W.= 292.1)



15 **24**

General procedure 1;

White solid;

T.L.C. System: DCM 100%, Rf: 0.51

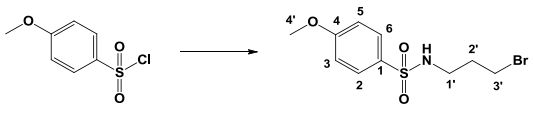
Yield: 1.02 g (89%)

^{1}H-NMR ($CDCl_3$), δ: 2.02-2.07 (m, 2H, H-2'), 2.45 (s, 3H, H-4'), 3.11-3.15 (m, 2H, H-1'), 3.44 (t, J= 6.4 Hz, 2H, H-3'), 4.77 (t, J= 6.4 Hz, 1H, NH), 7.34 (d, J= 8.3 Hz, 2H, H-aromatic), 7.78 (d, J= 8.3 Hz, 2H, H-aromatic).

^{13}C-NMR ($CDCl_3$), δ: 21.54 (CH_3, C-4'), 30.17, 32.33, 41.43 (CH_2, C-2', 1', 3'), 127.11, 129.83 (CH, C-aromatic), 136.74, 143.64 (C, C-4, 1).

***N*-(3-Bromo-propyl)-4-methoxy-benzenesulfonamide (25)[4]**

($C_{10}H_{14}BrNO_3S$; M.W.= 308.1)



16 **25**

General procedure 1;

Pale yellow oil;

T.L.C. System: DCM-*n*hexane 4:1 v/v, Rf: 0.25

Yield: 1.04 g (86%)

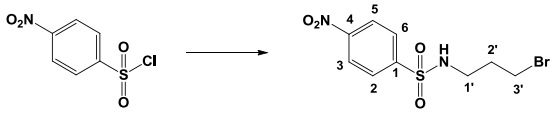
^{1}H-NMR ($CDCl_3$), δ: 2.04 (m, 2H, H-2'), 3.12 (q, J= 6.4 Hz, 2H, H-1'), 3.44 (t, J= 6.4 Hz, 2H, H-3'), 3.89 (s, 3H, H-4'), 4.77 (t, J= 6.4 Hz, 1H, NH), 7.01 (d, J= 8.8 Hz, 2H, H-aromatic), 7.83 (d, J= 8.8 Hz, 2H, H-aromatic).

^{13}C-NMR ($CDCl_3$), δ: 30.49, 32.53, 41.87 (CH_2, C-2', 1', 3'), 56.01 (CH_3, C-5'),

114.30, 125.36 (CH, C-aromatic), 130.87 (C, C-1), 165.56 (C, C-4).

***N*-(3-Bromo-propyl)-4-nitro-benzenesulfonamide (26)[4]**

($C_9H_{11}BrN_2O_4S$; M.W.= 323.1)



17 **26**

General procedure 1;

Pale yellow solid;

T.L.C. System: DCM 100%, Rf: 0.44

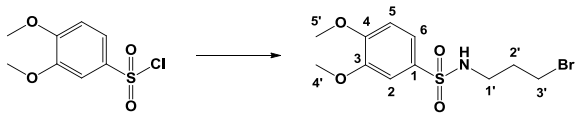
Yield: 0.94 g (73%)

^{1}H-NMR ($CDCl_3$), δ: 2.09 (m, 2H, H-2'), 3.22 (q, J= 6.3 Hz, 2H, H-1'), 3.45 (t, J= 6.3 Hz, 2H, H-3'), 5.03 (t, J= 6.3 Hz, 1H, NH), 8.09 (d, J= 8.7 Hz, 2H, H-aromatic), 8.40 (d, J= 8.7 Hz, 2H, H-aromatic).

^{13}C-NMR ($CDCl_3$), δ: 29.81, 32.25, 41.62 (CH_2, C-2', 1', 3'), 124.53, 128.35 (CH, C-aromatic), 145.67, 150.21 (C, C-1, 4).

***N*-(3-Bromo-propyl)-3,4-dimethoxy-benzenesulfonamide (27)**

($C_{11}H_{16}BrNO_4S$; M.W.= 338.2)



18 **27**

General procedure 1;

Pale yellow oil;

T.L.C. System: DCM-*n*hexane 4:1 v/v, Rf: 0.33

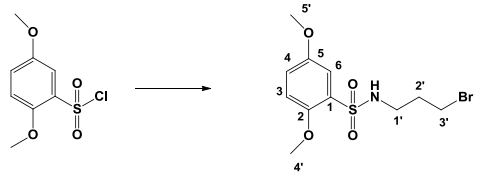
Yield: 0.82 g (61%)

^{1}H-NMR ($CDCl_3$), δ: 2.04 (m, 2H, H-2'), 3.12 (q, J= 6.4 Hz, 2H, H-1'), 3.44 (t, J= 6.4 Hz, 2H, H-3'), 3.94 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 4.88 (t, J= 6.4 Hz, 1H, NH), 6.96 (d, J= 8.5 Hz, 1H, H-5), 7.37 (d, J= 2.1 Hz, 1H, H-2), 7.51 (dd, J_1= 8.5 Hz, J_2= 2.1 Hz, 1H, H-6).

^{13}C-NMR ($CDCl_3$), δ: 30.20, 32.26, 41.45 (CH_2, C-2', 1', 3'), 56.21, 56.30 (CH_3, C-4', 5'), 109.59, 110.65, 121.07 (CH, C-5, 2, 6), 131.30, 149.30, 152.71 (C, C-1, 3, 4).

***N*-(3-Bromo-propyl)-2,5-dimethoxy-benzenesulfonamide (28)**

($C_{11}H_{16}BrNO_4S$; M.W.= 338.2)



19 **28**

General procedure 1;

White solid;

T.L.C. System: DCM-*n*hexane 4:1 v/v, Rf: 0.37

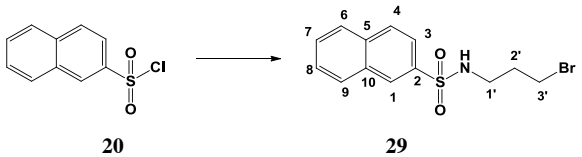
Yield: 0.94 g (70%)

^{1}H-NMR ($CDCl_3$), δ: 2.15 (m, 2H, H-2'), 3.17 (q, J= 6.4 Hz, 2H, H-1'), 3.56 (t, J= 6.4 Hz, 2H, H-3'), 3.79 (s, 3H, OC<u>H</u>$_3$), 3.93 (s, 3H, OC<u>H</u>$_3$), 5.21 (t, J= 6.4 Hz, 1H, N<u>H</u>), 6.97 (d, J= 8.9 Hz, 2H, H-3), 7.11 (dd, J_1= 8.9 Hz, J_2= 3.1 Hz, 2H, H-4), 7.53 (d, J= 3.1, 2H, H-6).

^{13}C-NMR ($CDCl_3$), δ: 30.12, 32.29, 41.37 (CH_2, C-2', 1', 3'), 56.14, 57.21 (CH_3, C-5', 6'), 113.93, 114.56, 120.22 (CH, C-3, 4, 6), 131.94 (C, C-1), 150.16, 153.31 (C, C-3, 4).

Naphthalene-2-sulfonic acid (3-bromo-propyl)-amide (29)[6]

($C_{13}H_{14}BrNO_2S$; M.W.= 328.2)



20 **29**

General procedure 1;

White solid;

T.L.C. System: DCM-*n*hexane 4:1 v/v, Rf: 0.43

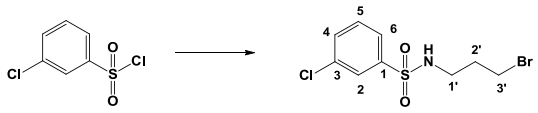
Yield: 1.16 g (89%)

^{1}H-NMR ($CDCl_3$), δ: 2.07 (m, 2H, H-2'), 3.20 (q, J= 6.4 Hz, 2H, H-1'), 3.45 (t, J= 6.4 Hz, 2H, H-3'), 4.85 (t, J= 6.4 Hz, 1H, NH), 7.67 (m, 2H, H-aromatic), 7.87 (dd, J_1= 8.6 Hz, J_2= 1.8 Hz, 1H, H-aromatic), 7.95 (d, J= 7.9 Hz, 1H, H-aromatic), 8.00 (m, 2H, H-aromatic), 8.48 (s, 1H, H-1).

^{13}C-NMR ($CDCl_3$), δ: 30.03, 32.31, 41.38 (CH_2, C-2', 1', 3'), 123.97, 127.53, 127.80, 128.41, 129.14, 129.36, 129.70 (CH, C-1, 3, 4, 6, 7, 8, 9), 129.14, 129.36, 129.70 (C, C-2, 5, 10).

N-(3-Bromo-propyl)-3-chloro-benzenesulfonamide (91)

($C_9H_{11}BrClNO_2S$; M.W.= 312.6)



82 **91**

General procedure 1;

Pale yellow oil;

T.L.C. System: DCM-*n*hexane 4:1 v/v, Rf: 0.32

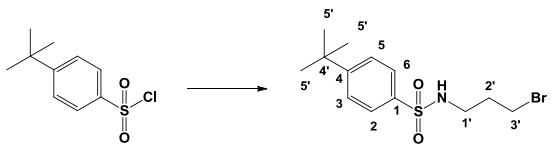
Yield: 0.92 g (75%)

^{1}H-NMR ($CDCl_3$), δ: 2.08 (m, 2H, H-2'), 3.18 (q, J= 6.4 Hz, 2H, H-1'), 3.45 (t, J= 6.4 Hz, 2H, H-3'), 4.85 (t, J= 6.4 Hz, 1H, NH), 7.50 (t, J= 7.8 Hz, 1H, H-5), 7.59 (dt, J_1= 7.8 Hz, J_2= 1.4 Hz, 1H, H-aromatic), 7.79 (dt, J_1= 7.8 Hz, J_2= 1.4 Hz, 1H, H-aromatic), 7.89 (t, J= 1.4 Hz, 1H, H-2).

^{13}C-NMR ($CDCl_3$), δ: 29.94, 32.31, 41.53 (CH_2, C-2', 1', 3'), 125.13, 127.18, 130.55, 132.98 (CH, C-2, 4, 5, 6), 135.46, 141.54 (C, C-1, 3).

N-(3-Bromo-propyl)-4-*tert*-butyl-benzenesulfonamide (92)

($C_{13}H_{20}BrNO_2S$; M.W.= 334.2)



83 **92**

General procedure 1;

White solid;

T.L.C. System: DCM-*n*hexane 4:1 v/v, Rf: 0.52

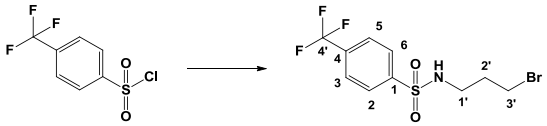
Yield: 1.02 g (77%)

^{1}H-NMR ($CDCl_3$), δ: 1.36 (s, 9H, H-5'), 2.05-2.09 (m, 2H, H-2'), 3.12-3.17 (m, 2H, H-1'), 3.44 (t, J= 6.4 Hz, 2H, H-3'), 4.93 (t, J= 6.4 Hz, 1H, NH), 7.55 (d, J= 8.6 Hz, 2H, H-aromatic), 7.82 (d, J= 8.6 Hz, 2H, H-aromatic).

^{13}C-NMR ($CDCl_3$), δ: 30.17 (CH_2, C-2'), 31.09 (CH_3, C-5'), 32.42 (CH_2, C-1'), 35.17 (C, C-4'), 41.44 (CH_2, C-3'), 126.20, 126.93 (CH, C-aromatic), 136.63 (C, C-4), 156.63 (C, C-1).

N-(3-Bromo-propyl)-4-trifluoromethyl-benzenesulfonamide (93)[7]

($C_{10}H_{11}BrF_3NO_2S$; M.W.= 346.1)



84 **93**

General procedure 1;

White solid;

T.L.C. System: DCM-*n*hexane 4:1 v/v, Rf: 0.38

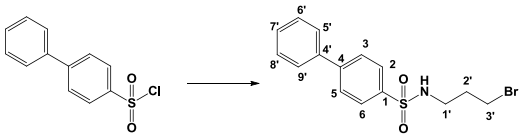
Yield: 1.10 g (80%)

^{1}H-NMR ($CDCl_3$), δ: 2.06-2.10 (m, 2H, H-2'), 3.17-3.21 (m, 2H, H-1'), 3.45 (t, J= 6.4 Hz, 2H, H-3'), 4.98 (t, J= 6.4 Hz, 1H, NH), 7.82 (d, J= 8.4 Hz, 2H, H-aromatic), 8.04 (d, J= 8.4 Hz, 2H, H-aromatic).

^{13}C-NMR ($CDCl_3$), δ: 29.86, 32.29, 41.55 (CH_2, C-2', 1', 3'), 126.42, 127.59 (CH, C-aromatic), 134.48, 134.74, 143.43 (C, C-1, 4, 4').

Biphenyl-4-sulfonic acid (3-bromo-propyl)-amide (94)

($C_{15}H_{16}BrNO_2S$; M.W.= 354.2)



85 **94**

General procedure 1;

Colourless oil;

T.L.C. System: DCM-*n*hexane 4:1 v/v, Rf: 0.26

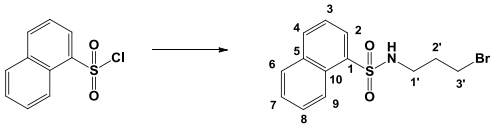
Yield: 0.95 g (67%)

^{1}H-NMR ($CDCl_3$), δ: 2.07-2.12 (m, 2H, H-2'), 3.18-3.23 (m, 2H, H-1'), 3.47 (t, J= 6.3 Hz, 2H, H-3'), 4.87 (t, J= 6.3 Hz, 1H, NH), 7.45 (t, J= 7.1 Hz, 1H, H-7'), 7.48-7.53 (m, 2H, H-aromatic), 7.62-7.65 (m, 2H, H-aromatic), 7.76 (d, J= 8.5 Hz, 2H, H-aromatic), 7.97 (d, J= 8.5 Hz, 2H, H-aromatic).

^{13}C-NMR ($CDCl_3$), δ: 30.14, 32.39, 41.52 (CH_2, C-2', 1', 3'), 127.34, 127.60, 127.85, 128.55, 129.08 (CH, C-aromatic), 138.30, 139.25, 145.80 (C, C-aromatic).

Naphthalene-1-sulfonic acid (3-bromo-propyl)-amide (95)[9]

($C_{13}H_{14}BrNO_2S$; M.W.= 328.2)



86 **95**

General procedure 1;

Colourless oil;

T.L.C. System: DCM-*n*hexane 4:1 v/v, Rf: 0.39

Yield: 1.44 g (95%)

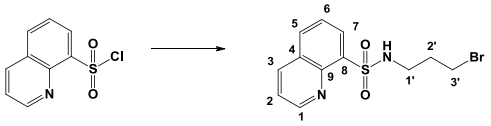
^{1}H-NMR ($CDCl_3$), δ: 1.91 (m, 2H, H-2'), 3.08 (q, J= 6.3 Hz, 2H, H-1'), 3.26 (t, J= 6.3 Hz, 2H, H-3'), 5.68 (t, J= 6.3 Hz, 1H, NH), 7.52 (t, J= 7.7 Hz, 1H, H-aromatic), 7.58 (m, 1H, H-aromatic), 7.64 (m, 1H, H-aromatic), 7.93 (d, J= 8.1 Hz, 1H, H-aromatic), 8.06 (d, J= 8.2 Hz, 1H, H-aromatic), 8.28 (dd, J_1= 7.3 Hz, J_2= 1.1 Hz, 1H, H-aromatic),

8.72 (d, J= 8.5 Hz, 1H, H-aromatic).

^{13}C-NMR ($CDCl_3$), δ: 30.32, 32.36, 41.45 (CH_2, C-2', 1', 3'), 124.24, 124.59, 127.20 (CH, C-aromatic), 128.51 (C, C-aromatic), 128.83, 129.52, 129.92 (CH, C-aromatic), 134.41, 134.47 (C, C-aromatic), 134.61 (CH, C-aromatic).

Quinoline-8-sulfonic acid (3-bromo-propyl)-amide (96)[9]

($C_{12}H_{13}BrN_2O_2S$; M.W.= 329.2)



87 **96**

General procedure 1;

White crystals;

T.L.C. System: DCM-*n*hexane 4:1 v/v, Rf: 0.48

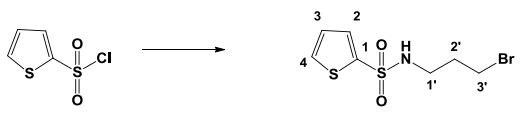
Yield: 0.96 g (73%)

^{1}H-NMR ($CDCl_3$), δ: 2.05 (m, 2H, H-2'), 3.03 (q, J= 6.4 Hz, 2H, H-1'), 3.45 (t, J= 6.1 Hz, 2H, H-3'), 6.52 (t, J= 6.4 Hz, 1H, NH), 7.59 (dd, J_1= 8.3 Hz, J_2= 4.3 Hz, 1H, H-aromatic), 7.68 (t, J= 7.7 Hz, 1H, H-aromatic), 8.09 (dd, J_1= 8.3 Hz, J_2= 1.4 Hz, 1H, H-aromatic), 8.31 (dd, J_1= 8.3 Hz, J_2= 1.7 Hz, 1H, H-aromatic), 8.45 (dd, J_1= 7.7 Hz, J_2= 1.4 Hz, 1H, H-aromatic), 9.05 (dd, J_1= 4.3 Hz, J_2= 1.7 Hz, 1H, H-aromatic).

^{13}C-NMR ($CDCl_3$), δ: 30.40, 32.49, 41.56 (CH_2, C-2', 1', 3'), 122.36, 125.77 (CH, C-aromatic), 128.83 (C, C-aromatic), 131.24, 133.44 (CH, C-aromatic), 135.69 (C, C-aromatic), 137.17 (CH, C-aromatic), 143.18 (C, C-aromatic), 151.37 (CH, C-aromatic).

Thiophene-2-sulfonic acid (3-bromo-propyl)-amide (97)[8]

($C_7H_{10}BrNO_2S_2$; M.W.= 284.1)



88 **97**

General procedure 1;

Pale yellow oil;

T.L.C. System: DCM-*n*hexane 4:1 v/v, Rf: 0.52

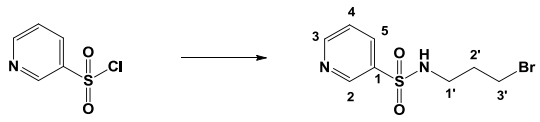
Yield: 1.01 g (89%)

^{1}H-NMR ($CDCl_3$), δ: 2.09 (m, 2H, H-2'), 3.24 (q, J= 6.1 Hz, 2H, H-1'), 3.46 (t, J= 6.1 Hz, 2H, H-3'), 4.94 (bs, 1H, NH), 7.12 (m, 1H, H-aromatic), 7.64 (m, 2H, H-aromatic).

^{13}C-NMR ($CDCl_3$), δ: 30.10, 32.22, 41.73 (CH_2, C-2', 1', 3'), 127.51, 132.09, 132.37 (CH, C-aromatic), 140.63 (C, C-1).

Pyridine-3-sulfonic acid (3-bromo-propyl)-amide (98)

($C_8H_{11}BrN_2O_2S$; M.W.= 279.1)



89 **98**

General procedure 1;

Yellow oil;

T.L.C. System: DCM-*n*hexane 4:1 v/v, Rf: 0.36.

Purification: flash column chromatography (DCM- *n*hexane 50:50 v/v increasing to DCM- *n*hexane 100:0 v/v).

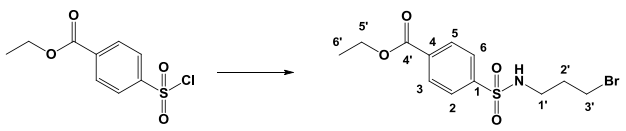
Yield: 0.62 g (56%)

^{1}H-NMR ($CDCl_3$), δ: 1.97 (m, 2H, H-2'), 3.08 (q, J= 6.3, 2H, H-1'), 3.34 (t, J=6.3, 2H, H-3'), 6.56 (t, J= 6.3, 1H, NH), 7.44 (dd, J_1= 8.1 Hz, J_2= 4.9 Hz, 1H, H-4), 8.11 (dt, J_1= 8.1 Hz, J_2= 1.9 Hz, 1H, H-5), 8.71 (dd, J_1= 4.9 Hz, J_2= 1.4 Hz, 1H, H-3), 8.97 (d, J= 1.9 Hz, 1H, H-2).

^{13}C-NMR ($CDCl_3$), δ: 30.35, 32.26, 41.29 (CH_2, C-2', 1', 3'), 124.19, 135.04 (CH, C-4, 5), 136. 74 (C, C-1), 147.58, 153.01 (CH, C-3, 2).

4-(3-Bromo-propylsulfamoyl)-benzoic acid ethyl ester (99)

($C_{12}H_{16}BrNO_4S$; M.W.= 350.2)



90 **99**

General procedure 1;

Yellow oil;

T.L.C. System: DCM-*n*hexane 4:1 v/v, Rf: 0.32.

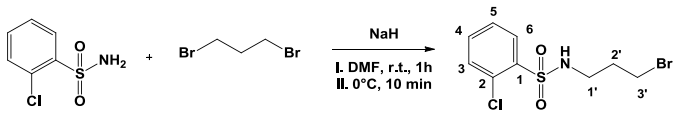
Yield: 0.92 g (66%)

^{1}H-NMR ($CDCl_3$), δ: 1.34 (t, J= 7.1 Hz, 3H, H-6'), 1.97 (m, 2H, H-2'), 3.07 (q, J= 6.3, 2H, H-1'), 3.35 (t, J=6.3, 2H, H-3'), 4.34 (q, J= 7.1 Hz, 2H, H-5'), 5.73 (t, J= 6.3, 1H, NH), 7.89 (d, J= 8.3 Hz, 2H, H-aromatic), 8.10 (d, J= 8.3 Hz, 2H, H-aromatic).

^{13}C-NMR ($CDCl_3$), δ: 14.21 (CH_3, C-6'), 30.05, 32.45, 41.49, 61.63 (CH_2, C-2', 1', 3', 5'), 127.64, 130.52 (CH, C-aromatic), 134.14, 143.62 (C, C-1, 4), 165.24 (C, C-4').

N-(3-Bromo-propyl)-2-chloro-benzenesulfonamide (115)[8]

($C_9H_{11}BrClNO_2S$; M.W.= 312.6)



112 **113** **115**

A mixture of 2-chlorobenzenesulfonamide (**112**) (1 g, 5.2 mmol) and NaH (0.2088 g, 5.2 mmol) in anhydrous DMF (7 mL) was stirred under nitrogen atmosphere for 1 h at r.t. The reaction mixture was then cooled to 0°C with an ice bath and added of 1,3-dibromopropane (**113**) (3.2 mL, 31.3 mmol) before being stirred for 10 min. under ice-cooling. The organic solvent and the excess **113** were removed under high vacuum. The crude residue was suspended in DCM (25 mL), washed with water (2 x 50 mL) and with brine (50 mL). The organic layer was then dried over $MgSO_4$ and dried under vacuum. The crude residue was purified by flash column chromatography (DCM-*n*hexane 50:50 v/v increasing to DCM-*n*hexane 80:20 v/v) to give pure *N*-(3-bromo-propyl)-2-chloro-benzenesulfonamide (**115**) as a colourless oil.

T.L.C. System: DCM-*n*hexane 4:1 v/v, Rf: 0.51.

Yield: 0.6382 g (39%)

^{1}H-NMR ($CDCl_3$), δ: 2.01 (m, 2H, H-2'), 3.08 (q, J= 6.4 Hz, 2H, H-1'), 3.40 (t, J= 6.4 Hz, 2H, H-3'), 5.47 (t, J= 6.4 Hz, 1H, NH), 7.41 (m, 1H, H-aromatic), 7.51 (d, J=4.4 Hz, 2H, H-aromatic), 8.07 (d, J=7.8 Hz, 1H, H-aromatic).

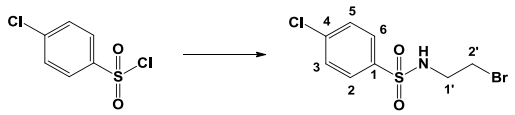
^{13}C-NMR ($CDCl_3$), δ: 30.30, 32.35, 41.48 (CH_2, C-2', 1', 3'), 127.41, 131.01 (CH, C-

aromatic), 131.38 (C, C-aromatic), 132.07, 134.05 (CH, C-aromatic), 136.87 (C, C-aromatic).

6.2.4 *N*-(2-Bromoethyl)arylsulfonamides (85-87)

***N*-(2-Bromo-ethyl)-4-chloro-benzenesulfonamide (38)[10]**

($C_8H_9BrClNO_2S$; M.W.= 298.5)



13 **38**

General procedure 2;

White solid;

T.L.C. System: DCM-*n*hexane 4:1 v/v, Rf: 0.25.

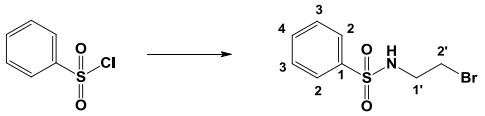
Yield: 1.08 g (87%)

^{1}H-NMR ($CDCl_3$), δ: 3.39-3.46 (m, 4H, H-1', 2'), 5.04 (bs, 1H, NH), 7.53 (d, J= 8.6 Hz, 2H, H-aromatic), 7.84 (d, J= 8.6 Hz, 2H, H-aromatic).

^{13}C-NMR ($CDCl_3$), δ: 31.56, 44.56 (CH_2, C-1', 2'), 128.48, 129.59 (CH, C-aromatic), 138.47, 139.56 (C, C-1, 4).

***N*-(2-Bromo-ethyl)-benzenesulfonamide (39)[11]**

($C_8H_{10}BrNO_2S$; M.W.= 264.1)



14 **39**

General procedure 2;

White solid;

T.L.C. System: DCM-*n*hexane 4:1 v/v, Rf: 0.52.

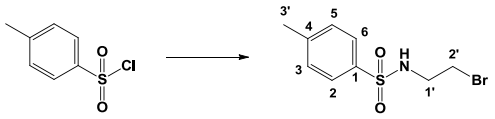
Yield: 0.88 g (80%)

^{1}H-NMR ($CDCl_3$), δ: 3.39-3.43 (m, 4H, H-1', 2'), 5.11 (bs, 1H, NH), 7.54-7.58 (m, 2H, H-3), 7.60-7.64 (m, 1H, H-4), 7.90 (d, J= 8.0, 2H, H-2).

^{13}C-NMR ($CDCl_3$), δ: 31.48, 44.58 (CH_2, C-1', 2'), 126.98, 129.31, 132.99 (CH, C-3, 4, 2), 139.89 (C, C-1).

***N*-(2-Bromo-ethyl)-4-methyl-benzenesulfonamide (40)[12]**

($C_9H_{12}BrNO_2S$; M.W.= 278.1)



15 **40**

General procedure 3;

White solid;

T.L.C. System: DCM-*n*hexane 4:1 v/v, Rf: 0.59.

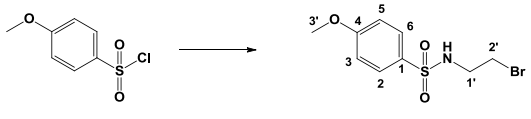
Yield: 0.96 g (83%)

^{1}H-NMR ($CDCl_3$), δ: 2.46 (s, 3H, H-3'), 3.40 (m, 4H, H-1', 2'), 4.96 (bs, 1H, NH), 7.34 (d, J= 8.2 Hz, 2H, H-aromatic), 7.78 d, J= 8.2 Hz, 2H, H-aromatic).

^{13}C-NMR ($CDCl_3$), δ: 21.54 (CH_3, C-3'), 31.70, 44.55 (CH_2, C-1', 2'), 127.05, 129.89 (CH, C-aromatic), 136.93, 143.86 (C, C-4, 1).

***N*-(2-Bromo-ethyl)-4-methoxy-benzenesulfonamide (41)**

($C_9H_{15}BrNO_3S$; M.W.= 294.1)



16 **41**

General procedure 2;

White solid;

T.L.C. System: DCM-*n*hexane 4:1 v/v, Rf: 0.28.

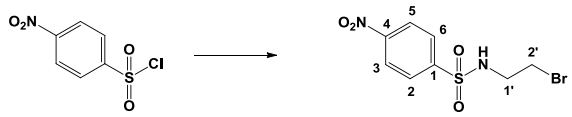
Yield: 0.92 g (75%)

^{1}H-NMR ($CDCl_3$), δ: 3.40 (m, 4H, H-1', 2'), 3.90 (s, 3H, H-3'), 4.92 (bs, 1H, NH), 7.01 (d, J= 8.8 Hz, 2H, H-aromatic), 7.83 (d, J= 8.8 Hz, 2H, H-aromatic).

^{13}C-NMR ($CDCl_3$), δ: 33.40, 44.50 (CH_2, C-1', 2'), 56.78 (CH_3, C-3'), 117.27, 127.84 (CH, C-aromatic), 131.33, 165.21 (C, C-1, 4).

***N*-(2-Bromo-ethyl)-4-nitro-benzenesulfonamide (42)[13]**

($C_8H_9BrN_2O_4S$; M.W.= 309.1)



17 **42**

General procedure 2;

White solid;

T.L.C. System: DCM-*n*hexane 4:1 v/v, Rf: 0.33.

Purification: flash column chromatography (DCM-*n*hexane 50:50 v/v increasing to DCM-*n*hexane 100:0).

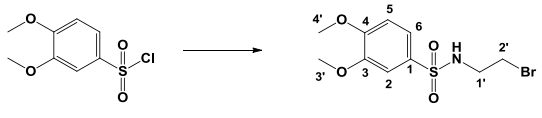
Yield: 0.84 g (65%)

^{1}H-NMR ($CDCl_3$), δ: 3.47 (m, 4H, H-1', 2'), 5.11 (bs, 1H, NH), 8.10 (d, J= 8.9 Hz, 2H, H-aromatic), 8.41 (d, J= 8.9 Hz, 2H, H-aromatic).

^{13}C-NMR ($CDCl_3$), δ: 31.47, 44.67 (CH_2, C-1', 2'), 124.55, 128.29 (CH, C-aromatic), 145.87, 150.26 (C, C-1, 4).

***N*-(2-Bromo-ethyl)-3,4-dimethoxy-benzenesulfonamide (43)[7]**

($C_{10}H_{14}BrNO_4S$; M.W.= 324.1)



18 **43**

General procedure 2;

White solid;

T.L.C. System: DCM-*n*hexane 4:1 v/v, Rf: 0.41.

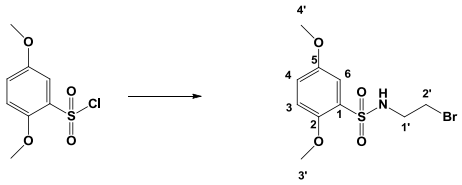
Yield: 0.5649 g (83%)

^{1}H-NMR ($CDCl_3$), δ: 3.41 (m, 4H, H-1', 2'), 3.96 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3), 4.96 (bs, 1H, NH), 6.97 (d, J= 8.4 Hz, 1H, H-5), 7.36 (d, J= 2.2 Hz, 1H, H-2), 7.52 (dd, J_1= 8.4 Hz, J_2= 2.2 Hz, 1H, H-6).

^{13}C-NMR ($CDCl_3$), δ: 32.94, 44.72 (CH_2, C-1', 2'), 56.47, 56.75 (CH_3, C-3', 4'),112.60, 115.22, 119.46 (CH, C-5, 2, 6), 132.53 (C, C-1), 147.24, 151.19 (C, C-3, 4).

***N*-(2-Bromo-ethyl)-2,5-dimethoxy-benzenesulfonamide (44)**

($C_{10}H_{14}BrNO_4S$; M.W.= 324.1)



19 **44**

General procedure 2;

White solid;

T.L.C. System: DCM-*n*hexane 4:1 v/v, Rf: 0.47.

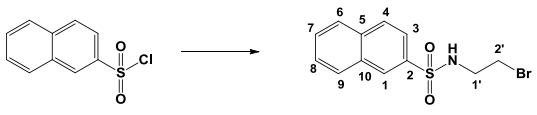
Yield: 1.1 g (81%)

^{1}H-NMR ($CDCl_3$), δ: 3.37 (m, 4H, H-1', 2'), 3.82 (s, 3H, OC<u>H</u>$_3$), 3.97 (s, 3H, OC<u>H</u>$_3$), 5.55 (bs, 1H, N<u>H</u>), 6.99 (d, J= 8.9 Hz, 1H, H-3), 7.10 (dd, J_1= 8.9 Hz, J_2= 2.6 Hz, 1H, H-4), 7.44 (d, J= 2.6, Hz, 1H, H-6).

^{13}C-NMR ($CDCl_3$), δ: 31.65, 45.10 (CH_2, C-1', 2'), 56.06, 56.87 (CH_3, C-3', 4'), 113.56, 114.38, 120.59 (CH, C-3, 4, 6), 127.64 (C, C-1), 150.11, 153.27 (C, C-2, 5).

Naphthalene-2-sulfonic acid (2-bromo-ethyl)-amide (45)

($C_{12}H_{12}BrNO_2S$; M.W.= 314.1)



20 **45**

General procedure 2;

White solid;

T.L.C. System: DCM-*n*hexane 4:1 v/v, Rf: 0.62.

Yield: 0.57 g (87%)

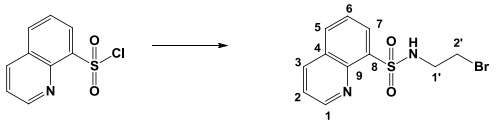
^{1}H-NMR ($CDCl_3$), δ: 3.44 (m, 4H, H-1', 2'), 5.16 (bs, 1H, N<u>H</u>), 7.67 (m, 2H, H-aromatic), 7.87 (dd, J_1= 8.6 Hz, J_2= 1.8 Hz, 1H, H-aromatic), 7.95 (d, J= 7.9 Hz, 1H, H-aromatic), 8.00 (m, 2H, H-aromatic), 8.48 (s, 1H, H-1).

^{13}C-NMR ($CDCl_3$), δ: 33.40, 44.56 (CH_2, C-1', 2'), 122.98, 125.93, 126.07, 126.59, 128.74, 128.96, 129.11 (CH, C-1, 3, 4, 6, 7, 8, 9), 130.14, 130.46, 130.71 (C, C-2, 5,

10).

Quinoline-8-sulfonic acid (2-bromo-ethyl)-amide (117)[10]

($C_{11}H_{11}BrN_2O_2S$; M.W.= 315.1)



87 **117**

General procedure 2;

White solid;

T.L.C. System: DCM-*n*hexane 4:1 v/v, Rf: 0.40.

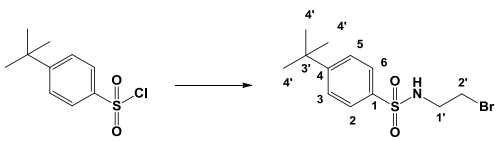
Yield: 0.43 g (65%)

^{1}H-NMR ($CDCl_3$), δ: 3.30 (q, J= 6.1 Hz, 2H, H-1'), 3.38 (t, J= 6.1, 4H, H-2'), 6.91 (t, J= 6.1 Hz, 1H, NH), 7.67 (m, 2H, H-aromatic), 7.56 (dd, J_1= 8.2 Hz, J_2= 4.3 Hz, 1H, H-aromatic), 7.64 (t, J= 7.7 Hz, 1H, H-aromatic), 8.07 (dd, J_1= 8.2 Hz, J_2= 1.2 Hz, 1H, H-aromatic), 8.29 (dd, J_1= 8.3 Hz, J_2= 1.6 Hz, 1H, H-aromatic), 8.39 (dd, J_1= 7.2 Hz, J_2= 1.2 Hz, 1H, H-aromatic), 9.03 (dd, J_1= 4.3 Hz, J_2= 1.6 Hz, 1H, H-aromatic).

^{13}C-NMR ($CDCl_3$), δ: 31.43, 45.31 (CH_2, C-1', 2'), 122.47, 125.06 (CH, C-aromatic), 128.53 (C, C-aromatic), 129.44, 131.14 (CH, C-aromatic), 132.79 (C, C-aromatic), 135.05 (CH, C-aromatic), 143.02 (C, C-aromatic), 151.45 (CH, C-aromatic).

***N*-(2-Bromo-ethyl)-4-*tert*-butyl-benzenesulfonamide (147)[7]**

($C_9H_{12}BrNO_2S$; M.W.= 320.25)



83 **147**

General procedure 2;

White solid;

T.L.C. System: DCM- *n*hexane 4:1 v/v, Rf: 0.60.

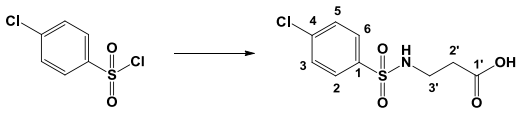
Yield: 1.04 g (78%)

^{1}H-NMR ($CDCl_3$), δ: 1.36 (s, 9H, H-4'), 3.37-3.45 (m, 4H, H-1', 2'), 5.13 (t, J= 6.1 Hz, 1H, NH), 7.55 (d, J= 8.7 Hz, 2H, H-aromatic), 7.81 (d, J= 8.7 Hz, 2H, H-aromatic).
^{13}C-NMR ($CDCl_3$), δ: 31.07 (CH_3, C-4'), 31.07 (CH_2), 35.18 (C, C-3'), 44.57 (CH_2), 126.26, 126.58 (CH, C-aromatic), 136.77, 156.83 (C, C-4, 1).

6.2.5 3-Arylsulfonylamino propionic acids (55-61, 57a, 153, 154)

3-(4-Chloro-benzenesulfonylamino)-propionic acid (55)[14]

($C_9H_{10}ClNO_4S$; M.W.= 263.6)



13 **55**

General procedure 3;

White solid;

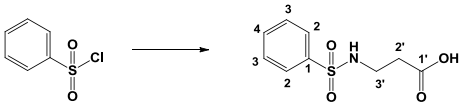
Yield: 1.14 g (64%)

^{1}H-NMR (DMSO-d$_6$), δ: 2.36 (t, J= 6.9 Hz, 2H, H-2'), 2.92-2.98 (m, 2H, H-3'), 7.68 (d, J= 8.4 Hz, 2H, H-aromatic), 7.75 (bs, 1H, NH), 7.80 (d, J= 8.4 Hz, 2H, H-aromatic).

^{13}C-NMR (DMSO-d$_6$), δ: 34.10, 38.52 (CH_2, C-2', 1'), 128.46, 129.34 (CH, C-aromatic), 137.26, 139.12 (C, C-1, 4), 172.15 (C, C-1').

3-Benzenesulfonylamino-propionic acid (56)[15]

($C_9H_{11}NO_4S$; M.W.= 229.2)



14 **56**

General procedure 3;

White solid;

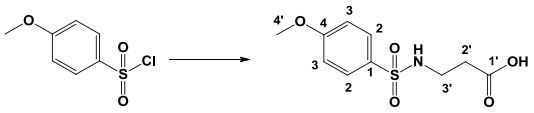
Yield: 0.81 g (52%)

^{1}H-NMR (DMSO-d$_6$), δ: 2.36 (t, J= 7.0 Hz, 2H, H-2'), 2.92-2.96 (m, 2H, H-3'), 7.62-7.68 (m, 3H, H-aromatic), 7.61 (bs, 1H, NH), 7.78-7.82 (m, 2H, H-aromatic).

^{13}C-NMR (DMSO-d$_6$), δ: 34.12, 38.54 (CH_2, C-2', 1'), 126.47, 129.20, 132.41 (CH, C-2, 3, 4), 140.17 (C, C-1), 172.20 (C, C-1').

3-(4-Methoxy-benzenesulfonylamino)-propionic acid (57)[14]

($C_{10}H_{13}NO_5S$; M.W.= 259.2)



16 **57**

General procedure 3;

White solid;

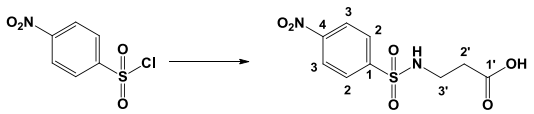
Yield: 1.26 g (72%)

^{1}H-NMR ($CDCl_3$), δ: 2.64 (t, J= 5.8 Hz, 2H, H-2'), 3.21 (m, 2H, H-3'), 3.90 (s, 3H, H-4'), 5.72 (bs, 1H, NH), 7.01 (d, J= 8.7, 2H, H-aromatic), 7.83 (d, J= 8.7 Hz, 2H, H-aromatic), 12.31 (s, 1H, COOH).

^{13}C-NMR ($DMSO-d_6$), δ: 34.02, 38.47 (CH_2, C-2', 1'), 55.56 (CH_3, C-4'), 114.33, 131.49 (CH, C-aromatic), 131.49, 162.16 (C, C-1, 4), 172.47 (C, C-1').

3-(4-Nitro-benzenesulfonylamino)-propionic acid (58)[14]

($C_9H_{10}N_2O_6S$; M.W.= 274.2)



17 **58**

General procedure 3;

Pale yellow solid;

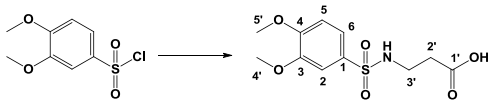
Yield: 0.94 g (51%)

^{1}H-NMR ($DMSO-d_6$), δ: 2.38 (t, J= 6.9 Hz, 2H, H-2'), 3.02 (m, 2H, H-3'), 8.05 (d, J= 8.9 Hz, 2H, H-aromatic), 8.08 (bs, 1H, NH), 8.42 (d, J= 8.9, 2H, H-aromatic), 12.39 (s, 1H, COOH).

^{13}C-NMR ($DMSO-d_6$), δ: 34.12, 38.56 (CH_2, C-2', 1'), 124.57, 128.09 (CH, C-aromatic), 145.88, 149.57 (C, C-1, 4), 172.08 (C, C-1').

3-(3,4-Dimethoxy-benzenesulfonylamino)-propionic acid (59)[16]

($C_{11}H_{15}NO_6S$; M.W.= 289.3)



18 **59**

General procedure 3;

White solid;

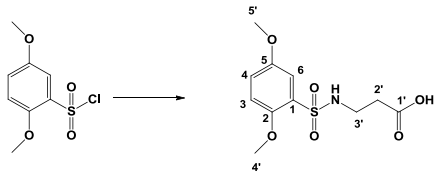
Yield: 1.14 g (58%)

^{1}H-NMR (DMSO-d_6), δ: 2.35 (t, J= 7.0 Hz, 2H, H-2'), 2.91 (m, 2H, H-3'), 3.82 (s, 3H, OC<u>H</u>$_3$), 3.84 (s, 3H, OC<u>H</u>$_3$), 7.13 (d, J= 8.5 Hz, 1H, H-5), 7.30 (d, J= 2.1 Hz, 1H, H-2), 7.37 (dd, J_1= 8.5 Hz, J_2= 2.1 Hz, 1H, H-6), 7.49 (t, J= 5.7 Hz, 1H, N<u>H</u>), 12.25 (s, 1H, COO<u>H</u>).

^{13}C-NMR (DMSO-d_6), δ: 34.06, 38.55 (CH_2, C-2', 1'), 55.71, 55.78 (CH_3, C-4', 5'), 109.37, 111.11, 120.23 (CH, C-5, 2, 6), 131.68, 148.68, 151.86 (C, C-1, 3, 4), 172.29 (C, C-1').

3-(2,5-Dimethoxy-benzenesulfonylamino)-propionic acid (60)[16]

($C_{11}H_{15}NO_6S$; M.W.= 289.3)



19 **60**

General procedure 3;

White solid;

Yield: 1.22 g (64%)

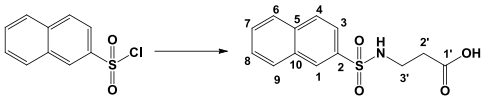
^{1}H-NMR ($CDCl_3$), δ: 2.61 (t, J= 5.9 Hz, 2H, H-2'), 3.21 (m, 2H, H-3'), 3.84 (s, 3H, OC<u>H</u>$_3$), 3.95 (s, 3H, OC<u>H</u>$_3$), 5.78 (t, J= 6.6 Hz, 1H, N<u>H</u>), 7.00 (d, J= 8.9 Hz, 1H, H-3), 7.11 (dd, J_1= 8.9 Hz, J_2= 3.0 Hz, 1H, H-4), 7.47 (d, J= 3.0 Hz, 1H, H-6), 11.89 (s, 1H, COO<u>H</u>).

^{13}C-NMR (DMSO-d_6), δ: 33.88, 38.65 (CH_2, C-1', 2'), 55.73, 56.43 (CH_3, C-4', 5'),

114.21, 114.24, 119.49 (CH, C-3, 4, 6), 128.16, 150.21, 152.28 (C, C-1, 2, 5), 172.42 (C, C-1').

3-(Naphthalene-2-sulfonylamino)-propionic acid (61)[17]

($C_{13}H_{13}NO_4S$; M.W.= 279.3)



20 **61**

General procedure 3;

White solid;

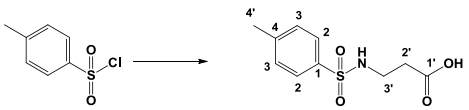
Yield: 1.16 g (61%)

^{1}H-NMR ($CDCl_3$), δ: 2.38 (t, J= 7.1 Hz, 2H, H-2'), 2.99 (m, 2H, H-3'), 7.69 (m, 3H, H-aromatic, NH), 7.82 (m, 2H, H-aromatic), 8.05 (d, J= 8.1 Hz, 1H, H-aromatic), 8.15 (m, 2H, H-aromatic), 8.45 (s, 1H, H-1), 12.22 (bs, 1H, COOH).

^{13}C-NMR (DMSO-d_6), δ: 34.16, 38.59 (CH_2, C-1', 2'), 123.97, 127.53, 127.80, 128.41, 129.14, 129.36, 129.70 (CH, C-aromatic), 131.70, 134.12, 137.20 (C, C-2, 5, 10), 172.21 (C, C-1').

3-(Toluene-4-sulfonylamino)-propionic acid (57a)[14]

($C_{10}H_{13}NO_4S$; M.W.= 243.2)



15 **57a**

General procedure 3;

White solid;

Yield: 0.95 g (58%)

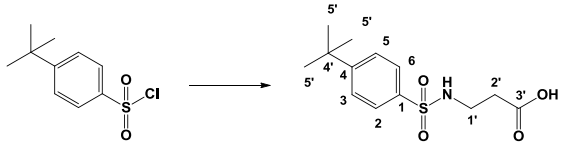
^{1}H-NMR ($CDCl_3$), δ: 2.35 (t, J= 7.1 Hz, 2H, H-2'), 2.39 (s, 3H, H-4'), 2.89-2.94 (m, 2H, H-3'), 7.40 (d, J= 8.1 Hz, 2H, H-aromatic), 7.57 (t, J= 5.7 Hz, 1H, NH), 7.68 (d, J= 8.1 Hz, 2H, H-aromatic), 12.25 (s, 1H, COOH).

^{13}C-NMR (DMSO-d_6), δ: 20.92 (CH_3, C-4'), 34.10, 38.53 (CH_2, C-2', 1'), 126.54,

129.61 (CH, C-aromatic), 137.30, 142.64 (C, C-1, 4), 172.22 (C, C-1').

3-(4-*tert*-Butyl-benzenesulfonylamino)-propionic acid (153)

($C_{13}H_{19}NO_4S$; M.W.= 285.36)



83 **153**

General procedure 3;

White solid;

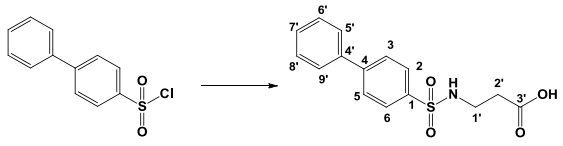
Yield: 1.04 g (54%)

^{1}H-NMR ($CDCl_3$), δ: 1.31 (s, 9H, H-5'), 2.38 (t, J= 7.1 Hz, 2H, H-2'), 2.90-2.94 (m, 2H, H-1'), 7.59 (t, J= 5.4 Hz, 1H, NH), 7.62 (d, J= 8.5 Hz, 2H, H-aromatic), 7.72 (d, J= 8.5 Hz, 2H, H-aromatic), 12.26 (s, 1H, COOH).

^{13}C-NMR (DMSO-d_6), δ: 30.78 (CH_3, C-5'), 34.16 (CH_2), 34.69 (C, C-4'), 38.57 (CH_2), 126.01, 126.40 (CH, C-aromatic), 137.27, 155.35 (C, C-1, 4), 172.25 (C, C-1').

3-(Biphenyl-4-sulfonylamino)-propionic acid (154)

($C_{15}H_{15}NO_4S$; M.W.= 305.3)



85 **154**

General procedure 3;

White solid;

Yield: 0.93 g (45%)

^{1}H-NMR ($CDCl_3$), δ: 2.40 (t, J= 7.0 Hz, 2H, H-2'), 2.96-3.00 (m, 2H, H-1'), 7.45 (t, J= 7.4 Hz, 1H, H-7'), 7.50-7.54 (m, 2H, H-aromatic), 7.70-7.70 (m, 3H, 2H-aromatic, NH), 7.87 (d, J= 8.5 Hz, 2H, H-aromatic), 7.90 (d, J= 8.5 Hz, 2H, H-aromatic), 12.28 (bs, 1H, COOH).

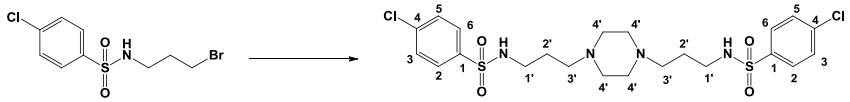
^{13}C-NMR (DMSO-d_6), δ: 34.18, 38.60 (CH_2, C-1', 2'), 127.05, 127.19, 127.40, 128.45,

129.09 (CH, C-aromatic), 138.53, 138.94, 143.92 (C, C-1, 4, 4'), 172.23 (C, C-1').

6.2.6 *N,N'*-(3,3'-(Piperazine-1,4-di*yl*)bis(propane-3,1-di*yl*))diarylsulfonamides (12, 30-36, 100-108, 111)

***N,N'*-(3,3'-(Piperazine-1,4-di*yl*)bis(propane-3,1-di*yl*))bis(4-chlorobenzene-sulfonamide) (12)**

($C_{22}H_{30}Cl_2N_4O_4S_2$; M.W.= 549.5)



22 **12**

General procedure 4;

Pale yellow solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.61.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 96:4 v/v).

Yield: 0.21 g (64%)

Melting Point: 168-170°C

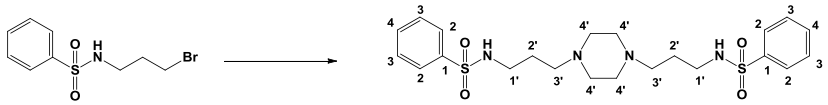
MS $(ESI)^+$: 549.0, 551.0 $[M+H]^+$

^{1}H-NMR ($CDCl_3$), δ: 1.65-1,71 (m, 4H, H-2'), 2.41-2.54 (m, 12H, H-3', 4'), 3.09 (t, J= 5.6 Hz, 4H, H-1'), 7.34 (bs, 2H, NH), 7.51 (d, J= 8.6 Hz, 4H, H-aromatic), 7.80 (d, J= 8.6 Hz, 4H, H-aromatic).

^{13}C-NMR (DMSO-d_6), δ: 26.02, 40.84, 52.58, 54.74 (CH_2, C-1', 2', 3', 4'), 128.42, 129.31 (CH, C-aromatic), 137.13, 139.39 (C, C-1, 4).

***N,N'*-(3,3'-(Piperazine-1,4-di*yl*)bis(propane-3,1-di*yl*))dibenzene-sulfonamide (23)**

($C_{22}H_{32}N_4O_4S_2$; M.W.= 480.6)



23 **30**

General procedure 4;

White solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.46.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 96:4 v/v).

Yield: 0.18 g (64%)

Melting Point: 136-138°C

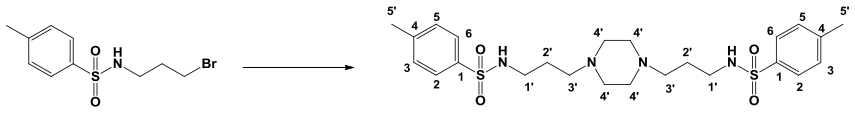
MS (ESI)$^+$: 481.1 $[M+H]^+$

^{1}H-NMR ($CDCl_3$), δ: 1.60 (m, 4H, H-2'), 2.38 (m, 12H, H-3', 4'), 3.04 (t, J= 5.8 Hz, 4H, H-1'), 7.10 (bs, 2H, NH), 7.50 (m, 4H, H-3), 7.56 (m, 2H, H-4), 7.83 (m, 4H, H-2).

^{13}C-NMR ($CDCl_3$), δ: 24.09, 44.04, 53.00, 57.95 (CH_2, C-1', 2', 3', 4'), 126.88, 129.03, 132.65 (CH, C-3, 4, 2), 140.16 (C, C-1).

***N,N'*-(3,3'-(Piperazine-1,4-di*yl*)bis(propane-3,1-di*yl*))bis(4-methylbenzene-sulfonamide) (31)[18]**

($C_{24}H_{36}N_4O_4S_2$; M.W.= 508.6)



24 **31**

General procedure 4;

White solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.67.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 95:5 v/v).

Yield: 0.20 g (68%)

Melting Point: 176-178°C (lit. 181-183°C)[18]

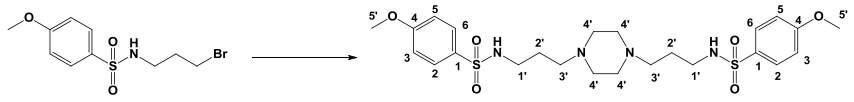
MS (ESI)$^+$: 509.1 $[M+H]^+$

^{1}H-NMR ($CDCl_3$), δ: 1.65 (m, 4H, H-2'), 2.46 (bs, 18H, H-3', 4', 5'), 3.07 (t, J= 5.6 Hz, 4H, H-1'), 7.12 (bs, 2H, NH), 7.32 (d, J= 8.2 Hz, 4H, H-aromatic), 7.74 (d, J= 8.2 Hz, 4H, H-aromatic).

^{13}C-NMR (DMSO-d_6), δ: 20.91 (CH_3, C-5'), 26.02, 40.91, 52.60, 54.88 (CH_2, C-1', 2', 3', 4'), 126.50, 129.56 (CH, C-aromatic), 137.80 (C, C-4), 142.47 (C, C-1).

***N,N'*-(3,3'-(Piperazine-1,4-di*yl*)bis(propane-3,1-di*yl*))bis(4-methoxybenzene-sulfonamide) (32)**

($C_{24}H_{36}N_4O_6S_2$; M.W.= 540.6)



25 **32**

General procedure 4;

White solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.72.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 91:9 v/v).

Yield: 0.19 g (60%)

Melting Point: 172-174°C

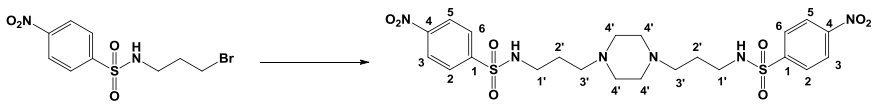
MS $(ESI)^+$: 541.1 $[M+H]^+$

^{1}H-NMR (DMSO-d_6), δ: 1.47 (m, 4H, H-2'), 2.18 (bs, 12H, H-3', 4'), 2.72 (m, 4H, H-1'), 3.83 (s, 6H, H-5'), 7.11 (d, J= 8.3 Hz, 4H, H-aromatic), 7.41 (t, J= 5.6 Hz, 2H, NH), 7.70 (d, J= 8.3 Hz, 4H, H-aromatic).

^{13}C-NMR (DMSO-d_6), δ: 25.63, 39.78, 51.90, 54.38 (CH_2, C-1', 2', 3', 4'), 56.01 (CH_3, C-5'), 114.44, 126.56 (CH, C-aromatic), 131.80 (C, C-1), 165.27 (C, C-4).

***N,N'*-(3,3'-(Piperazine-1,4-di*yl*)bis(propane-3,1-di*yl*))bis(4-nitrobenzene-sulfonamide) (33)**

($C_{22}H_{30}N_6O_8S_2$; M.W.= 570.6)



26 **33**

General procedure 4;

Pale yellow solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.59.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 91:9

v/v).

Yield: 0.14 g (43%)

Melting Point: 226-228°C

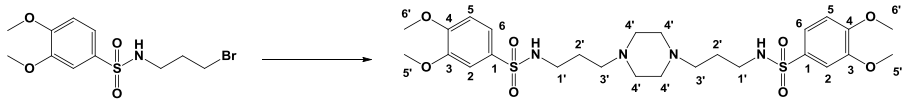
MS $(ESI)^+$: 571.1 $[M+H]^+$

^{1}H-NMR (DMSO-d$_6$), δ: 1.49 (m, 4H, H-2'), 2.18 (m, 12H, H-3', 4'), 2.82 (t, J= 6.8 Hz, 4H, H-1'), 7.98 (bs, 2H, NH), 8.03 (d, J= 8.7 Hz, 4H, H-aromatic), 8.42 (d, J= 8.7 Hz, 4H, H-aromatic).

^{13}C-NMR (DMSO-d$_6$), δ: 26.11, 40.87, 52.58, 54.66 (CH_2, C-1', 2', 3', 4'), 124.57, 128.02 (CH, C-aromatic), 146.14, 149.50 (C, C-1, 4).

***N,N'*-(3,3'-(Piperazine-1,4-di*yl*)bis(propane-3,1-di*yl*))bis(3,4-dimethoxybenzene-sulfonamide) (34)**

($C_{26}H_{40}N_4O_8S_2$; M.W.= 600.7)



27 **34**

General procedure 4;

Yellow solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.70.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 95:5 v/v).

Yield: 0.23 g (65%)

Melting Point: 152-154°C

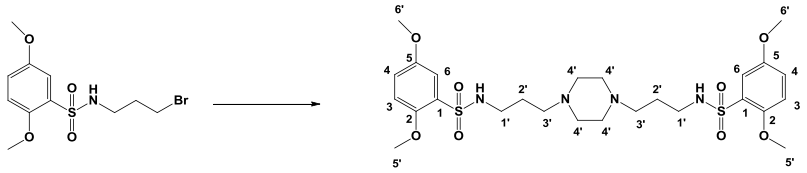
MS $(ESI)^+$: 601.1 $[M+H]^+$

^{1}H-NMR ($CDCl_3$), δ: 1.66 (m, 4H, H-2'), 2.47 (m, 12H, H-3', 4'), 3.06 (t, J= 5.7 Hz, 4H, H-1'), 3.93 (s, 6H, OCH_3), 3.96 (s, 6H, OCH_3), 6.53 (bs, 2H, NH), 6.95 (d, J= 8.4 Hz, 2H, H-5), 7.33 (d, J= 2.1, 2H, H-2), 7.46 (dd, J_1= 8.4 Hz, J_2= 2.1 Hz, 2H, H-6).

^{13}C-NMR ($CDCl_3$), δ: 24.14, 44.09, 53.06, (CH_2, C-2', 3', 4'), 56.18, 56.25 (CH_3, C-5', 6'), 57.79 (CH_2, C-1'), 109.76, 110.59, 120.77 (CH, C-5, 2, 6), 131.93 (C, C-1), 149.09, 152.37 (C, C-3, 4).

***N,N'*-(3,3'-(Piperazine-1,4-di*yl*)bis(propane-3,1-di*yl*))bis(2,5-dimethoxybenzene-sulfonamide) (35)**

($C_{26}H_{40}N_4O_8S_2$; M.W.= 600.7)



28 **35**

General procedure 4;

White solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.73.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 95:5 v/v).

Yield: 0.25 g (71%)

Melting Point: 170-172°C

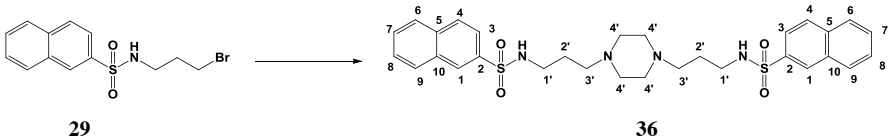
MS $(ESI)^+$: 601.1 $[M+H]^+$

^{1}H-NMR ($CDCl_3$), δ: 1.71 (m, 4H, H-2'), 2.55 (bs, 12H, H-5', 6'), 3.00 (t, J= 6.2 Hz, 4H, H-1'), 3.83 (s, 6H, $OC\underline{H}_3$), 3.95 (s, 6H, $OC\underline{H}_3$), 6.22 (bs, 2H, N<u>H</u>), 7.00 (d, J= 8.9 Hz, 2H, H-3), 7.08 (dd, J_1= 8.9 Hz, J_2= 3.1 Hz, 2H, H-4), 7.47 (d, J= 3.1, 2H, H-6).

^{13}C-NMR ($CDCl_3$), δ: 25.35, 44.4, 52.56, 55.45 (CH_2, C-2', 3', 4', 1'), 56.04, 57.27 (CH_3, C-5', 6'), 114.16, 114.89, 120.22 (CH, C-3, 4, 6), 132.02 (C, C-1), 150.41, 153.37 (C, C-3, 4).

***N,N'*-(3,3'-(Piperazine-1,4-di*yl*)bis(propane-3,1-di*yl*))dinaphthalene-2-sulfonamide (36)**

($C_{30}H_{36}N_4O_4S_2$; M.W.= 580.7)



29 **36**

General procedure 4;

White solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.62.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 96:4 v/v).

Yield: 0.27 g (79%)

Melting Point: 186-188°C

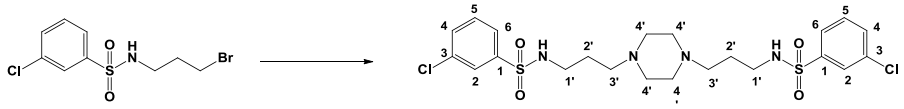
MS $(ESI)^+$: 581.1 $[M+H]^+$

^{1}H-NMR ($CDCl_3$), δ: 1.66 (m, 4H, H-2'), 2.46 (bs, 12H, H-3', 4'), 3.13 (t, J= 5.5 Hz, 4H, H-1'), 7.32 (bs, 2H, NH), 7.66 (m, 4H, H-aromatic), 7.83 (dd, J_1= 8.7 Hz, J_2= 1.8 Hz, 2H, H-aromatic), 7.95 (d, J= 7.9, 2H, H-aromatic), 7.99 (m, 4H, H-aromatic), 8.44 (s, 2H, H-1).

^{13}C-NMR ($DMSO-d_6$), δ: 25.97, 40.88, 52.43, 54.72 (CH_2, C-2', 3', 4', 1'), 122.24, 127.33, 127.51, 127.77, 128.60, 129.11, 129.33 (CH, C-aromatic), 131.70, 134.08, 137.41 (C, C-2, 5, 10).

***N,N'*-(3,3'-(Piperazine-1,4-di*yl*)bis(propane-3,1-di*yl*))bis(3-chlorobenzene-sulfonamide) (100)**

($C_{21}H_{36}Cl_2N_4O_4S_2$; M.W.= 549.5)



91 **100**

General procedure 4;

White solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.66.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 97:3 v/v).

Yield: 0.26 g (79%)

Melting Point: 156-158°C

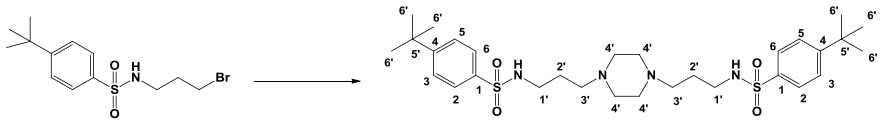
MS $(ESI)^+$: 549.0, 551.0 $[M+H]^+$

^{1}H-NMR ($DMSO-d_6$), δ: 1.48 (m, 4H, H-2'), 2.20 (m, 12H, H-3', 4'), 2.79 (m, 4H, H-1'), 7.64 (t, J= 7.8 Hz, 2H, H-aromatic), 7.74 (m, 6H, 4x H-aromatic, 2 x NH), 7.78 (t, J= 1.8 Hz, 2H, H-2).

^{13}C-NMR (DMSO-d$_6$), δ: 26.03, 40.86, 52.57, 54.70 (CH_2, C-2', 3', 4', 1'), 125.17, 126.04, 131.30, 132.29, 133.79 (CH, C-aromatic), 133.79, 142.46(C, C-1, 3).

***N,N'*-(3,3'-(Piperazine-1,4-di*yl*)bis(propane-3,1-di*yl*))bis(4-*tert*butylbenzene-sulfonamide) (101)**

($C_{30}H_{48}N_4O_4S_2$; M.W.= 592.8)



92 **101**

General procedure 4;

White solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.59.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 97:3 v/v).

Yield: 0.29 g (82%)

Melting Point: 188-190°C

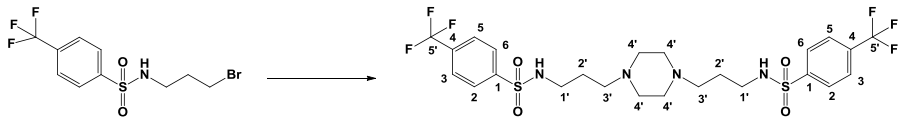
MS $(ESI)^+$: 593.3 $[M+H]^+$

^{1}H-NMR (DMSO-d$_6$), δ: 1.31 (s, 18H, H-6'), 1.46 (m, 4H, H-2'), 2.16 (m, 12H, H-3', 4'), 2.75 (m, 4H, H-1'), 7.49 (t, J= 5.8 Hz, 2H, NH), 7.60 (d, J= 8.4 Hz, 4H, H-aromatic), 7.70 (d, J= 8.4 Hz, 4H, H-aromatic).

^{13}C-NMR (DMSO-d$_6$), δ: 26.03 (CH_2, C-2'), 30.79 (CH_3, C-6'), 34.77 (C, C-5'), 40.87, 52.55, 54.82 (CH_2, C-3', 4', 1'), 125.93, 126.35 (CH, C-aromatic), 137.68, 155.19 (C, C-4,1).

***N,N'*-(3,3'-(Piperazine-1,4-di*yl*)bis(propane-3,1-di*yl*))bis(4-(trifluoromethyl)-benzenesulfonamide) (102)**

($C_{24}H_{30}F_6N_4O_4S_2$; M.W.= 616.6)



93 **102**

General procedure 4;

White solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.65.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 96:4 v/v).

Yield: 0.22 g (61%)

Melting Point: 184-186°C

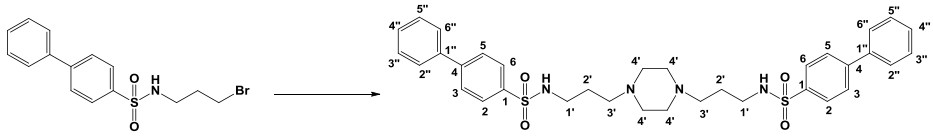
MS $(ESI)^+$: 617.1 $[M+H]^+$

^{1}H-NMR (DMSO-d$_6$), δ: 1.48 (m, 4H, H-2'), 2.16 (bs, 12H, H-3', 4'), 2.81 (m, 4H, H-1'), 7.49 (bs, 2H, NH), 7.99 (m, 8H, H-aromatic).

^{13}C-NMR (DMSO-d$_6$), δ: 26.02, 40.80, 52.52, 54.61 (CH_2, C-2', 3', 4', 1'), 122.41 (C, C-5'), 126.40, 127.44 (CH, C-aromatic), 132.20, 144.50 (C, C-4,1).

***N,N'*-(3,3'-(Piperazine-1,4-di*yl*)bis(propane-3,1-di*yl*))bis(biphenyl-sulfonamide) (103)**

($C_{34}H_{40}N_4O_4S_2$; M.W.= 632.8)



94 **103**

General procedure 4;

White solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.59.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 97:3 v/v).

Yield: 0.76 g (87%)

Melting Point: 183-185°C

MS $(ESI)^+$: 633.2 $[M+H]^+$

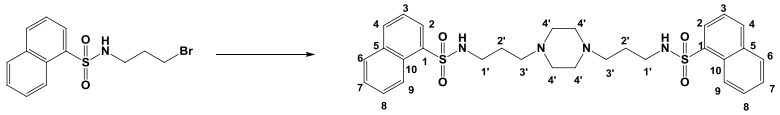
^{1}H-NMR (DMSO-d$_6$), δ: 1.46-1.50 (m, 4H, H-2'), 2.12-2.22 (m, 12H, CH_2), 3.51-3.56 (m, 4H, CH_2), 7.41-7.44 (m, 2H, H-4''), 7.49-7.53 (m, 4H, H-aromatic), 7.62 (bs, 2H, NH), 7.71-7.75 (m, 4H, H-aromatic), 7.84-7.89 (m, 8H, H-aromatic).

^{13}C-NMR (DMSO-d$_6$), δ: 26.04, 40.91, 52.58, 54.81 (CH_2, C-1', 2', 3', 4'), 127.01,

127.15, 127.33, 128.42, 129.08 (CH, C-aromatic), 138.53, 139.25, 143.80 (C, C-1, 4, 1'').

***N,N'*-(3,3'-(Piperazine-1,4-di*yl*)bis(propane-3,1-di*yl*))dinaphthalene-2-sulfonamide (104)**

($C_{30}H_{36}N_4O_4S_2$; M.W.= 580.7)



95 **104**

General procedure 4;

White solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.67.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 96:4 v/v).

Yield: 0.31 g (91%)

Melting Point: 216-218°C

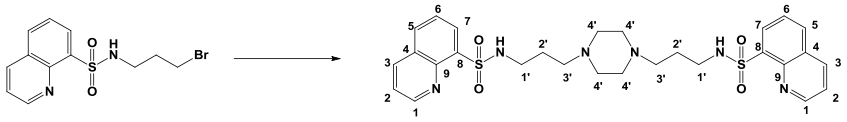
MS $(ESI)^+$: 581.2 $[M+H]^+$

^{1}H-NMR (DMSO-d$_6$), δ: 1.36 (m, 4H, H-2'), 1.93 (bs, 8H, H-4'), 1.99 (t, J= 6.8 Hz, 4H, H-3'), 2.79 (m, 4H, H-1'), 7.67 (m, 6H, H-aromatic), 7.91 (bs, 2H, NH), 8.10 (m, 4H, H-aromatic), 8.22 (d, J= 8.2 Hz, 2H, H-aromatic), 8.64 (d, J= 8.4 Hz, 2H, H-aromatic).

^{13}C-NMR (DMSO-d$_6$), δ: 25.88, 40.73, 52.33, 54.65 (CH_2, C-2', 3', 4', 1'), 124.45, 124.64, 126.77 (CH, C-aromatic), 127.56 (C, C-aromatic), 127.73, 128.54, 128.91, 133.62 (CH, C-aromatic), 133.87, 135.49 (C, C-aromatic).

***N,N'*-(3,3'-(Piperazine-1,4-di*yl*)bis(propane-3,1-di*yl*))diquinoline-8-sulfonamide (105)**

($C_{28}H_{34}N_6O_4S_2$; M.W.= 582.7)



96 **105**

General procedure 4;

White solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.73.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 96:4 v/v).

Yield: 0.26 g (76%)

Melting Point: 200-202°C

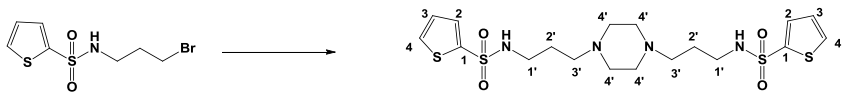
MS $(ESI)^+$: 583.2 $[M+H]^+$

^{1}H-NMR ($CDCl_3$), δ: 1.67 (m, 4H, H-2'), 2.37 (bs, 12H, H-3', 4'), 2.95 (m, 4H, H-1'), 6.79 (bs, 2H, NH), 7.59 (dd, J_1= 8.4, J_2= 4.2, 2H, H-aromatic), 7.68 (t, J= 7.6 Hz, 2H, H-aromatic), 8.08 (d, J=8.0 Hz, 2H, H-aromatic), 8.31 (d, J= 8.0 Hz, 2H, H-aromatic), 8.46 (d, J= 7.1 Hz, 2H, H-aromatic), 9.04 (m, 2H, H-aromatic).

^{13}C-NMR ($CDCl_3$), δ: 26.13, 42.47, 52.92, 56.08 (CH_2, C-2', 3', 4', 1'), 122.22, 125.75 (CH, C-aromatic), 128.80 (C, C-aromatic), 131.44, 133.19 (CH, C-aromatic), 136.09 (C, C-aromatic), 136.99 (CH, C-aromatic), 143.46 (C, C-8), 151.16 (CH, C-aromatic).

***N,N'*-(3,3'-(Piperazine-1,4-di*yl*)bis(propane-3,1-di*yl*))dithiophene-2-sulfonamide (106)**

($C_{18}H_{28}N_4O_4S_4$; M.W.= 492.6)



97 **106**

General procedure 4;

White solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.45.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 96:4 v/v).

Yield: 0.24 g (83%)

Melting Point: 138-140°C

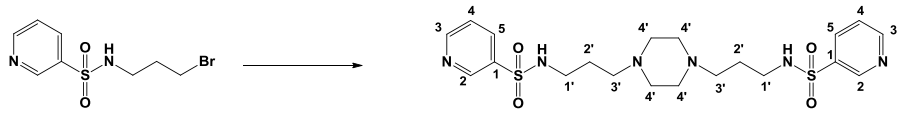
MS $(ESI)^+$: 493.0 $[M+H]^+$

^{1}H-NMR (DMSO-d$_6$), δ: 1.52 (m, 4H, H-2'), 2.24 (m, 12H, H-3', 4'), 2.86 (m, 4H, H-1'), 7.18 (dd, J_1= 5.1, J_2= 3.7, 2H, H-3), 7.57 (dd, J_1= 3.7, J_2= 1.3, 2H, H-aromatic), 7.79 (bs, 2H, NH), 7.92 (dd, J_1= 5.1, J_2= 1.3, 2H, H-aromatic).

^{13}C-NMR (DMSO-d$_6$), δ: 25.88, 41.22, 52.62, 54.92 (CH_2, C-2', 3', 4', 1'), 127.61, 131.38, 132.29 (CH, C-2, 3, 4), 141.44 (C, C-1).

***N,N'*-(3,3'-(Piperazine-1,4-di*yl*)bis(propane-3,1-di*yl*))dipyridine-3-sulfonamide (107)**

($C_{20}H_{30}N_6O_4S_2$; M.W.= 482.6)



98 **107**

General procedure 4;

Pale yellow solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.52.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 91:9 v/v).

Yield: 0.18 g (63%)

Melting Point: 140-142°C

MS $(ESI)^+$: 483.1 $[M+H]^+$

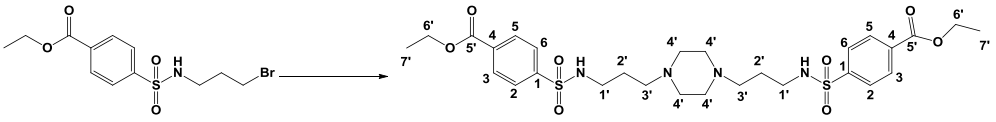
^{1}H-NMR (DMSO-d$_6$), δ: 1.50 (m, 4H, H-2'), 2.20 (bs, 12H, H-3', 4'), 2.82 (bs, 4H, H-1'), 7.65 (dd, J_1= 8.0 Hz, J_2= 4.9 Hz, 2H, H-4), 7.85 (bs, 2H, NH), 8.16 (dt, J_1= 8.0 Hz, J_2= 1.9 Hz, 2H, H-5), 8.82 (dd, J_1= 4.9 Hz, J_2= 1.9 Hz, 2H, H-3), 8.94 (d, J= 2.2 Hz, 2H, H-2).

^{13}C-NMR (DMSO-d$_6$), δ: 26.05, 40.80, 52.50, 54.65 (CH_2, C-2', 3', 4', 1'), 124.26,

134.46 (CH, C-4, 5), 136.88 (C, C-1), 146.99, 152.93 (CH, C-3, 2).

Ethyl 3-(*N*-(3-(4-(3-(4-carboxyphenylensulfonamido)propyl) piperazin-1-*yl*)propyl) sulfamoyl)benzoate (108)

($C_{28}H_{40}N_4O_8S_2$; M.W.= 624.7)



99 **108**

General procedure 4;

White solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.56.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 96:4 v/v).

Yield: 0.15 g (41%)

Melting Point: 170-172°C

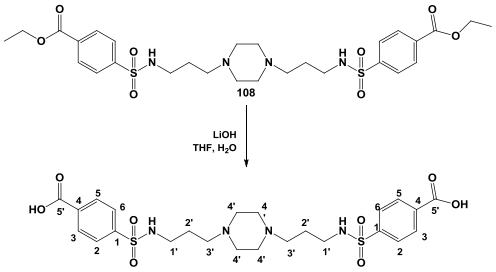
MS $(ESI)^+$: 625.2 $[M+H]^+$

^{1}H-NMR ($CDCl_3$), δ: 1.44 (t, J= 7.1 Hz, 6H, H-7'), 1.67 (m, 4H, H-2'), 2.47 (m, 12H, H-3', 4'), 3.11 (t, J= 5.6 Hz, 4H, H-1'), 4.44 (q, J= 7.1 Hz, 4H, H-6'), 7.45 (bs, 2H, NH), 7.92 (d, J= 8.5 Hz, 4H, H-aromatic), 8.19 (d, J= 8.5 Hz, 4H, H-aromatic).

^{13}C-NMR ($CDCl_3$), δ: 14.27 (CH_3, C-7'), 23.81, 44.39, 53.05, 57.97, 61.68 (CH_2, C-2', 3', 4', 1', 6'), 126.88, 130.21 (CH, C-aromatic), 133.99, 144.15 (C, C-4, 1), 165.22 (C, C-6').

3-(*N*-(3-(4-(3-(4-Carboxyphenylensulfonamido)propyl)piperazin-1-*yl*)-propyl)-sulfamoyl)benzoic acid (111)

($C_{24}H_{32}N_4O_8S_2$; M.W.= 568.6)



111

Compound **108** (0.15 g, 0.25 mmol) was dissolved 3 mL of THF. LiOH monohydrate (0.07 g, 1.7 mmol) was dissolved in 4 mL of distilled water and added to the THF solution. The reaction was stirred o.n. at 80°C. The organic solvent was then removed at reduced pressure and the water residue was acidified to pH 5 with 1M HCl solution. The resulting precipitate was filtered, washed with water and dried under vacuum to afford pure 3-(N-(3-(4-(3-(4-carboxyphenylensulfonamido)propyl)piperazin-1-yl)-propyl)sulfamoyl)benzoic acid **111** as a white solid.

Yield: 0.07 g (51%)

Melting Point: > 260°C

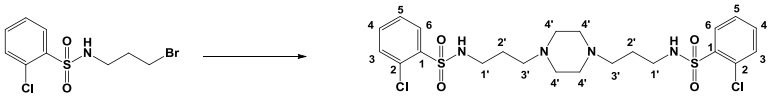
MS $(ESI)^+$: 591.1 $[M+Na]^+$

Sodium salt ^{1}H-NMR (D_2O), δ: 1.48 (m, 4H, H-2'), 2.15 (t, J= 7.8 Hz, 4H, H-3'), 2.31 (bs, 8H, H-4'), 2.69 (t, J= 7.1 Hz, 4H, H-1'), 7.72 (d, J= 8.1 Hz, 4H, H-aromatic), 7.88 (d, J= 8.1 Hz, 4H, H-aromatic).

Sodium salt ^{13}C-NMR (D_2O), δ: 27.83, 43.54, 51.57, 55.62 (CH_2, C-2', 3', 4', 1'), 126.20, 129.17 (CH, C-aromatic), 138.69, 145.22 (C, C-4, 1), 174.53 (C, C-6').

***N,N'*-(3,3'-(Piperazine-1,4-di*yl*)bis(propane-3,1-di*yl*))bis(2-chlorobenzene-sulfonamide) (116)**

($C_{21}H_{36}Cl_2N_4O_4S_2$; M.W.= 549.5)



115 **116**

General procedure 4;

White solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.79.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 98:2 v/v).

Yield: 0.26 g (80%)

Melting Point: 162-164°C

MS $(ESI)^+$: 549.1, 551.1 $[M+H]^+$

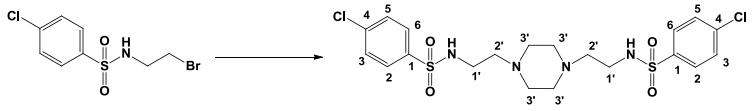
^{1}H-NMR (DMSO-d$_6$), δ: 1.49 (m, 4H, H-2'), 2.17 (m, 12H, H-3', 4'), 2.85 (m, 4H, H-1'), 7.54 (td, J_1= 7.4 Hz, J_2= 1.5 Hz, 2H, H-aromatic), 7.65 (m, 4H, H-aromatic), 7.88 (t, J= 5.1 Hz, 2H, NH), 7.96 (dd, J_1= 7.9 Hz, J_2= 1.3 Hz, 2H, H-aromatic).

^{13}C-NMR (DMSO-d$_6$), δ: 25.78, 41.02, 52.52, 54.93 (CH_2, C-2', 3', 4', 1'), 127.63, 130.51 (CH, C-aromatic), 130.59 (C, C-aromatic), 131.71, 133.90 (CH, C-aromatic), 137.83 (C, C-aromatic).

6.2.7 *N,N'*-(2,2'-(Piperazine-1,4-diyl)bis(ethane-2,1-diyl)) diarylsulfonamides (46-53, 118)

***N,N'*-(2,2'-(Piperazine-1,4-diyl)bis(ethane-2,1-diyl))bis(4-chlorobenzene sulfonamide) (46)**

($C_{20}H_{26}Cl_2N_4O_4S_2$; M.W.= 521.4)



38 **46**

General procedure 5;

White solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.66.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 95:5 v/v).

Yield: 0.14 g (45%)

Melting Point: 218-220°C

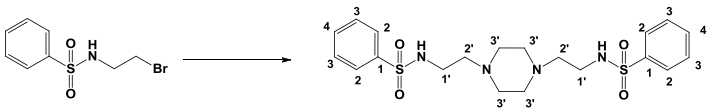
MS $(ESI)^+$: 521.0, 523.0 $[M+H]^+$

^{1}H-NMR ($CDCl_3$), δ: 2.17, (bs, 8H, H-3'), 2.43 (t, J= 5.5, 4H, H-2'), 3.08 (m, 4H, H-1'), 5.14 (bs, 2H, NH), 7.51 (d, J= 8.5 Hz, 4H, H-aromatic), 7.82 (d, J= 8.5 Hz, 4H, H-aromatic).

^{13}C-NMR (DMSO-d_6), δ: 40.97, 52.84, 54.95 (CH_2, C-1', 2', 3'), 128.67, 129.75 (CH, C-aromatic), 137.95, 139.82 (C, C-1, 4).

***N,N'*-(2,2'-(Piperazine-1,4-diyl)bis(ethane-2,1-diyl))dibenzenesulfonamide (47)**

($C_{20}H_{28}N_4O_4S_2$; M.W.= 452.5)



39 **47**

General procedure 5;

White solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.40.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 97:3 v/v).

Yield: 0.12 g (52%)

Melting Point: 158-160°C

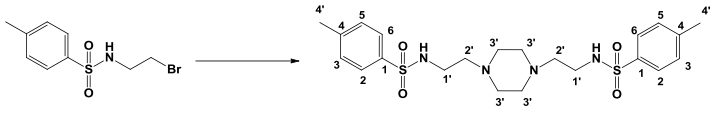
MS $(ESI)^+$: 453.1 $[M+H]^+$

^{1}H-NMR ($CDCl_3$), δ: 2.17, (s, 8H, H-3'), 2.39 (t, J= 5.8, 4H, H-2'), 3.00 (m, 4H, H-1'), 5.13 (bs, 2H, NH), 7.53 (m, 4H, H-3), 7.60 (tt, J_1= 7.3 Hz, J_2= 2.1 Hz, 2H, H-4), 7.88 (m, 4H, H-2).

^{13}C-NMR ($CDCl_3$), δ: 39.16, 52.27, 55.44 (CH_2, C-1', 2', 3'), 127.07, 129.06, 132.64 (CH, C-3, 4, 2), 139.64 (C, C-1).

***N,N'*-(2,2'-(Piperazine-1,4-diyl)bis(ethane-2,1-diyl))bis(4-methylbenzene-sulfonamide) (48)[19]**

($C_{22}H_{32}N_4O_4S_2$; M.W.= 480.6)



40 **48**

General procedure 5;

White solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.59.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 98:2 v/v).

Yield: 0.25 g (89%)

Melting Point: 182-184°C (lit. 185-187°C)[19]

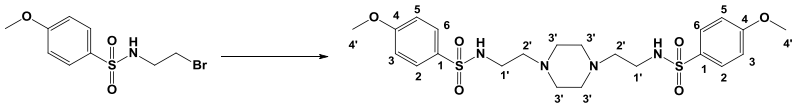
MS $(ESI)^+$: 481.1 $[M+H]^+$

^{1}H-NMR ($CDCl_3$), δ: 2.24, (bs, 8H, H-3'), 2.39 (t, J= 5.7, 4H, H-2'), 2.45 (s, 6H, H-4'), 2.98 (m, 4H, H-1'), 5.10 (bs, 2H, NH), 7.32 (d, J= 8.2 Hz, 4H, H-aromatic), 7.57 (d, J= 8.2 Hz).

^{13}C-NMR (DMSO-d_6), δ: 20.91 (CH_3, C-4'), 40.05, 52.43, 56.74 (CH_2, C-1', 2', 3'), 126.49, 129.52 (CH, C-aromatic), 137.68, 142.46 (C, C-4, 1).

***N,N'*-(2,2'-(Piperazine-1,4-diyl)bis(ethane-2,1-diyl))bis(4-methoxy-benzenesulfonamide) (49)**

($C_{22}H_{32}N_4O_6S_2$; M.W.= 512.6)



41 **49**

General procedure 5;

White solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.45.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 96:4 v/v).

Yield: 0.23 g (78%)

Melting Point: 170-172°C

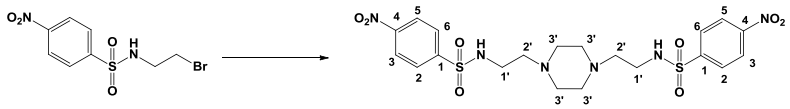
MS $(ESI)^+$: 513.1 $[M+H]^+$

^{1}H-NMR ($CDCl_3$), δ: 2.24, (bs, 8H, H-3'), 2.40 (t, J= 5.4, 4H, H-2'), 2.97 (m, 4H, H-1'), 3.89 (s, 6H, H-4'), 5.07 (bs, 2H, NH), 6.99 (d, J= 8.8 Hz, 4H, H-aromatic), 7.81 (d, J= 8.8 Hz, 4H, H-aromatic).

^{13}C-NMR ($CDCl_3$), δ: 39.12, 52.32, 55.49 (CH_2, C-1', 2', 3'), 55.63 (CH_3, C-4'), 114.19, 129.23 (CH, C-aromatic), 131.19, 162.89 (C, C-1, 4).

***N,N'*-(2,2'-(Piperazine-1,4-diyl)bis(ethane-2,1-diyl))bis(4-nitrobenzene-sulfonamide) (50)**

($C_{20}H_{26}N_4O_8S_2$; M.W.= 542.5)



42 **50**

General procedure 5;

Pale yellow solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.68.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 97:3

v/v).

Yield: 0.20 g (63%)

Melting Point: 212-214°C

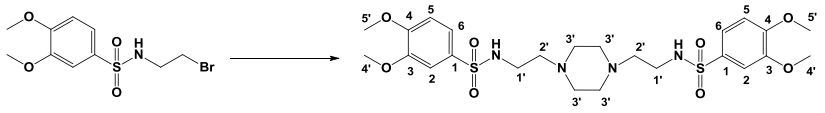
MS $(ESI)^+$: 543.0 $[M+H]^+$

^{1}H-NMR (DMSO-d$_6$), δ: 2.18, (bs, 8H, H-3'), 2.55 (t, J= 6.6, 4H, H-2'), 2.98 (t, J= 6.6 Hz, 4H, H-1'), 7.92 (bs, 2H, NH), 8.05 (d, J= 8.8 Hz, 4H, H-aromatic), 8.40 (d, J= 8.8 Hz).

^{13}C-NMR (DMSO-d$_6$), δ: 40.06, 52.33, 56.83 (CH_2, C-1', 2', 3'), 124.47, 127.98 (CH, C-aromatic), 146.45, 149.44 (C, C-1, 4).

***N,N'*-(2,2'-(Piperazine-1,4-diyl)bis(ethane-2,1-diyl))bis(3,4-dimethoxy-benzenesulfonamide) (51)**

($C_{24}H_{36}N_4O_8S_2$; M.W.= 572.6)



43 **51**

General procedure 5;

White solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.53.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 96:4 v/v).

Yield: 0.25 g (74%)

Melting Point: 152-154°C

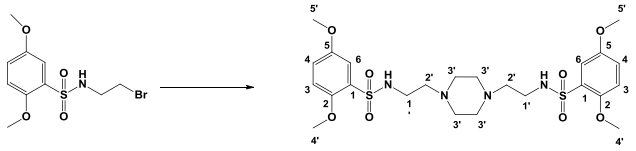
MS $(ESI)^+$: 573.1 $[M+H]^+$

^{1}H-NMR ($CDCl_3$), δ: 2.26, (bs, 8H, H-3'), 2.40 (t, J= 5.6, 4H, H-2'), 2.99 (m, 4H, H-1'), 3.94 (s, 6H, OCH_3), 3.96 (s, 6H, OCH_3), 5.11 (bs, 2H, NH), 6.94 (m, 2H, H-aromatic), 7.34 (m, 2H, H-aromatic), 7.49 (m, 2H, H-aromatic).

^{13}C-NMR ($CDCl_3$), δ: 39.19, 52.35, 56.21 (CH_2, C-1', 2', 3'), 56.28, 56.30 (CH_3, C-4', 5'), 109.66, 110.51, 121.03 (CH, C-2, 5, 6), 131.27, 149.19, 152.58 (C, C-1, 3, 4).

***N,N'*-(2,2'-(Piperazine-1,4-diyl)bis(ethane-2,1-diyl))bis(2,5-dimethoxy-benzenesulfonamide) (52)**

($C_{24}H_{36}N_4O_8S_2$; M.W.= 572.6)



44 **52**

General procedure 5;

White solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.47.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 95:5 v/v).

Yield: 0.26 g (77%)

Melting Point: 176-178°C

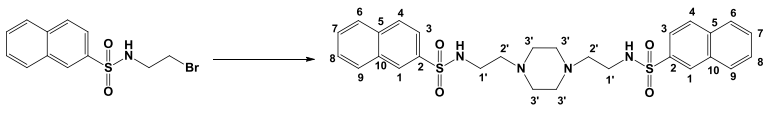
MS $(ESI)^+$: 573.1 $[M+H]^+$

^{1}H-NMR ($CDCl_3$), δ: 2.31, (bs, 8H, H-3'), 2.42 (t, J= 5.6, 4H, H-2'), 2.98 (m, 4H, H-1'), 3.83 (s, 6H, $OC\underline{H}_3$), 3.92 (s, 6H, $OC\underline{H}_3$), 5.58 (t, J= 4.9 Hz, 2H, N<u>H</u>), 6.96 (d, J= 9.1 Hz, 2H, H-3), 7.08 (dd, J_1=9.1 Hz, J_2= 3.1 Hz, 2H, H-4), 7.47 (d, J= 3.1 Hz, 2H, H-6).

^{13}C-NMR ($CDCl_3$), δ: 39.85, 52.64, 56.04 (CH_2, C-3', 2', 1'), 56.05, 57.08 (CH_3, C-4', 5'), 113.74, 114.86, 120.35 (CH, C-3, 4, 6), 127.83, 150.25, 153.35 (C, C-1, 2, 5).

***N,N'*-(2,2'-(Piperazine-1,4-diyl)bis(ethane-2,1-diyl))dinaphthalene-2-sulfonamide (53)**

($C_{28}H_{32}N_4O_4S_2$; M.W.= 552.7)



45 **53**

General procedure 5;

White solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.56.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 97:3 v/v).

Yield: 0.27 g (82%)

Melting Point: 176-178°C

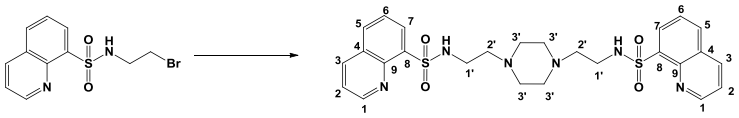
MS (ESI)$^+$: 553.1 $[M+H]^+$

^{1}H-NMR ($CDCl_3$), δ: 2.18, (bs, 8H, H-3'), 2.37 (t, J= 5.8, 4H, H-2'), 3.01 (m, 4H, H-1'), 5.22 (bs, 2H, NH), 7.66 (m, 4H, H-aromatic), 7.82 (dd, J_1= 8.8 Hz, J_2= 1.8 Hz, 2H, H-aromatic), 7.92 (d, J= 8.1 Hz, 2H, H-aromatic), 7.97 (m, 4H, H-aromatic), 8.45 (s, 2H, H-1).

^{13}C-NMR ($CDCl_3$), δ: 39.17, 52.26, 55.46 (CH_2, C-3', 2', 1'), 122.22, 127.65, 127.91, 128.56, 128.84, 129.18, 129.42 (CH, C-aromatic), 131.98, 134.66, 137.32 (C, C-2, 5, 10).

***N,N'*-(2,2'-(Piperazine-1,4-di*yl*)bis(ethane-2,1-di*yl*))diquinoline-8-sulfonamide (118)**

($C_{26}H_{30}N_6O_4S_2$; M.W.= 554.6)



117 **118**

General procedure 5;

White solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.52.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 97:3 v/v).

Yield: 0.17 g (52%)

Melting Point: charring > 220°C

MS (ESI)$^+$: 555.1 $[M+H]^+$

^{1}H-NMR ($CDCl_3$), δ: 1.89, (bs, 8H, H-3'), 2.29 (t, J= 5.8, 4H, H-2'), 2.97 (q, J= 5.8 Hz, 4H, H-1'), 5.22 (t, J= 5.8 Hz, 2H, NH), 7.55 (dd, J_1= 8.6 Hz, J_2= 4.2 Hz, 2H, H-aromatic), 7.68 (t, J= 7.8 Hz, 2H, H-aromatic), 8.07 (dd, J_1= 8.1 Hz, J_2= 1.2 Hz, 2H, H-aromatic), 8.28 (dd, J_1= 8.4 Hz, J_2= 1.6 Hz, 2H, H-aromatic), 8.45 (dd, J_1= 7.2 Hz, J_2=

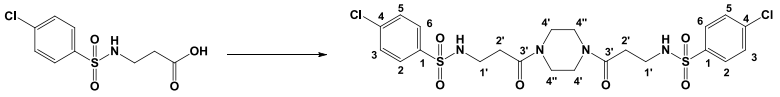
1.4 Hz, 2H, H-aromatic), 9.02 (dd, J_1= 4.3 Hz, J_2= 1.7 Hz, 2H, H-aromatic).

^{13}C-NMR ($CDCl_3$), δ: 38.97, 51.86, 55.10 (CH_2, C-3', 2', 1'), 121.78, 124.95 (CH, C-aromatic), 127.70 (C, C-aromatic), 130.94, 132.87 (CH, C-aromatic), 135.59 (C, C-aromatic), 136.39 (CH, C-aromatic), 142.76 (C, C-8), 150.66 (CH, C-aromatic).

6.2.8 *N,N'*-(3,3'-(Piperazine-1,4-di*yl*)bis(3-oxopropane-3,1-di*yl*))diaryl sulfonamides (62-68)

***N,N'*-(3,3'-(Piperazine-1,4-di*yl*)bis(3-oxopropane-3,1-di*yl*))bis(4-chlorobenzene-sulfonamide) (62)**

($C_{22}H_{26}Cl_2N_4O_6S_2$; M.W.= 576.1)



55 **62**

General procedure 6;

White solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.76.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 98:2 v/v).

Yield: 0.14 g (41%)

Melting Point: 206-208°C

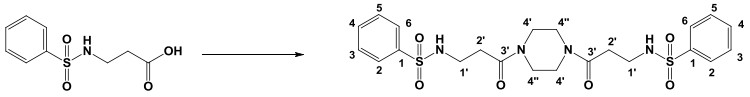
MS $(ESI)^+$: 598.9, 600.9 $[M+Na]^+$

^{1}H-NMR ($CDCl_3$), δ: 2.62 (t, J= 5.2 Hz, 4H, H-2'), 3.33 (m, 4H, H-1'), 3.43 (m, 4H, C$\underline{H}_2$), 3.56 (m, 4H, C$\underline{H}_2$), 7.1 (bs, 2H, N$\underline{H}$), 7.52 (d, J= 8.5 Hz, 4H, H-aromatic), 7.83 (d, J= 8.5 Hz, 4H, H-aromatic).

^{13}C-NMR (DMSO-d_6), δ: 32.58, 32.60, 38.85, 40.62, 40.94, 44.33, 44.60 (CH_2, C-2', 1', 4', 4''), 128.48, 129.32 (CH, C-aromatic), 137.22, 139.21 (C, C-1, 4), 168.60 (C, C-3').

***N,N'*-(3,3'-(Piperazine-1,4-di*yl*)bis(3-oxopropane-3,1-di*yl*)dibenzenesulfonamide (63)**

($C_{22}H_{28}N_4O_6S_2$; M.W.= 508.1)



56 **63**

General procedure 6;

White solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.57.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 97:3 v/v).

Yield: 0.17 g (57%)

Melting Point: 194-196°C

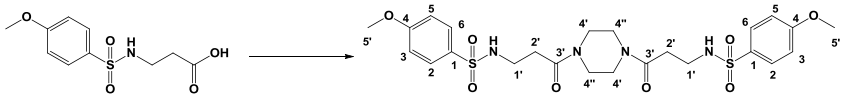
MS (ESI)$^+$: 531.0 $[M+Na]^+$

^{1}H-NMR ($CDCl_3$), δ: 2.59 (bs, 4H, H-2'), 3.26 (m, 4H, H-1'), 3.40 (m, 4H, C$\underline{H}_2$), 3.60 (m, 4H, C$\underline{H}_2$), 5.55 (bs, 2H, N$\underline{H}$), 7.54 (m, 4H, H-aromatic), 7.59 (m, 2H, H-4), 7.89 (m, 4H, H-aromatic).

^{13}C-NMR (DMSO-d_6), δ: 32.56, 32.62, 38.89, 40.61, 40.92, 44.32, 44.61 (CH_2, C-1', 2', 4', 4''), 126.46, 129.20, 132.38 (CH, C-aromatic), 140.28 (C, C-1), 168.64 (C, C-3').

***N,N'*-(3,3'-(Piperazine-1,4-di*yl*)bis(3-oxopropane-3,1-di*yl*))bis(4-methoxybenzene-sulfonamide) (64)**

($C_{24}H_{32}N_4O_8S_2$; M.W.= 568.2)



57 **64**

General procedure 6;

White solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.65.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 98:2 v/v).

Yield: 0.15 g (46%)

Melting Point: 178-180°C

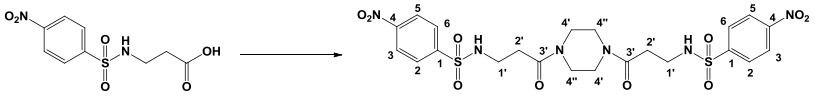
MS (ESI)$^+$: 591.1 $[M+Na]^+$

^{1}H-NMR ($CDCl_3$), δ: 2.59 (t, J= 5.6 Hz, 4H, H-2'), 3.23 (m, 4H, H-1'), 3.42 (m, 4H, C$\underline{H}_2$), 3.60 (m, 4H, C$\underline{H}_2$), 3.89 (s, 6H, H-5'), 5.42 (bs, 2H, N$\underline{H}$), 7.00 (d, J= 8.8 Hz, 4H, H-aromatic), 7.82 (d, J= 8.8 Hz, 4H, H-aromatic).

^{13}C-NMR ($CDCl_3$), δ: 33.03, 33.16, 39.01, 39.07, 41.22, 44.79, 44.96 (CH_2, C-2', 1', 4', 4''), 55.64 (CH_3, C-5'), 114.32, 129.10 (CH, C-aromatic), 131.72, 162.89 (C, C-1, 4), 168.88 (C, C-3').

***N,N'*-(3,3'-(Piperazine-1,4-diyl)bis(3-oxopropane-3,1-diyl))bis(4-nitrobenzene-sulfonamide) (65)**

($C_{22}H_{26}N_4O_{10}S_2$; M.W.= 598.1)



58 **65**

General procedure 6;

Pale yellow solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.70.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 95:5 v/v).

Yield: 0.12 g (35%)

Melting Point: charring > 270°C

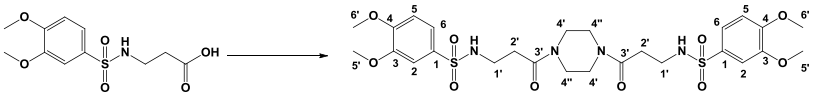
MS $(ESI)^+$: 621.0 $[M+Na]^+$

^{1}H-NMR ($CDCl_3$), δ: 2.66 (t, J= 5.3 Hz, 4H, H-2'), 3.29 (m, 4H, H-1'), 3.46 (m, 4H, C<u>H</u>$_2$), 3.65 (m, 4H, C<u>H</u>$_2$), 5.76 (bs, 2H, N<u>H</u>), 8.08 (d, J= 8.9 Hz, 4H, H-aromatic), 8.39 (d, J= 8.9 Hz, 4H, H-aromatic).

^{13}C-NMR (DMSO-d_6), δ: 32.18, 32.34, 38.85, 40.02, 40.44, 44.31, 44.58 (CH_2, C-2', 1', 4', 4''), 124.57, 128.08 (CH, C-aromatic), 145.95, 149.56 (C, C-1, 4), 168.70 (C, C-3').

***N,N'*-(3,3'-(Piperazine-1,4-diyl)bis(3-oxopropane-3,1-diyl))bis(3,4-dimethoxy-benzenesulfonamide) (66)**

($C_{26}H_{36}N_4O_{10}S_2$; M.W.= 628.2)



59 **66**

General procedure 6;

White solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.65.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 98:2 v/v).

Yield: 0.17 g (47%)

Melting Point: 102-104°C

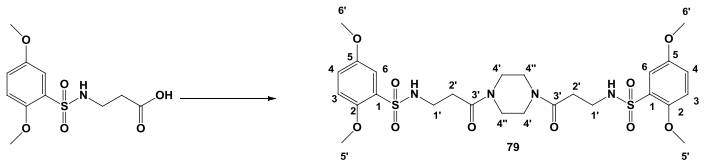
MS (ESI)$^+$: 651.1 $[M+Na]^+$

^{1}H-NMR (CDCl$_3$), δ: 2.80 (bs, 4H, C$\underline{H}_2$), 3.21 (m, 4H, C$\underline{H}_2$), 3.55 (m, 4H, C$\underline{H}_2$), 3.95 (m, 12H, OC$\underline{H}_3$), 4.04 (bs, 4H, C$\underline{H}_2$), 5.25 (bs, 1H, $\underline{N}$H), 5.54 (bs, 1H, $\underline{N}$H), 6.97 (m, 2H, H-aromatic), 7.33 (m, 2H, H-aromatic), 7.49 (m, 2H, H-aromatic).

^{13}C-NMR (CDCl$_3$), δ: 33.05, 33.27, 38.81, 39.08, 41.19, 41.34, 44.95, 45.09 (CH_2, C-1', 2', 4', 4''), 56.20, 56.27, 56.36, 56.49 (CH_3, C-5', 6'), 109.57, 109.70, 110.62, 110.68, 120.85, 120.93 (CH, C-aromatic), 129.96, 131.59, 149.25, 149.52, 152.00, 152.76 (C, C-1, 3, 4), 169.84, 171.89 (C, C-3').

***N,N'*-(3,3'-(Piperazine-1,4-di*yl*)bis(3-oxopropane-3,1-di*yl*))bis(2,5-dimethoxy-benzenesulfonamide) (67)**

($C_{26}H_{36}N_4O_{10}S_2$; M.W.= 628.2)



60 **67**

General procedure 6;

White solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.63.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 97:3 v/v).

Yield: 0.19 g (52%)

Melting Point: 90-92°C

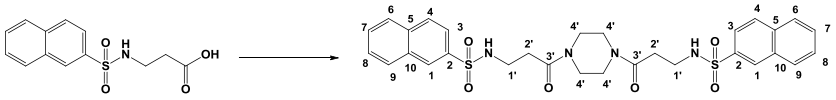
MS (ESI)$^+$: 651.1 $[M+Na]^+$

^{1}H-NMR (CDCl$_3$), δ: 2.55 (t, J= 6.0 Hz, 4H, H-2'), 3.22 (m, 4H, H-1'), 3.37 (m, 4H, H-4', 4''), 3.60 (m, 4H, C$\underline{H}_2$), 3.84 (s, 6H, OC$\underline{H}_3$), 3.97 (s, 6H, OC$\underline{H}_3$), 5.92 (t, J= 6.1 Hz, 2H, N$\underline{H}$), 7.00 (d, J= 9.0 Hz, 2H, H-3), 7.09 (dd, J_1= 9.0 Hz, J_2= 3.0 Hz, 2H, H-4), 7.46 (d, J= 3.0 Hz, H-6).

^{13}C-NMR (DMSO-d$_6$), δ: 32.21, 32.51, 38.90, 40.52, 40.79, 44.23, 44.47 (CH_2, C-1', 2', 4', 4''), 55.76, 56.53 (CH_3, C-5', 6'), 114.15, 114.26, 119.50 (CH, C-3, 4, 6), 128.22, 150.17, 152.34 (C, C-1, 2, 5), 168.92 (C, C-3').

***N,N'*-(3,3'-(Piperazine-1,4-di*yl*)bis(3-oxopropane-3,1-di*yl*))dinaphthalene-2-sulfonamide (68)**

($C_{30}H_{32}N_4O_6S_2$; M.W.= 608.2)



61 **68**

General procedure 6;

White solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.79.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 97:3 v/v).

Yield: 0.19 g (54%)

Melting Point: 228-230°C

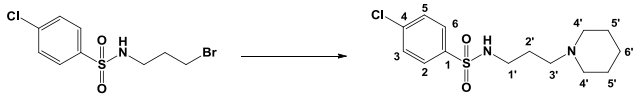
MS $(ESI)^+$: 631.0 $[M+Na]^+$

^{1}H-NMR (CDCl$_3$), δ: 2.55 (m, 4H, C$\underline{H}_2$), 3.28 (m, 8H, C$\underline{H}_2$), 3.52 (4H, m, C$\underline{H}_2$), 5.61 (bs, 2H, N$\underline{H}$), 7.66 (m, 4H, H-aromatic), 7.86 (dd, J_1= 8.7 Hz, J_2= 1.7 Hz, 2H, H-aromatic), 7.93 (d, J= 7.9 Hz, 2H, H-aromatic), 7.99 (m, 4H, H-aromatic), 8.45 (s, 2H, H-1).

^{13}C-NMR (DMSO-d$_6$), δ: 32.59, 32.63, 38.90, 40.54, 40.83, 44.25, 44.49 (CH_2, C-1', 2', 4', 4''), 122.27, 127.36, 127.55, 127.79, 128.67, 129.13, 129.36 (CH, C-aromatic), 131.69, 134.11, 137.32 (C, C-2, 5, 10), 168.58 (C, C-3').

6.2.9 *N*-(3-(Piperidin-1-*yl*)propyl)arylsulfonamides (69, 70)

4-Chloro-*N*-(3-piperidin-1-*yl*-propyl)-benzenesulfonamide (69)
($C_{14}H_{21}ClN_2O_2S$; M.W.= 316.8)



22 **69**

General procedure 7;

Pale yellow oil;

T.L.C. System: DCM-MeOH 95:5 v/v, Rf: 0.30.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 93:7 v/v).

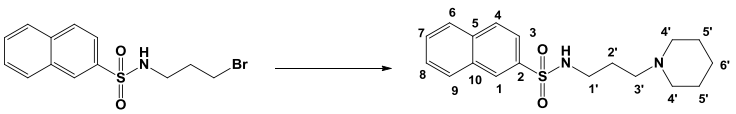
Yield: 0.28 g (75%)

MS $(ESI)^+$: 317.1, 319.1 $[M+H]^+$

^{1}H-NMR ($CDCl_3$), δ: 1.50 (bs, 2H, C<u>H</u>$_2$), 1.67 (m, 6H, C<u>H</u>$_2$), 2.43 (m, 6H, C<u>H</u>$_2$), 3.09 (t, J= 5.3 Hz, 2H, H-1'), 7.50 (d, J= 8.5 Hz, 2H, H-aromatic), 7.82 (d, J= 8.5 Hz, 2H, H-aromatic), 8.03 (bs, 1H, N<u>H</u>).

^{13}C-NMR ($CDCl_3$), δ: 23.60, 24.00, 25.70, 44.32, 54.39, 58.71 (CH_2, C-1', 2', 3', 4', 5', 6'), 128.42, 129.21 (CH, C-aromatic), 138.61, 138.98 (C, C-1, 4).

***N*-(3-Piperidin-1-*yl*-propyl)-naphthalene-2-sulfonamide (70)**
($C_{18}H_{24}N_2O_2S$; M.W.= 332.5)



29 **70**

General procedure 7;

Brownish oil;

T.L.C. System: DCM-MeOH 95:5 v/v, Rf: 0.37.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 95:5 v/v).

Yield: 0.28 g (71%)

MS (ESI)$^+$: 333.1 $[M+H]^+$

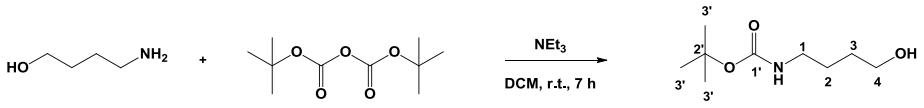
^{1}H-NMR ($CDCl_3$), δ: 1.48 (bs, 2H, C<u>H</u>$_2$), 1.63 (m, 6H, C<u>H</u>$_2$), 2.34 (m, 6H, C<u>H</u>$_2$), 3.11 (t, J= 5.7 Hz, 2H, H-1'), 7.64 (m, 2H, H-aromatic), 7.86 (dd, J_1= 8.6 Hz, J_2= 1.8 Hz, 1H, H-aromatic), 7.93 (d, J= 7.9 Hz, 1H, H-aromatic), 7.98 (m, 3H, 2 x H-aromatic, N<u>H</u>), 8.45 (s, 1H, H-1).

^{13}C-NMR ($CDCl_3$), δ: 23.61, 24.20, 26.03, 44.73, 54.47, 59.00 (CH_2, C-1', 2', 3', 4', 5', 6'), 122.43, 127.43, 127.89, 128.16, 128.50, 129.13, 129.25 (CH, C-aromatic), 132.23, 134.67, 137.16 (C, C-1, 5, 10).

6.2.10 *N,N'*-(4,4'-(Piperazine-1,4-diyl)bis(butane-4,1-diyl))bis(4-chlorobenzene-sulfonamide) (73)

(4-Hydroxy-butyl)-carbamic acid *tert*-butyl ester (78)[20]

($C_9H_{19}NO_3$; M.W.= 189.3)



71 **77** **78**

Di-*tert*-butyl dicarbonate (**77**) (2.69 g, 12.3 mmol) was dissolved at °C in 3 mL of anhydrous DCM and added dropwise to a stirred solution of 4-amino-1-butanol (**71**) (1g, 11.2 mmol) and triethylamine (1.7 mL, 12.3 mmol), in 6 mL of anhydrous DCM. The resulting mixture was stirred at r.t. for 7 h. The reaction mixture was then diluted with DCM and washed with saturated NH_4Cl solution (2 x 25 mL) and brine (25 mL). The organic extract was dried over $MgSO_4$ and the solvent was removed at reduced pressure to afford pure 4-hydroxy-butyl)-carbamic acid *tert*-butyl ester (**78**) as a pale yellow oil.

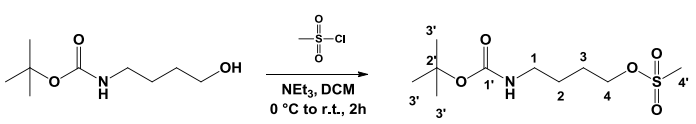
Yield: 1.97 g (93%)

^{1}H-NMR ($CDCl_3$), δ: 1.44 (s, 9H, H-3', 4', 5'), 1.58 (m, 4H, H-2, 3), 2.05 (bs, 1H, O<u>H</u>), 3.15 (m, 2H, C<u>H</u>$_2$), 3.66 (m, 2H, C<u>H</u>$_2$), 4.70 (bs, 1H, N<u>H</u>).

^{13}C-NMR ($CDCl_3$), δ: 26.60 (CH_2, C-aliphatic), 28.41 (CH_3, C-3', 4', 5'), 29.71, 40.29 62.34 (CH_2, C-aliphatic), 79.17 (C, C-2'), 156.15 (C, C-1').

Methanesulfonic acid 4-*tert*-butoxycarbonylamino-butyl ester (79)[21]

($C_{10}H_{21}NO_5S$; M.W.= 267.3)



78 **79**

To a solution of Boc-4-aminobutanol (**78**) (1 g, 5.3 mmol) and triethylamine (1.5 mL, 10.6 mmol) in 7 mL of anhydrous DCM was added dropwise methane sulfonyl chloride (0.66 g, 5.8 mmol) over a period of 10 min at 0°C. The mixture was then stirred for an additional 1 h at 0°C and 1 h at r.t. The reaction mixture was diluted with DCM (20 mL)

and washed with cold saturated $NaHCO_3$ solution (2 x 30 mL). The organic layer was dried over $MgSO_4$ and the solvent was removed under vacuum to give pure methanesulfonic acid 4-*tert*-butoxycarbonylamino-butyl ester (**79**) as a yellow oil.

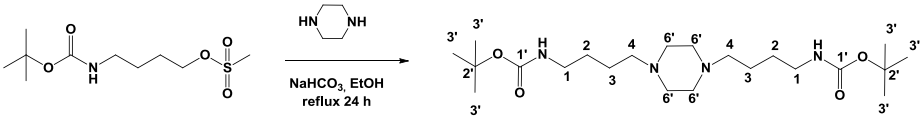
Yield: 1.21 g (86%)

^{1}H-NMR ($CDCl_3$), δ: 1.44 (s, 9H, H-3'), 1.61 (m, 2H, C<u>H</u>$_2$), 1.79 (m, 2H, C<u>H</u>$_2$), 3.01 (s, 3H, H-6'), 3.16 (m, 2H, C<u>H</u>$_2$), 4.25 (t, J= 6.4 Hz, 2H, H-4), 4.64 (bs, 1H, N<u>H</u>).

^{13}C-NMR ($CDCl_3$), δ: 26.26, 26.40 (CH_2, C-2, 3), 28.38 (CH_3, C-3'), 39.75 (CH_3, C-6'), 42.54, 69.62 (CH_2, C-1, 4), 79.24 (C, C-2'), 155.99 (C, C-1').

{4-[4-(4-*tert*-Butoxycarbonylamino-butyl)-piperazin-1-yl]-butyl}-carbamic acid *tert*-butyl ester (80)

($C_{22}H_{44}N_4O_4$; M.W.= 428.6)



79 **80**

Piperazine (0.17 g, 2.1 mmol) and $NaHCO_3$ (0.38, 4.5 mmol) were suspended in 3 mL of absolute ethanol and added dropwise of **79** (1.21 g, 4.5 mmol) diluted in 5 mL of ethanol. The reaction mixture was stirred under reflux for 24 h. The solvent was evaporated under vacuum, and the residue was suspended in DCM (30 mL) and washed with saturated $NaHCO_3$ solution (2 x 30 mL) and brine (30 mL). The organic phase was evaporated at reduced pressure after drying over $MgSO_4$ to give {4-[4-(4-*tert*-Butoxycarbonylamino-butyl)-piperazin-1-yl]-butyl}-carbamic acid *tert*-butyl ester **80** as a yellow waxy solid.

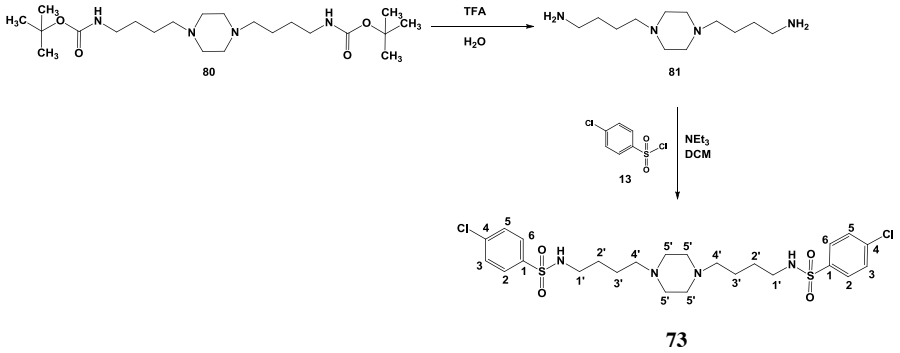
Yield: 0.63 g (70%)

^{1}H-NMR ($CDCl_3$), δ: 1.37 (m, 18 H, H-3'), 1.63 (m, 8H, C<u>H</u>$_2$), 2.32 (bs, 4H, C<u>H</u>$_2$), 2.56 (bs, 8H, C<u>H</u>$_2$), 3.33 (t, J= 5.3 Hz, 4H, H-1), 5.56 (bs, 2H, N<u>H</u>).

^{13}C-NMR ($CDCl_3$), δ: 24.40, 25.74 (CH_2, C-aliphatic), 28.48, 28.56 (CH_3, C-3'), 40.53, 53.05, 58.05 (CH_2, C-aliphatic), 78.86 (C, C-2'), 156.05 (C, C-1').

***N,N'*-(4,4'-(Piperazine-1,4-di*yl*)bis(butane-4,1-di*yl*))bis(4-chlorobenzene-sulfonamide) (73)**

($C_{24}H_{34}N_4O_4S_2$; M.W.= 577.6)



80 81 13 73

Compound **80** (0.63 g, 1.5 mmol) was dissolved at 0°C in a mixture of 2 mL of TFA and 0.2 mL of distilled water. The reaction mixture was left stirring at r.t for 30 min., then the volume was reduced under vacuum and the remaining suspension was poured dropwise into 5 mL of ice-cooled diethyl ether. The resulting white precipitate was left in the fridge o.n. before being filtered and washed with cold diethyl ether.

The white solid was suspended with 4-chlorobenzenesulfonyl chloride (**13**) (0.63 g, 3.0 mmol) in 7 mL of anhydrous DCM; the resulting mixture was treated dropwise with triethylamine (1.3 mL, 9.5 mmol) under ice-cooling and stirred for 1 h at r.t.

The reaction mixture was then diluted with DCM and washed with water (2 x 30 mL) and brine (30 mL). The organic solvent was removed under vacuum after drying over $MgSO_4$ and the crude residue was purified by flash column chromatography (DCM:MeOH 100:0 v/v increasing to 91:9 v/v) to afford pure *N,N'*-(4,4'-(piperazine-1,4-di*yl*)bis(butane-4,1-di*yl*))bis(4-chlorobenzenesulfonamide) (**73**) as a white solid.

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.64.

Yield: 0.12 g (15%)

Melting Point: 182-184°C

MS $(ESI)^+$: 577.1. 579.1 $[M+H]^+$

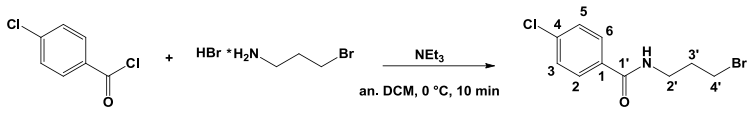
^{1}H-NMR ($CDCl_3$), δ: 1.63 (m, 8H, C<u>H</u>$_2$), 2.50 (bs, 4H, C<u>H</u>$_2$), 2.77 (bs, 8H, C<u>H</u>$_2$), 2.96 (t, J= 5.3 Hz, 4H, H-1'), 7.49 (d, J= 8.6 Hz, 4H, H-aromatic), 7.81 (d, J= 8.6 Hz, 4H, H-aromatic).

^{13}C-NMR ($CDCl_3$), δ: 24.17, 28.29, 42.88, 51.81, 57.72 (CH_2, C-1', 2', 3', 4', 5'), 128.43, 129.29 (CH, C-aromatic), 138.66, 139.28 (C, C-1, 4).

6.2.11 *N,N'*-(3,3'-(Piperazine-1,4-diyl)bis(propane-3,1-diyl))bis(4-chlorobenzamide) (122)

N-(3-Bromopropyl)-4-chlorobenzamide (120)

($C_{10}H_{11}BrClNO$; M.W.= 276.6)



119 **21** **120**

4-Chlorobenzoyl chloride (**119**) (0.5 g, 2.9 mmol) and 3-bromopropylamine hydrobromide (**32**) (0.69 g, 3.2 mmol) were suspended in anhydrous DCM (8 mL) under a nitrogen atmosphere. Triethylamine (0.9 mL, 6.6 mmol) was then added dropwise under ice-cooling and the reaction mixture was left stirring at 0°C for 10 min. and then at r.t. for 20 min.The reaction mixture was then diluted with DCM (20 mL) and washed with a 2M hydrochloric acid solution (2 x 30 mL) and brine (30 mL). The organic layer was dried over $MgSO_4$ and the solvent was removed under vacuum to afford pure *N*-(3-bromopropyl)-4-chlorobenzamide (**120**) as a white solid.

T.L.C. System: DCM- *n*hexane 4:1 v/v, Rf: 0.53.

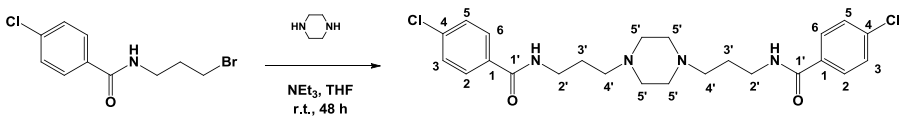
Yield: 0.63 g (78%)

^{1}H-NMR ($CDCl_3$), δ: 2.23 (m, 2H, H-3'), 3.51 (t, J= 6.4 Hz, 2H, H-4'), 3.64 (m, 2H, H-2'), 6.38 (bs, 1H, NH), 7.43 (d, J= 8.6 Hz, 2H, H-aromatic), 7.73 (d, J= 8.6 Hz, 2H, H-aromatic).

^{13}C-NMR ($CDCl_3$), δ: 30.97, 32.01, 38.81 (CH_2, C-3', 4', 2'), 128.31, 128.89 (CH, C-aromatic), 132.71, 137.87 (C, C-1, 4), 166.65 (C, C-1').

N,N'-(3,3'-(Piperazine-1,4-diyl)bis(propane-3,1-diyl))bis(4-chlorobenzamide) (122)

($C_{24}H_{30}Cl_2N_4O_2$; M.W.= 477.4)



120 **122**

A mixture of compound **120** (0.5 g, 1.8 mmol), piperazine (0.07 g, 0.8 mmol) and

triethylamine (0.24 mL, 1.7 mmol) in 7 mL of anhydrous THF was stirred under nitrogen atmosphere for 48 h. at r.t. The reaction mixture was then diluted with DCM (20 mL), washed with saturated $NaHCO_3$ solution (2 x 30 mL) and brine (30 mL) and dried over $MgSO_4$. The organic solvent was removed under vacuum and the crude residue was purified by flash column chromatography (DCM-MeOH 100:0 v/v increasing to DCM-MeOH 91:9 v/v) to afford pure *N,N'*-(3,3'-(piperazine-1,4-di*yl*)bis(propane-3,1-diyl))bis(4-chlorobenzamide) (**122**) as a white solid.

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.63.

Yield: 0.1375 g (36%)

Melting Point: 180-182°C

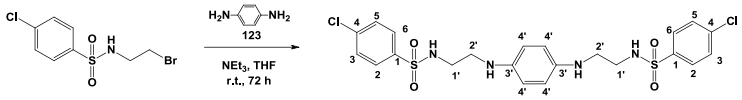
MS (ESI^+): 477.1, 479.1 $[M+H]^+$

^{1}H-NMR ($CDCl_3$), δ: 1.82 (m, 4H, H-3'), 2.56 (m, 12H, H-4', 5'), 3.59 (m, 4H, H-2'), 7.42 (d, J= 8.5 Hz, 4H, H-aromatic), 7.78 (d, J= 8.5 Hz, 4H, H-aromatic), 8.05 (bs, 2H, N<u>H</u>).

^{13}C-NMR (DMSO-d_6), δ: 26.10, 37.92, 52.75, 55.64 (CH_2, C-2', 3', 4', 5'), 128.28, 129.02 (CH, C-aromatic), 133.37, 135.79 (C, C-1, 4), 164.99 (C, C-1').

6.2.12 *N,N'*-(2,2'-(1,4-Phenylenebis(azanediyl))bis(ethane-2,1-diyl))bis(4-chlorobenzene-sulfonamide) (125)

($C_{22}H_{24}Cl_2N_4O_4S_2$; M.W.= 543.5)



38 **125**

A mixture of compound **38** (1.05 g, 3.5 mmol), 4-phenylendiamine (**123**) (0.16 g, 1.5 mmol) and triethylamine (0.47 mL, 3.4mmol) in 13 mL of anhydrous THF was stirred under nitrogen atmosphere for 72 h at r.t. The reaction mixture was then diluted with DCM (35 mL), washed with saturated $NaHCO_3$ solution (2 x 30 mL) and brine (30 mL) and dried over $MgSO_4$. The organic solvent was removed under vacuum and the crude residue was purified by flash column chromatography (*n*hexane-EtOAc 100:0 v/v increasing to *n*hexane-EtOAc 20:80 v/v) to afford pure *N,N'*-(2,2'-(1,4-phenylenebis(azanediyl))bis(ethane-2,1-diyl))bis(4-chlorobenzenesulfonamide) (**125**) as a light brown solid.

T.L.C. System: EtOAc-*n*hexane 8:2 v/v, Rf: 0.46.

Yield: 0.13 g (17%)

Melting Point: 162-164°C

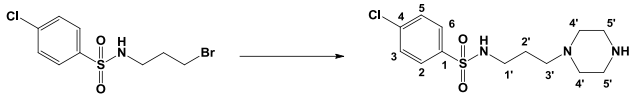
MS $(ESI)^+$: 543.0, 545.0 $[M+H]^+$

^{1}H-NMR (DMSO-d_6), δ: 2.87 (t, J= 6.5 Hz, 4H, C<u>H</u>$_2$), 2.97 (m, 4H, C<u>H</u>$_2$), 4.70 (t, J= 6.1 Hz, 2H, N<u>H</u>), 6.32 (s, 4H, H-4'), 7.65 (d, J= 8.6 Hz, 4H, H-aromatic), 7.79 (d, J= 8.6 Hz, 4H, H-aromatic), 7.81 (bs, 2H, N<u>H</u>).

^{13}C-NMR (DMSO-d_6), δ: 41.84, 43.63 (CH_2, C-1', 2'), 113.74 (CH, C-4'), 128.39, 129.31 (CH, C-aromatic), 137.20, 139.33, 139.64 (C, C-1, 4, 3').

6.2.13 *N*-(3-Piperazin-1-*yl*-propyl)-arylsulfonamides (139-143)

4-Chloro-N-(3-piperazin-1-yl-propyl)-benzenesulfonamide) (139)
($C_{13}H_{20}ClN_3O_2S$; M.W.= 317.1)



22 **139**

General procedure 8;

Pale yellow solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.73.

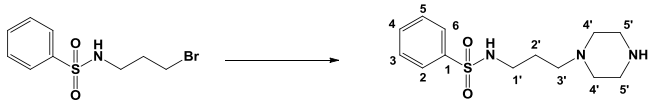
Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 96:4 v/v).

Yield: 1.37g (56%)

^{1}H-NMR ($CDCl_3$), δ: 1.61-1.67 (m, 2H, H-2'), 2.35-2.46 (m, 6H, CH_2), 2.91 (t, J= 4.8 Hz, 4H, CH_2), 3.07 (t, J= 5.8 Hz, 2H, CH_2), 7.48 (d, J= 8.7 Hz, 2H, H-aromatic), 7.79 (d, J= 8.7 Hz, 2H, H-aromatic).

^{13}C-NMR ($CDCl_3$), δ: 23.70, 44.25, 44.41, 45.88, 46.09, 54.18, 58.58 (CH_2, C-1', 2', 3', 4', 5'), 128.40, 129.25 (CH, C-aromatic), 138.70, 138.94 (C, C-1, 4).

***N*-(3-Piperazin-1-*yl*-propyl)-benzenesulfonamide (140)[22]**
($C_{13}H_{21}N_3O_2S$; M.W.= 283.3)



23 **140**

General procedure 8;

Pale yellow oil;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.26.

Purification: flash column chromatography (DCM:MeOH:NEt_3 100:0:0 v/v increasing to DCM:MeOH:NEt_3 90:9:1 v/v).

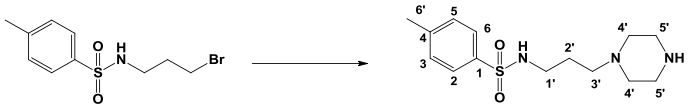
Yield: 1.55 g (71%)

^{1}H-NMR ($CDCl_3$), δ: 1.60-1.66 (m, 2H, H-2'), 2.37-2.43 (m, 6H, CH_2), 2.92 (t, J=3. 4.8 Hz, 4H, CH_2), 3.08 (t, J= 5.7 Hz, 2H, CH_2), 7.50-7.54 (m, 2H, H-aromatic), 7.57 (tt, J_1= 7.3 Hz, J_2= 1.2 Hz, 1H, H-4), 7.87 (d, J= 7.2 Hz, 2H, H-2, 6).

^{13}C-NMR ($CDCl_3$), δ: 23.85, 44.16, 45.80, 45.98, 53.43, 54.00, 58.46 (CH_2, C-1', 2', 3', 4', 5'), 126.91, 128.98, 132.32 (CH, C-aromatic), 140.30 (C, C-1).

4-Methyl-*N*-(3-Piperazin-1-*yl*-propyl)-benzenesulfonamide (141)

($C_{14}H_{23}N_3O_2S$; M.W.= 297.4)



24 **141**

General procedure 8;

Pale yellow waxy solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.23.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 90:9:1 v/v).

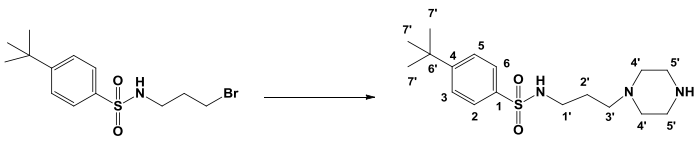
Yield: 1.35 g (59%)

^{1}H-NMR ($CDCl_3$), δ: 1.49-1.54 (m, 2H, H-2'), 2.24-2.30 (m, 6H, CH_2), 2.32 (s, 3H, H-6'), 2.80 (t, J= 4.7 Hz, 4H, CH_2), 2.92 (t, J= 6.1 Hz, 2H, CH_2), 7.21 (d, J= 8.1 Hz, 2H, H-aromatic), 7.64 (d, J= 8.1 Hz, 2H, H-aromatic).

^{13}C-NMR ($CDCl_3$), δ: 21.39 (CH_3, C-6'), 24.21, 43.56, 45.80, 53.91, 57.94 (CH_2, C-1', 2', 3', 4', 5'), 126.86, 129.52 (CH, C-aromatic), 137.25, 142.93 (C, C-1, 4).

4-*tert*-Butyl-*N*-(3-piperazin-1-*yl*-propyl)-benzenesulfonamide (142)

($C_{17}H_{29}N_3O_2S$; M.W.= 339.5)



92 **142**

General procedure 8;

Yellow oil;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.24.

Purification: flash column chromatography (DCM:MeOH:NEt_3 100:0:0 v/v increasing to 90:9:1 v/v).

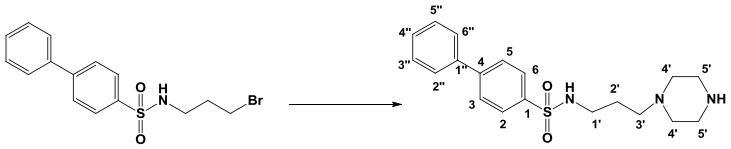
Yield: 2.61 g (71%)

^{1}H-NMR ($CDCl_3$), δ: 1.36 (s, 9H, H-7'), 1.62-1.67 (m, 2H, H-2'), 2.37-2.56 (m, 6H, CH_2), 2.91 (t, J= 4.8 Hz, 4H, CH_2), 3.09 (t, J=5.8 Hz, 2H, CH_2), 7.52 (d, J= 8.5 Hz, 2H, H-aromatic), 7.78 (d, J= 8.5 Hz, 2H, H-aromatic).

^{13}C-NMR ($CDCl_3$), δ: 23.88 (CH_2, C-2'), 31.12 (CH_3, C-7'), 35.09 (C, C-6'), 44.18, 46.16, 54.29, 58.58 (CH_2, C-3', 4', 1', 5'), 125.91, 126.80 (CH, C-aromatic), 137.27, 156.02 (C, C-4,1).

Biphenyl-4-sulfonic acid (3-piperazin-1-*yl*-propyl)-amide (143)

($C_{19}H_{25}N_3O_2S$; M.W.= 359.5)



94 **143**

General procedure 8;

Colourless oil;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.33.

Purification: flash column chromatography (DCM:MeOH:NEt_3 100:0:0 v/v increasing to 90:9:1 v/v).

Yield: 1.13g (41%)

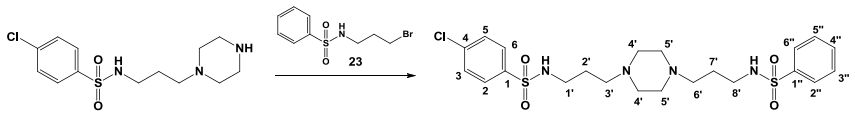
^{1}H-NMR ($CDCl_3$), δ: 1.62-1.67 (m, 2H, H-2'), 2.35-2.43 (m, 6H, CH_2), 2.91 (t, J= 4.7 Hz, 4H, CH_2), 3.10 (t, J= 5.8 Hz, 2H, CH_2), 7.40 (tt, J_1= 7.3 Hz, J_2= 2.1 Hz, 1H, H-4''), 7.45-7.49 (m, 2H, H-aromatic), 7.60 (d, J= 7.2 Hz, 2H, H-aromatic), 7.71 (d, J= 8.5 Hz, 2H, H-aromatic), 7.91 (d, J= 8.5 Hz, 2H, H-aromatic).

^{13}C-NMR ($CDCl_3$), δ: 24.05, 44.00, 45.97, 54.09, 58.31 (CH_2, C-2', 3', 4', 1', 5'), 126.99, 127.59, 127.91, 128.41, 129.04 (CH, C-aromatic), 138.92, 139.33, 145.20 (C, C-aromatic).

6.2.14 *N*-(3-(4-(3-Arylsulfonylamino-propyl)-piperazin-1-*yl*)-propyl)-aryl sulfonamides (126-138)

***N*-{3-[4-(3-Benzenesulfonylamino-propyl)-piperazin-1-*yl*]-propyl}-4-chloro-benzenesulfonamide (126)**

($C_{22}H_{31}ClN_4O_4S_2$; M.W.= 515.1)



139 **126**

General procedure 9;

White solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.59.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 97:3 v/v).

Yield: 0.35 g (68%)

Melting Point: 128-130°C

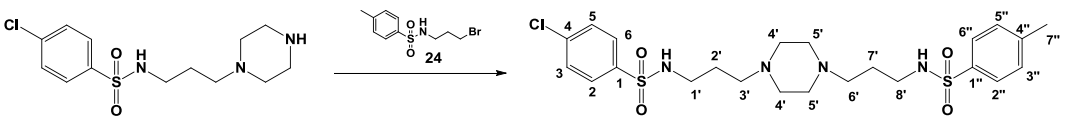
MS $(ESI)^+$: 515.1, 517.1 $[M+H]^+$

^{1}H-NMR (DMSO-d$_6$), δ: 1.44-1.51 (m, 4H, H-2', 7'), 2.11-2.26 (m, 12H, CH_2), 2.74-2.79 (m, 4H, CH_2), 7.56-7.62 (m, 3H, 2H-aromatic, NH), 7.61-7.64 (m, 1H, H-aromatic), 7.66-7.70 (m, 3H, 2H-aromatic, NH), 7.78 (d, J= 8.3 Hz, 2H, H-aromatic).

^{13}C-NMR (DMSO-d$_6$), δ: 26.02, 26.05, 40.85, 40.93, 52.59, 54.74, 54.86 (CH_2), 126.41, 128.42, 129.15, 129.31, 132.26 (CH, C-aromatic), 137.13, 139.39, 140.50 (C, C-1, 4, 4'').

4-Chloro-*N*-(3-(4-(3-(4-methylbenzenesulfonylamido)propyl)-piperazin-1-*yl*)-propyl)benzenesulfonamide (127)

($C_{23}H_{33}ClN_4O_4S_2$; M.W.= 529.1)



139 **127**

General procedure 9;

White solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.57.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 96:4 v/v).

Yield: 0.45 g (86%)

Melting Point: 172-174°C

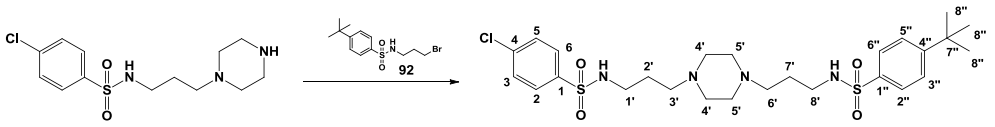
MS $(ESI)^+$: 529.1, 531.1 $[M+H]^+$

^{1}H-NMR (DMSO-d$_6$), δ: 1.43-1.51 (m, 4H, H-2', 7'), 2.10-2.28 (m, 12H, CH_2), 2.38 (s, 3H, H-7''), 2.69-2.81 (m, 4H, CH_2), 7.39 (d, J= 8.1 Hz, 2H, H-aromatic),7.48 (t, J= 5.6 Hz, 1H, NH), 7.64-7.71 (m, 5H, 4H-aromatic, NH), 7.79 (d, J= 8.7 Hz, 2H, H-aromatic).

^{13}C-NMR (DMSO-d$_6$), δ: 20.91 (CH_3, C-7''), 26.02, 40.85, 40.92, 52.59, 54.75, 54.89 (CH_2), 126.50, 128.42, 129.30, 129.55 (CH, C-aromatic), 137.13, 137.62, 139.40, 142.46 (C, C-aromatic).

4-*tert*-Butyl-*N*-(3-(4-(3-(4-chlorophenylsulfonylamido)propyl)-piperazin-1-*yl*)-propyl)benzenesulfonamide (128)

($C_{26}H_{39}ClN_4O_4S_2$; M.W.= 571.2)



General procedure 9;

White solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.44.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 97:3 v/v).

Yield: 0.34 g (60%)

Melting Point: 130-132°C

MS $(ESI)^+$: 571.2, 573.2 $[M+H]^+$

Microanalysis: Calculated for $C_{26}H_{39}ClN_4O_4S_2$ (571.2); Theoretical: %C = 54.67, %H =

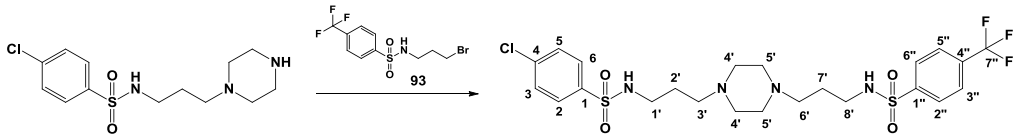
6.88, %N = 9.80; Found: %C = 54.51, %H = 6.41, %N = 9.61.

^{1}H-NMR (DMSO-d$_6$), δ: 1.30 (s, 9H, H-8''), 1.41-1.53 (m, 4H, H-2', 7'), 2.11-2.35 (m, 12H, CH_2), 2.72-2.80 (m, 4H, CH_2), 7.49 (t, J= 5.5 Hz, 1H, NH), 7.60 (d, J= 8.5 Hz, 2H, H-aromatic), 7.65-7.72 (m, 5H, 4H-aromatic, NH), 7.78 (d, J= 8.5 Hz, 2H, H-aromatic).

^{13}C-NMR (DMSO-d$_6$), δ: 26.03 (CH_2), 30.79 (CH_3, C-8''), 34.77 (C, C-7''), 40.84, 40.88, 52.55, 52.60, 54.75, 54.82 (CH_2), 125.93, 126.35, 128.42, 129.31 (CH, C-aromatic), 137.13, 137.68, 139.40, 155.19 (C, C-aromatic).

4-Chloro-*N*-(3-(4-(3-(4-(trifluoromethyl)phenylsulfonylamido)propyl)-piperazin-1-*yl*)-propyl)benzenesulfonamide (129)

($C_{23}H_{30}ClF_3N_4O_4S_2$; M.W.= 583.1)



139 **129**

General procedure 9;

White solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.61.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 96:4 v/v).

Yield: 0.49 g (85%)

Melting Point: 158-160°C

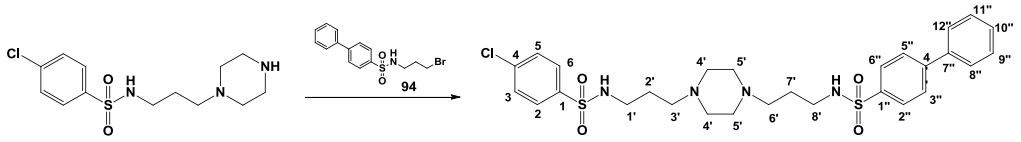
MS (ESI)$^+$: 583.1, 585.1 $[M+H]^+$

^{1}H-NMR (DMSO-d$_6$), δ: 1.44-1.51 (m, 4H, H-2', 7'), 2.11-2.27 (m, 12H, CH_2), 2.74-2.83 (m, 4H, CH_2), 7.67 (d, J= 8.5 Hz, 2H, H-aromatic), 7.69 (bs, 1H, NH), 7.78 (d, J= 8.5 Hz, 2H, H-aromatic), 7.86 (bs, NH), 7. 94-8.03 (m, 4H, H-aromatic).

^{13}C-NMR (DMSO-d$_6$), δ: 26.02, 40.84, 52.56, 54.65, 54.72 (CH_2), 126.38, 126.41, 126.44 (CH, C-3'', 6''), 127.44, 128.41, 129.29 (CH, C-aromatic), 131.95, 131.98, 137.13, 139.40, 144.50 (C, C-aromatic).

_N_-(3-(4-(3-(4-Chlorophenylsulfonylamido)propyl)-piperazin-1-_yl_)-propyl)biphenyl-4-sulfonamide (130)

($C_{28}H_{35}ClN_6O_8S_2$; M.W.= 591.2)



139 **130**

General procedure 9;

White solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.63.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 96:4 v/v).

Yield: 0.42 g (71%)

Melting Point: 138-140°C

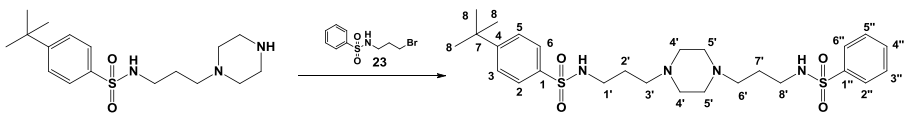
MS $(ESI)^+$: 591.1, 593.1 $[M+H]^+$

^{1}H-NMR (DMSO-d_6), δ: 1.42-1.54 (m, 4H, H-2', 7'), 2.15-2.31 (m, 12H, CH_2), 2.72-2.81 (m, 4H, CH_2), 7.44 (t, J= 5.7 Hz, 1H, NH), 7.50-7.54 (m, 2H, H-aromatic), 7.62 (t, J= 5.7 Hz, NH), 7.65-7.79 (m, 3H, H-aromatic), 7.74 (d, J= 7.1 Hz, 2H, H-aromatic), 7.78 (d, J= 8.6 Hz, 2H, H-aromatic), 7.85 (d, J= 8.6 Hz, 2H, H-aromatic), 7.89 (d, J= 8.6 Hz, 2H, H-aromatic).

^{13}C-NMR (DMSO-d_6), δ: 26.01, 26.07, 40.84, 40.92, 52.58, 54.73, 54.82 (CH_2), 127.02, 127.15, 127.35, 128.42, 129.08, 129.30 (CH, C-aromatic), 137.13, 138.54, 139.26, 139.39, 143.80 (C, C-aromatic).

4-_tert_-Butyl-_N_-(3-(4-(3-(phenylsulfonylamido)propyl)-piperazin-1-_yl_)-propyl)benzenesulfonamide (131)

($C_{26}H_{40}N_4O_4S_2$; M.W.= 536.7)



142 **131**

General procedure 9;

White solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.55.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 96:4 v/v).

Yield: 0.29 g (54%)

Melting Point: 134-136°C

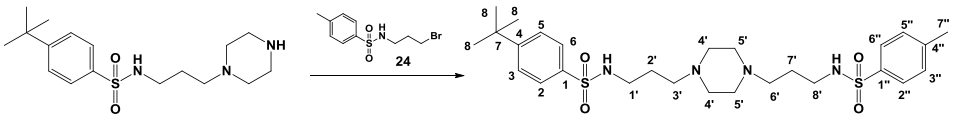
MS $(ESI)^+$: 537.2 $[M+H]^+$

^{1}H-NMR (DMSO-d$_6$), δ: 1.30 (s, 9H, H-8), 1.43-1.49 (m, 4H, H-2', 7'), 2.11-2.26 (m, 12H, CH_2), 2.72-2.78 (m, 4H, CH_2), 7.49 (t, J= 5.6 Hz, 1H, NH), 7.58-7.62 (m, 5H, 4H-aromatic, NH), 7.66-7.69 (m, 1H, H-aromatic), 7.70 (d, J= 8.5 Hz, 2H, H-aromatic), 7.78 (d, J= 7.8 Hz, 2H, H-aromatic).

^{13}C-NMR (DMSO-d$_6$), δ: 26.03, 26.06 (CH_2, H-2', 7'), 30.79 (CH_3, C-8), 34.77 (C, C-7), 40.88, 40.92, 52.56, 52.60, 54.82, 54.86 (CH_2), 125.93, 126.35, 126.41, 129.15, 132.26 (CH, C-aromatic), 137.68, 140.51, 155.20 (C, C-aromatic).

4-*tert*-Butyl-*N*-(3-(4-(3-(4-methylphenylsulfonylamido)propyl)-piperazin-1-*yl*)-propyl)benzenesulfonamide (132)

($C_{26}H_{42}N_4O_4S_2$; M.W.= 550.7)



142 **132**

General procedure 9;

White solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.64.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 96:4 v/v).

Yield: 0.46 g (83%)

Melting Point: 155-157°C

MS $(ESI)^+$: 573.2 $[M+H]^+$

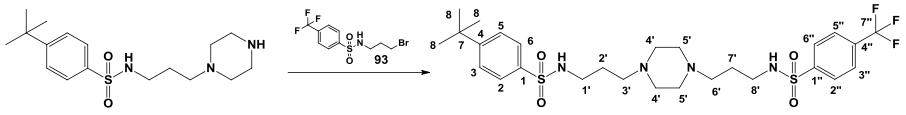
^{1}H-NMR (DMSO-d$_6$), δ: 1.30 (s, 9H, H-8), 1.43-1.49 (m, 4H, H-2', 7'), 2.09-2.24 (m,

12H, CH_2), 2.38 (CH_3, C-7''), 2.70-2.78 (m, 4H, CH_2), 7.39 (d, J= 8.1 Hz, 2H, H-aromatic), 7.49 (bs, 2H, NH), 7.60 (d, J= 8.5 Hz, 2H, H-aromatic), 7.66 (d, J= 8.1 Hz, 2H, H-aromatic), 7.70 (d, J= 8.5 Hz, 2H, H-aromatic).

^{13}C-NMR (DMSO-d$_6$), δ: 20.91 (CH_3, C-7''), 26.04 (CH_2), 30.79 (CH_3, C-8), 34.77 (C, C-7), 40.89, 40.92, 52.56, 52.61, 54.84, 54.90 (CH_2), 125.93, 126.35, 126.50, 129.55 (CH, C-aromatic), 137.63, 137.69, 142.44, 155.18 (C, C-aromatic).

4-*tert*-Butyl-*N*-(3-(4-(3-(4-(trifluoromethyl)phenylsulfonylamido)propyl)-piperazin-1-*yl*)-propyl)benzenesulfonamide (133)

($C_{27}H_{39}F_3N_4O_4S_2$; M.W.= 604.7)



142 **133**

General procedure 9;

White solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.76.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 95:5 v/v).

Yield: 0.42 g (70%)

Melting Point: 156-158°C

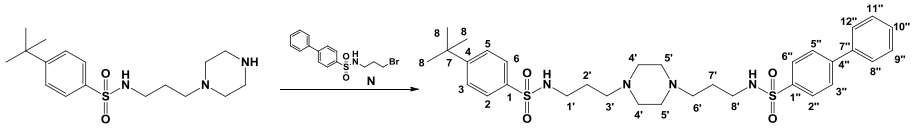
MS $(ESI)^+$: 605.2 $[M+H]^+$

^{1}H-NMR (DMSO-d$_6$), δ: 1.30 (s, 9H, H-8), 1.42-1.51 (m, 4H, H-2', 7'), 2.09-2.26 (m, 12H, CH_2), 2.73-2.77 (m, 2H, CH_2), 2.78-2.82 (m, 2H, CH_2), 7.49 (t, J= 5.5 Hz, 1H, NH), 7.60 (d, J= 8.4 Hz, 2H, H-aromatic), 7.70 (d, J= 8.4 Hz, 2H, H-aromatic), 7.86 (bs, 1H, NH), 7.97-9.01 (m, 4H, H-aromatic).

^{13}C-NMR (DMSO-d$_6$), δ: 26.02 (CH_2), 30.78 (CH_3, C-8), 34.76 (C, C-7), 40.81, 40.87, 52.53, 52.56, 54.64, 54.80 (CH_2), 125.92, 126.35 (CH, C-aromatic), 126.41, 126.44 (CH, C-3'', 6''), 127.44 (CH, C-aromatic), 132.20, 137.68, 144.50, 155.19 (C, C-7'', C-aromatic).

***N*-(3-(4-(3-(4-*tert*-Butylphenylsulfonylamido)propyl)-piperazin-1-*yl*)-propyl) biphenyl-4-sulfonamide (134)**

($C_{34}H_{44}N_4O_4S_2$; M.W.= 612.8)



142 **134**

General procedure 9;

White solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.62.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 97:3 v/v).

Yield: 0.36 g (58%)

Melting Point: 146-148°C

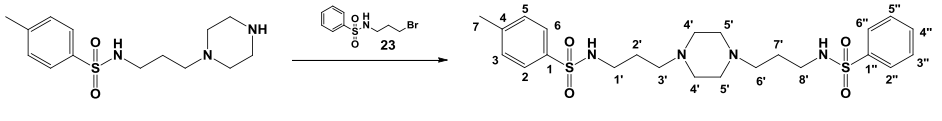
MS $(ESI)^+$: 613.2 $[M+H]^+$

^{1}H-NMR (DMSO-d$_6$), δ: 1.29 (s, 9H, H-8), 1.41-1.53 (m, 4H, H-2', 7'), 2.07-2.25 (m, 12H, CH_2), 2.71-2.83 (m, 4H, CH_2), 7.44 (t, J= 7.5 Hz, 1H, H-10''), 7.47-7.53 (m, 3H, 2H-aromatic, NH), 7.58-7.64 (m, 3H, 2H-aromatic, NH), 7.70 (d, J= 8.5 Hz, 2H, H-aromatic), 7.74 (d, J= 7.2 Hz, 2H, H-aromatic), 7.85 (d, J= 8.5 Hz, 2H, H-aromatic), 7.89 (d, J= 8.5 HZ, 2H, H-aromatic).

^{13}C-NMR (DMSO-d$_6$), δ: 26.01, 26.07 (CH_2, C-2', 7'), 30.78 (CH_3, C-8), 34.76 (C, C-7), 40.87, 40.92, 52.55, 52.59, 54.82 (CH_2), 125.92, 126.35, 127.01, 127.15, 127.34, 128.42, 129.08 (CH, C-aromatic), 137.68, 138.53, 139.26, 143.79, 155.19 (C, C-aromatic).

4-Methyl-*N*-(3-(4-(3-(phenylsulfonylamido)propyl)-piperazin-1-*yl*)-propyl)benzene sulfonamide (135)

($C_{23}H_{34}N_4O_4S_2$; M.W.= 494.6)



141 **135**

General procedure 5;

White solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.65.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 95:5 v/v).

Yield: 0.26 g (52%)

Melting Point: 137-139°C

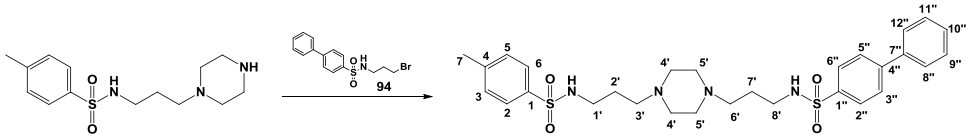
MS $(ESI)^+$: 495.2 $[M+H]^+$

^{1}H-NMR (DMSO-d$_6$), δ: 1.43-1.51 (m, 4H, H-2', 7'), 2.13-2.26 (m, 12H, CH_2), 2.38 (s, 3H, H-7), 2.70-2.79 (m, 4H, CH_2), 7.39 (d, J= 8.1 Hz, 2H, H-aromatic), 7.48 (t, J= 5.4 Hz, 1H, NH), 7.56-7.62 (m, 3H, 2H-aromatic, NH), 7.66 (d, J= 8.1 Hz, 2H, H-aromatic), 7.61-7.64 (m, 1H, H-aromatic), 7.78 (d, J= 7.5 Hz, 2H, H-aromatic).

^{13}C-NMR (DMSO-d$_6$), δ: 20.91 (CH_3, C-7), 26.01, 26.05 (CH_2, C-2', 7'), 40.93, 52.58, 54.87 (CH_2), 126.41, 126.50, 129.15, 129.55, 132.26 (CH, C-aromatic), 137.62, 140.51, 142.46 (C, C-aromatic).

***N*-(3-(4-(3-(4-Methylphenylsulfonylamido)propyl)-piperazin-1-*yl*)-propyl)biphenyl-4-sulfonamide (136)**

($C_{29}H_{38}N_4O_4S_2$; M.W.= 570.77)



141 **136**

General procedure 9;

White solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.58.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 96:4 v/v).

Yield: 0.38 g (67%)

Melting Point: 134-136°C

MS $(ESI)^+$: 571.2 $[M+H]^+$

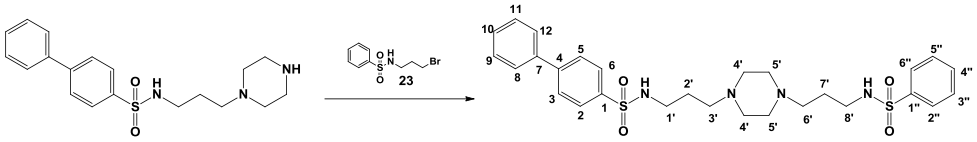
^{1}H-NMR (DMSO-d$_6$), δ: 1.42-1.54 (m, 4H, H-2', 7'), 2.13-2.25 (m, 12H, CH_2), 2.37

(s, 3H, H-7), 2.69-2.74 (m, 2H, CH_2), 2.78-2.83 (m, 2H, CH_2), 7.38 (d, J= 8.1 Hz, 2H, H-aromatic), 7.44 (t, J= 7.3 Hz, 1H, H-10''), 7.47 (t, J= 5.5 Hz, 1H, NH), 7.49-7.53 (m, 2H, H-aromatic), 7.63 (t, J= 5.5 Hz, 1H, NH), 7.66 (d, J= 8.2 Hz, 2H, H-aromatic), 7.72-7.75 (m, 2H, H-aromatic), 7.86 (d, J= 8.5 Hz, 2H, H-aromatic), 7.89 (d, J= 8.5 Hz, 2H, H-aromatic).

^{13}C-NMR (DMSO-d$_6$), δ: 20.91 (CH_3, C-7), 26.00, 26.06 (CH_2, C-2', 7'), 40.91, 52.58, 54.82, 54.87 (CH_2), 126.50, 127.01, 127.16, 127.34, 128.43, 129.08, 129.55 (CH, C-aromatic), 137.61, 138.53, 139.26, 142.46, 143.80 (C, C-aromatic).

N-(3-(4-(3-(Phenylsulfonamido)propyl)-piperazin-1-*yl*)-propyl)biphenyl-4-sulfonamide (137)

($C_{28}H_{36}N_4O_4S_2$; M.W.= 556.7)



General procedure 9;

White solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.47.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 97:3 v/v).

Yield: 0.39 g (71%)

Melting Point: 124-126°C

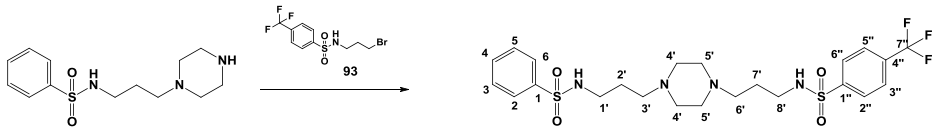
MS (ESI)$^+$: 557.2 $[M+H]^+$

^{1}H-NMR (DMSO-d$_6$), δ: 1.42-1.54 (m, 4H, H-2', 7'), 2.11-2.25 (m, 12H, CH_2), 2.73-2.77 (m, 2H, CH_2), 2.78-2.82 (m, 2H, CH_2), 7.44 (t, J= 7.3 Hz, 1H, H-aromatic), 7.50-7.53 (m, 2H, H-aromatic, NH), 7.56-7.61 (m, 3H, 2H-aromatic, NH), 7.61-7.66 (m, 2H, H-aromatic), 7.70 (d, J= 7.4 Hz, 2H, H-aromatic), 7.77-7.79 (m, 2H, H-aromatic), 7.85 (d, J= 8.5 Hz, 2H, H-aromatic), 7.89 (d, J= 8.5 Hz, 2H, H-aromatic).

^{13}C-NMR (DMSO-d$_6$), δ: 26.04, 40.92, 52.58, 54.82, 54.85 (CH_2), 126.41, 127.02, 127.15, 127.35, 128.43, 129.08, 129.14, 132.26 (CH, C-aromatic), 138.54, 139.26, 142.50, 143.80 (C, C-aromatic).

***N*-(3-(4-(3-(Phenylsulfonamido)propyl)-piperazin-1-*yl*)-propyl)-4-(trifluoromethyl) benzenesulfonamide (138)**

($C_{23}H_{31}F_3N_6O_4S_2$; M.W.= 548.6)



140 **138**

General procedure 9;

White solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.72.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 94:6 v/v).

Yield: 0.27 g (50%)

Melting Point: 119-121°C

MS $(ESI)^+$: 549.2 $[M+H]^+$

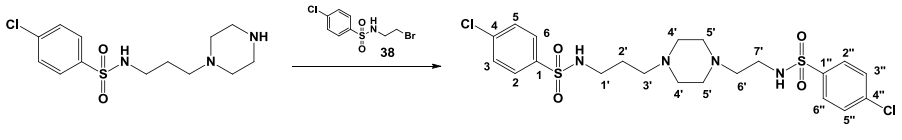
^{1}H-NMR (DMSO-d$_6$), δ: 1.43-1.51 (m, 4H, H-2', 7'), 2.11-2.23 (m, 12H, C<u>H</u>$_2$), 2.73-2.77 (m, 2H, C<u>H</u>$_2$), 2.78-2.82 (m, 2H, C<u>H</u>$_2$), 7.58-7.61 (m, 4H, 3H-aromatic, N<u>H</u>), 7.62-7.66 (m, 2H, 2H-aromatic), 7.77-7.79 (m, 2H, H-aromatic), 7.88 (bs, 1H, N<u>H</u>), 8.00 (s, 4H, H-aromatic).

^{13}C-NMR (DMSO-d$_6$), δ: 26.03, 40.81, 40.91, 52.53, 54.63, 54.82 (CH_2), 126.41, 126.45, 127.44, 129.14, 132.26 (CH, C-aromatic), 132.20, 137.68, 144.50 (C, C-7'', C-aromatic).

6.2.15 *N*-(3-(4-(2-(Arylsulfonamido)ethyl)piperazin-1-*yl*)propyl)arylsulfonamides (144-146)

4-Chloro-*N*-(3-(4-(2-(4-chlorophenylsulfonamido)ethyl)-piperazin-1-*yl*)-propyl) benzenesulfonamide (144)

($C_{21}H_{28}Cl_2N_4O_4S_2$; M.W.= 535.5)



139 **144**

General procedure 10;

White solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.46.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 97:3 v/v).

Yield: 0.38 g (72%)

Melting Point: 133-135°C

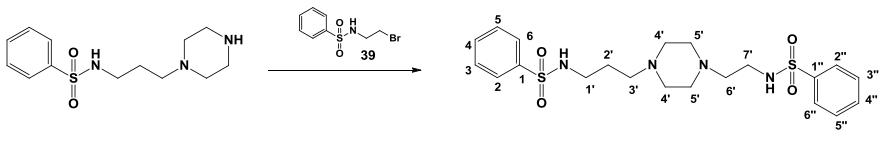
MS $(ESI)^+$: 535.1, 537.1 $[M+H]^+$

^{1}H-NMR (DMSO-d$_6$), δ: 1.43-1.51 (m, 2H, H-2'), 2.07-2.27 (m, 12H, CH_2), 2.73-2.80 (m, 2H, CH_2), 2.82-2.87 (m, 2H, CH_2), 7.58-7.73 (m, 6H, 4H-aromatic, 2NH), 7.76-7.83 (m, 4H, H-aromatic).

^{13}C-NMR (DMSO-d$_6$), δ: 26.00, 40.05, 40.82, 52.40, 52.56, 54.72, 56.78 (CH_2), 128.40, 128.42, 129.24, 129.30 (CH, C-aromatic), 137.07, 137.13, 139.38, 139.60 (C, C-1, 4, 4'').

***N*-(3-(4-(2-(Phenylsulfonamido)ethyl)-piperazin-1-*yl*)-propyl) benzenesulfonamide (145)**

($C_{21}H_{30}N_4O_4S_2$; M.W.= 466.6)



140 **145**

General procedure 10;

White solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.69.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 97:3 v/v).

Yield: 0.22 g (47%)

Melting Point: 135-137°C

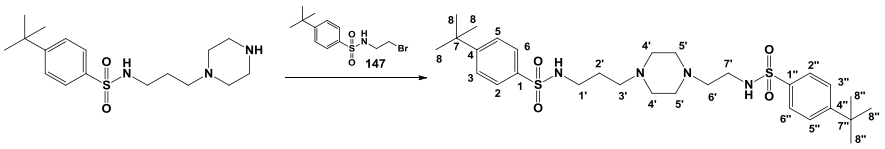
MS $(ESI)^+$: 467.1 $[M+H]^+$

^{1}H-NMR (DMSO-d$_6$), δ: 1.43-1.49 (m, 2H, H-2'), 2.14-2.28 (m, 12H, CH_2), 2.73-2.78 (m, 2H, CH_2), 2.81-2.86 (m, 2H, CH_2), 7.49 (bs, 1H, NH), 7.55-7.62 (m, 5H, 4H-aromatic, NH), 7.61-7.66 (m, 2H, H-aromatic), 7.76-7.82 (m, 4H, H-aromatic).

^{13}C-NMR (DMSO-d$_6$), δ: 26.04, 40.07, 40.91, 52.43, 52.58, 54.84, 56.75 (CH_2), 126.41, 129.12, 129.15, 132.27 (CH, C-aromatic), 140.49, 140.59 (C, C-4, 4'').

4-*tert*-Butyl-*N*-(3-(4-(2-(4-*tert*-butylphenylsulfonamido)ethyl)-piperazin-1-*yl*)-propyl) benzenesulfonamide (145)

($C_{29}H_{46}N_4O_4S_2$; M.W.= 578.8)



147 **145**

General procedure 10;

Pale yellow solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.55.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 96:4 v/v).

Yield: 0.31 g (53%)

Melting Point: 86-88°C

MS $(ESI)^+$: 579.2 $[M+H]^+$

^{1}H-NMR (DMSO-d$_6$), δ: 1.30 (s, 9H, H-tBu), 1.31 (s, 9H, H-tBu), 1.41-1.49 (m, 2H, H-2'), 2.05-2.21 (m, 12H, CH_2), 2.71-2.77 (m, 2H, CH_2), 2.78-2.84 (m, 2H, CH_2), 7.40 (bs, 1H, NH), 7.49 (t, J= 5.2 Hz, 1H, NH), 7.58-7.62 (m, 4H, H-aromatic), 7.68-7.73

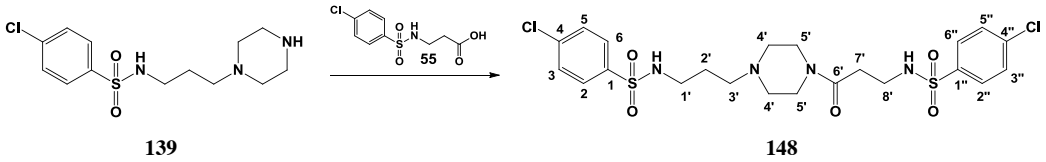
(m, 4H, H-aromatic).

^{13}C-NMR (DMSO-d_6), δ: 26.01 (CH_2, H-2'), 30.79 (CH_3, C-8, 8''), 39.02 (C, C-7, 7''), 40.08, 40.86, 52.41, 52.59, 54.81, 56.74 (CH_2), 125.90, 125.91, 125.92, 126.34 (CH, C-aromatic), 137.68, 137.74, 155.18 (C, C-aromatic).

6.2.16 *N*-(3-(4-(3-(Arylsulfonamido)propanoyl)piperazin-1-*yl*)-3-oxopropyl) aryl sulfonamides (148-152)

4-Chloro-*N*-(3-(4-(3-(4-chlorophenylsulfonamido)propanoyl)piperazin-1-*yl*)-3-oxopropyl)benzenesulfonamide (148)

($C_{22}H_{28}Cl_2N_4O_5S_2$; M.W.= 563.5)



139 **148**

General procedure 11;

White solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.48.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 97:3 v/v).

Yield: 0.26 g (46%)

Melting Point: 72-74°C

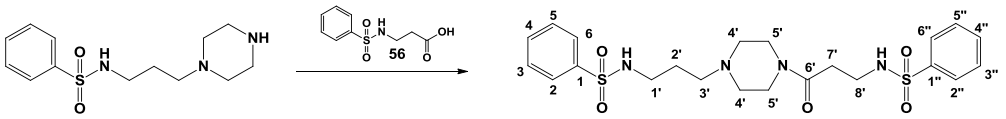
MS $(ESI)^+$: 563.1, 565.1 $[M+H]^+$

^{1}H-NMR ($CDCl_3$), δ: 1.64-1.70 (m, 2H, H-2'), 2.37-2.34 (m, 6H, CH_2), 2.54 (t, J= 5.2 Hz, 2H, CH_2), 3.07 (t, J= 5.7 Hz, 2H, CH_2), 3.18-3.24 (m, 2H, CH_2), 3.37-3.41 (m, 2H, CH_2), 3.54-3.60 (m, 2H, CH_2), 5.84 (bs, 1H, NH), 6.63 (bs, 1H, NH), 7.47-7.51 (m, 4H, H-aromatic), 7.78-7.82 (m, 4H, H-aromatic).

^{13}C-NMR ($CDCl_3$), δ: 24.74, 32.80, 39.22, 41.50, 43.32, 45.09, 52.61, 52.75, 57.08 (CH_2), 128.41, 128.45, 129.39, 129.43 (CH, C-aromatic), 138.72, 138.74, 138.96, 138.98 (C, C-aromatic), 169.41 (C, C-6').

_N_-(3-(4-(3-(Phenylsulfonamido)propanoyl)piperazin-1-_yl_)-3-oxopropyl)benzene sulfonamide (149)

($C_{22}H_{30}N_4O_5S_2$; M.W.= 494.6)



140 **149**

General procedure 11;

Colourless oil;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.54.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 97:3 v/v).

Yield: 0.18 g (37%)

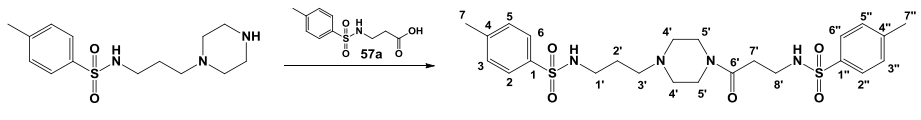
MS $(ESI)^+$: 495.1 $[M+H]^+$

^{1}H-NMR ($CDCl_3$), δ: 1.59-1.64 (m, 2H, H-2'), 2.29 (t, J= 4.7 Hz, 2H, CH_2), 2.32 (t, J= 4.7 Hz, 2H, CH_2), 2.37 (t, J= 6.0 Hz, 2H, CH_2), 2.49 (t, J= 5.6 Hz, 2H, CH_2), 3.04 (t, J= 6.0 Hz, 2H, CH_2), 3.17-3.21 (m, 2H, CH_2), 3.33 (t, J= 4.7 Hz, 2H, CH_2), 3.50-3.54 (m, 2H, CH_2), 5.80 (t, J= 6.2 Hz, 1H, NH), 6.66 (bs, 1H, NH), 7.48-7.52 (m, 4H, H-aromatic), 7.54-7.58 (m, 2H, H-aromatic), 7.82-7.86 (m, 4H, H-aromatic).

^{13}C-NMR ($CDCl_3$), δ: 24.80, 32.76, 39.22, 41.44, 43.16, 45.05, 52.54, 52.74, 56.87 (CH_2), 126.88, 126.89, 129.11, 129.16, 132.55, 132.59 (CH, C-aromatic), 140.11, 140.21 (C, C-aromatic), 169.43 (C, C-6').

4-Methyl-_N_-(3-(4-(3-(4-methylphenylsulfonamido)propanoyl)piperazin-1-_yl_)-3-oxopropyl)benzenesulfonamide (150)

($C_{24}H_{34}N_4O_5S_2$; M.W.= 522.6)



141 **150**

General procedure 11;

White solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.64.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 97:3 v/v).

Yield: 0.17 g (34%)

Melting Point: 149-151°C

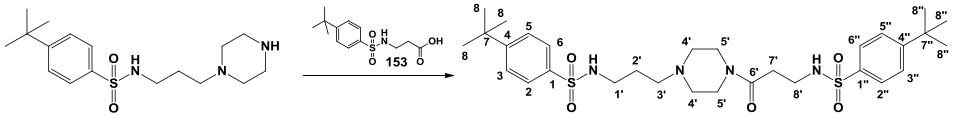
MS $(ESI)^+$: 523.2 $[M+H]^+$

^{1}H-NMR ($CDCl_3$), δ: 1.65-1.70 (m, 2H, H-2'), 2.36-2.42 (m, 6H, CH_2), 2.44 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 2.54 (t, J= 5.5 Hz, 2H, CH_2), 3.10 (t, J= 5.8 Hz, 2H, CH_2), 3.20-3.24 (m, 2H, CH_2), 3.40 (t, J= 4.7 Hz, 2H, CH_2), 3.59 (t, J= 4.7 Hz, 2H, CH_2), 5.52 (t, J= 6.7 Hz, 1H, NH), 6.40 (bs, 1H, NH), 7.31-7.35 (m, 4H, H-aromatic), 7.75 (d, J= 8.3 Hz, 2H, H-aromatic), 7.77 (d, J= 8.3 Hz, 2H, H-aromatic).

^{13}C-NMR ($CDCl_3$), δ: 21.20, 21.46 (CH_3, C-7, 7''), 24.61, 32.85, 39.16, 41.49, 43.56, 45.07, 52.72, 52.78, 57.42 (CH_2), 126.93, 126.99, 129.67, 129.74 (CH, C-aromatic), 137.10, 137.18, 143.27, 143.28 (C, C-aromatic), 169.45 (C, C-6').

4-*tert*-Butyl-*N*-(3-(4-(3-(4-*tert*-butylphenylsulfonamido)propanoyl)piperazin-1-*yl*)-3-oxopropyl)benzenesulfonamide (151)

($C_{30}H_{46}N_4O_5S_2$; M.W.= 606.8)



142 **151**

General procedure 11;

White solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.61.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 97:3 v/v).

Yield: 0.28 g (47%)

Melting Point: 189-191°C

MS $(ESI)^+$: 607.3 $[M+H]^+$

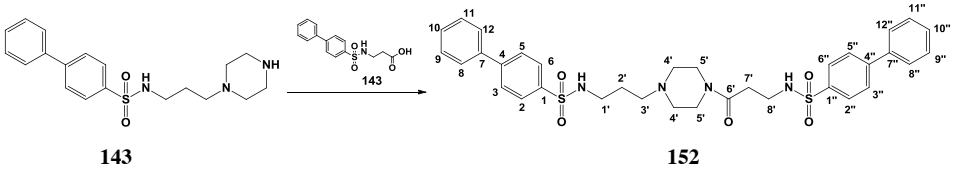
Microanalysis: Calculated for $C_{30}H_{46}N_4O_5S_2$ (606.8); Theoretical: %C = 59.38, %H = 7.64, %N = 9.23; Found: %C = 59.22, %H = 8.08, %N = 9.04.

^{1}H-NMR ($CDCl_3$), δ: 1.36 (s, 9H, H-tBu), 1.37 (s, 9H, H-tBu), 1.66-1.71 (m, 2H, H-2'), 2.37 (t, J= 5.0 Hz, 2H, CH_2), 2.42 (t, J= 5.0 Hz, 2H, CH_2), 2.46 (t, J= 5.9 Hz, 2H, CH_2), 2.57 (t, J= 5.6 Hz, 2H, CH_2), 3.11 (t, J= 5.9 Hz, 2H, CH_2), 3.21-3.25 (m, 2H, CH_2), 3.42 (t, J= 5.0 Hz, 2H, CH_2), 3.60 (t, J= 5.0 Hz, 2H, CH_2), 5.54 (t, J= 6.6 Hz, 1H, NH), 6.28 (bs, 1H, NH), 7.51-7.55 (m, 4H, H-aromatic), 7.78 (d, J= 8.5 Hz, 2H, H-aromatic), 7.80 (d, J= 8.5 Hz, 2H, H-aromatic).

^{13}C-NMR ($CDCl_3$), δ: 24.76 (CH_2, C-2'), 31.11 (CH_3, C-8, 8''), 32.97 (CH_2), 35.12 (C, C-7, 7''), 39.19, 41.49, 43.43, 45.08, 52.72, 52.82, 57.29 (CH_2), 126.03, 126.12, 126.80 (CH, C-aromatic), 137.05, 137.12, 156.29, 156.32 (C, C-aromatic), 169.46 (C, C-6').

N-(3-(4-(3-(Biphenyl-4-ylsulfonamido)propanoyl)piperazin-1-*yl*)-3-oxopropyl) biphenyl-4-sulfonamide (152)

($C_{34}H_{38}N_4O_5S_2$; M.W.= 646.8)



General procedure 11;

White solid;

T.L.C. System: DCM-MeOH 9:1 v/v, Rf: 0.54.

Purification: flash column chromatography (DCM:MeOH 100:0 v/v increasing to 97:3 v/v).

Yield: 0.30 g (47%)

Melting Point: 176-178°C

MS $(ESI)^+$: 647.2 $[M+H]^+$

^{1}H-NMR ($CDCl_3$), δ: 1.65-1.71 (m, 2H, H-2'), 2.35-2.39 (m, 4H, CH_2), 2.42 (t, J= 5.8 Hz, 2H, CH_2), 2.56 (t, J= 5.5 Hz, 2H, CH_2), 3.13 (t, J= 5.8 Hz, 2H, CH_2), 3.25-3.29 (m, 2H, CH_2), 3.40 (t, J= 4.6 Hz, 2H, CH_2), 3.59-3.61 (m, 2H, CH_2), 5.75 (t, J= 6.3 Hz, 1H, NH), 6.53 (bs, 1H, NH), 7.40- 7.46 (m, 2H, H-aromatic), 7.47-7.51 (m, 4H, H-aromatic), 7.60-7.64 (m, 4H, H-aromatic), 7.71-7.75 (m, 4H, H-aromatic), 7.91-7.96 (m, 4H, H-aromatic).

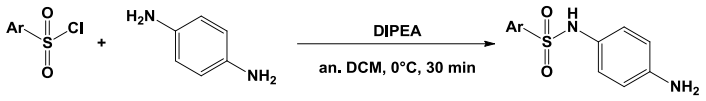
^{13}C-NMR ($CDCl_3$), δ: 24.77, 32.85, 39.28, 41.52, 43.42, 45.11, 52.70, 52.75, 57.16

(CH_2), 127.28, 127.31, 127.48, 127.50, 127.68, 128.06, 128.46, 128.53, 129.06, 129.10 (CH, C-aromatic), 138.75, 138.80, 139.24, 139.33, 145.44, 145.46 (C, C-aromatic), 169.46 (C, C-6').

6.3 Synthesis of *p*-phenylendiamine and ethylendiamine structures

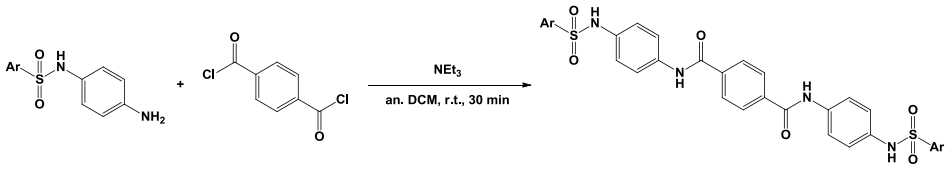
6.3.1 General procedures 12-15

General procedure 12: synthesis of *N*-(4-amino-phenyl)-arylsulfonamides



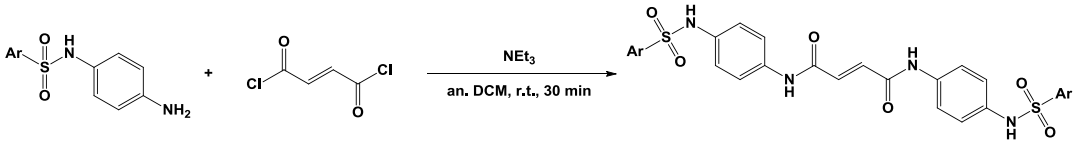
Arylsulfonyl chloride (5 mmol) and p-phenylendiamine (**123**) (1.62 g, 15 mmol) were suspended in 21 mL of anhydrous DCM under nitrogen atmosphere. The reaction mixture was cooled down to 0°C in an ice-bath and then treated dropwise with N,N-diisopropylethylamine (4.15 mL, 25 mmol) over a period of 10 min. The reaction was stirred for 30 min. under ice-cooling, then diluted with DCM (100 mL) and washed with water (2 x 100 mL). The organic solvent was removed under vaccum after drying over $MgSO_4$ and the crude residue was purified by flash column chromatography to give pure *N*-(4-amino-phenyl)-arylsulfonamides.

General procedure 13: synthesis of *N,N*'-bis-(4-arylsulfonylamino-phenyl)-terephthalamides



N-(4-Amino-phenyl)-arylsulfonamide (1.5 mmol) was dissolved in 6 mL of anhydrous DCM under N_2 atmosphere. Triethylamine (0.2 mL, 1.5 mmol) was added dropwise to the reaction mixture followed by terephthaloyl chloride (**167**) (0.10 g, 0.5 mmol) dissolved in 4 mL of anhydrous DCM. The reaction mixture was stirred at r.t. for 30 min., then diluted with DCM (25 mL), washed with 2M aqueous HCl solution (2 x 30 mL), saturated $NaHCO_3$ solution (2 x 30 mL), and finally with brine (30 mL). The organic phase was concentrated at reduced pressure after drying over $MgSO_4$ and the crude residue was purified by re-crystallisation from MeOH to afford pure *N,N*'-bis-(4-arylsulfonylamino-phenyl)-terephthalamides.

General procedure 14: synthesis of *N,N*'-bis-(4-arylsulfonylamino-phenyl)-fumaramides



N-(4-Amino-phenyl)-arylsulfonamide (1.5 mmol), was dissolved in 10 mL of anhydrous DCM under N_2 atmosphere. Triethylamine (0.2 mL, 1.5 mmol) was added dropwise to the reaction mixture followed by fumaryl chloride (**168**) (0.05 mL, 0.5 mmol). The reaction mixture was stirred at r.t. for 30 min., then diluted with DCM (25 mL), washed with 2M aqueous HCl solution (2 x 30 mL), saturated $NaHCO_3$ solution (2 x 30 mL), and finally with brine (30 mL). The organic phase was concentrated at reduced pressure after drying over $MgSO_4$ and the crude residue was purified by re-crystallisation from MeOH to afford pure *N,N*'-bis-(4-arylsulfonylamino-phenyl)-fumaramides.

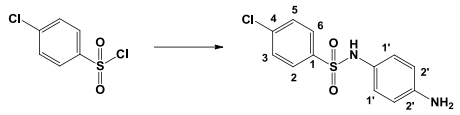
General procedure 15: synthesis of *N,N*'-bis-(4-(arylsulfonamido)phenyl)-succinamides

N-(4-Amino-phenyl)-arylsulfonamide (1.5 mmol) was dissolved in 10 mL of anhydrous DCM under N_2 atmosphere. Triethylamine (0.2 mL, 1.5 mmol) was added dropwise to the reaction mixture followed by succinyl chloride (**169**) (0.07 mL, 0.5 mmol). The reaction mixture was stirred at r.t. for 30 min, then diluted with DCM (20 mL), washed with 2M aqueous HCl solution (2 x 30 mL), saturated $NaHCO_3$ solution (2 x 30 mL), and finally with brine (30 mL). The organic phase was concentrated at reduced pressure after drying over $MgSO_4$ and the crude residue was purified by re-crystallisation from MeOH to afford pure *N,N*'-bis-(4-(arylsulfonamido)phenyl)-succinamides.

6.3.2 *N*-(4-Amino-phenyl)-arylsulfonamides (164-166, 177-181)

_N_-(4-Amino-phenyl)-4-chloro-benzenesulfonamide (164)[23]

($C_{12}H_{11}ClN_2O_2S$; M.W.= 282.7)



13 **164**

General procedure 12;

Pale yellow solid;

T.L.C. System: *n*hexane -EtOAc 3:7 v/v, Rf: 0.71.

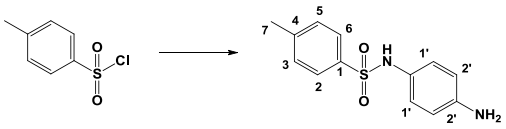
Yield: 1.06 g (75%)

^{1}H-NMR (CD_3OD), δ: 6.58 (d, J= 8.8 Hz, 2H, H-aromatic), 6.77 (d, J= 8.8 Hz, 2H, H-aromatic), 7.48 (d, J= 8.8 Hz, 2H, H-aromatic), 7.63 (d, J= 8.8 Hz, 2H, H-aromatic).

^{13}C-NMR (CD_3OD), δ: 116.66, 126.58 (CH, C-aromatic), 128.22 (C, C-aromatic), 130.05 (CH, C-aromatic), 139.75, 139.82, 147.40 (C, C-aromatic).

_N_-(4-Amino-phenyl)-4-methylbenzenesulfonamide (165)

($C_{13}H_{14}N_2O_2S$; M.W.= 262.3)



15 **165**

General procedure 12;

Yellow solid;

T.L.C. System: *n*hexane-EtOAc 4:6 v/v, Rf: 0.52.

Yield: 0.96 g (73%)

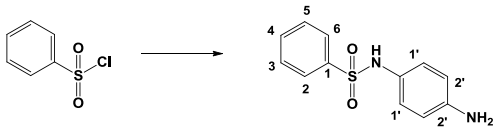
^{1}H-NMR (CD_3OD), δ: 2.38 (s, 3H, H-7), 6.56 (d, J= 8.7 Hz, 2H, H-aromatic), 6.77 (d, J= 8.7 Hz, 2H, H-aromatic), 7.26 (d, J= 8.3 Hz, 2H, H-aromatic), 7.54 (d, J= 8.3 Hz, 2H, H-aromatic).

^{13}C-NMR (CD_3OD), δ: 21.43 (CH_3, C-7), 116.66, 126.41, 128.40 (CH, C-aromatic),

128.72 (C, C-aromatic), 130.36 (CH, C-aromatic), 138.07, 144.66, 147.05 (C, C-aromatic).

***N*-(4-Amino-phenyl)-benzenesulfonamide (166)[24]**

($C_{12}H_{12}N_2O_2S$; M.W.= 248.3)



14 **166**

General procedure 12;

Pale pink solid;

T.L.C. System: *n*hexane -EtOAc 5:5 v/v, Rf: 0.35.

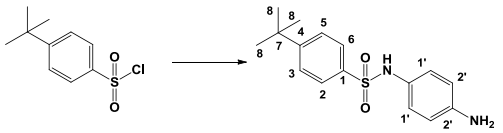
Yield: 0.95 g (77%)

^{1}H-NMR (CD_3OD), δ: 6.56 (d, J= 8.7 Hz, 2H, H-aromatic), 6.77 (d, J= 8.7 Hz, 2H, H-aromatic), 7.43-7.48 (m, 2H, H-aromatic), 7.56 (tt, J_1= 7.4 Hz, J_2= 1.2 Hz, 1H, H-4), 7.65-7.69 (m, 2H, H-aromatic).

^{13}C-NMR (CD_3OD), δ: 116.65, 126.59, 128.34 (CH, C-aromatic), 128.55 (C, C-aromatic), 129.84, 133.62 (CH, C-aromatic), 141.02, 147.17 (C, C-aromatic).

***N*-(4-Amino-phenyl)-4-*tert*-butylbenzenesulfonamide (177)**

($C_{16}H_{20}N_2O_2S$; M.W.= 304.4)



83 **177**

General procedure 12;

Yellow solid;

T.L.C. System: *n*hexane -EtOAc 3:7 v/v, Rf: 0.66.

Yield: 1.25 g (82%)

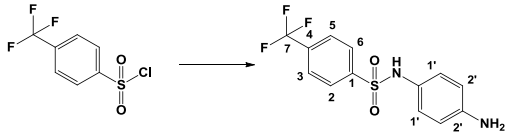
^{1}H-NMR (CD_3OD), δ: 1.33 (s, 9H, H-8), 6.57 (d, J= 8.6 Hz, 2H, H-aromatic), 6.79 (d, J= 8.6 Hz, 2H, H-aromatic), 7.50 (d, J= 8.6 Hz, 2H, H-aromatic), 7.60 (d, J= 8.6 Hz,

2H, H-aromatic).

^{13}C-NMR (CD_3OD), δ: 31.47 (CH_3, C-8), 35.97 (C, C-7), 116.69, 126.28, 126.82, 128.24 (CH, C-aromatic), 128.79, 138.12, 146.99, 157.52 (C, C-aromatic).

***N*-(4-Amino-phenyl)-4-(trifluoromethyl)benzenesulfonamide (178)[25]**

($C_{13}H_{11}F_3N_2O_2S$; M.W.= 316.3)



84 **178**

General procedure 12;

Yellow solid;

T.L.C. System: *n*hexane -EtOAc 3:7 v/v, Rf: 0.62.

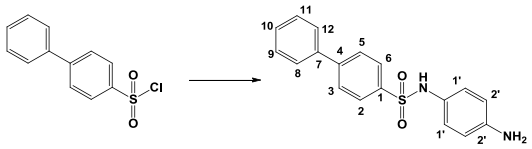
Yield: 1.47 g (93%)

^{1}H-NMR (CD_3OD), δ: 6.59 (d, J= 8.6 Hz, 2H, H-aromatic), 6.79 (d, J= 8.6 Hz, 2H, H-aromatic), 7.77 (d, J= 8.3 Hz, 2H, H-aromatic), 7.85 (d, J= 8.3 Hz, 2H, H-aromatic).

^{13}C-NMR (CD_3OD), δ: 116.88, 123.84 (CH, C-aromatic), 126.95, 126.98, 127.01 (CH, C-3, 5), 127.93 (C, C-aromatic), 129.01 (CH, C-aromatic), 134.58, 134.84, 135.10, 135.36 (C, C-7), 144.86, 147.53 (C, C-aromatic).

***N*-(4-Amino-phenyl)biphenyl-4-sulfonamide (179)[26]**

($C_{18}H_{16}N_2O_2S$; M.W.= 324.4)



85 **179**

General procedure 12;

Yellow solid;

T.L.C. System: *n*hexane -EtOAc 6:4 v/v, Rf: 0.44.

Yield: 1.11 g (69%)

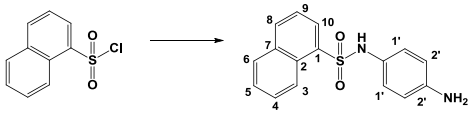
^{1}H-NMR (DMSO-d_6), δ: 4.95 (bs, 2H, $N\underline{H}_2$), 6.41 (d, J= 8.6 Hz, 2H, H-aromatic), 6.73

(d, J= 8.6 Hz, 2H, H-aromatic), 7.41-7.45 (m, 1H, H-10), 7.47-7.51 (m, 2H, H-aromatic), 7.71-7.74 (m, 4H, H-aromatic), 8.83 (d, J= 8.4 Hz, 2H, H-aromatic), 9.53 (bs, 1H, NH).

^{13}C-NMR (DMSO-d$_6$), δ: 113.99, 124.55 (CH, C-aromatic), 125.28 (C, C-aromatic), 126.99, 127.04, 127.40, 128.45, 129.06 (CH, C-aromatic), 138.37, 138.63, 143.75, 146.53 (C, C-aromatic).

***N*-(4-Aminophenyl)naphthalene-1-sulfonamide (180)[27]**

($C_{16}H_{14}N_2O_2S$; M.W.= 298.3)



86 **180**

General procedure 12;

Yellow solid;

T.L.C. System: *n*hexane -EtOAc 6:4 v/v, Rf: 0.63.

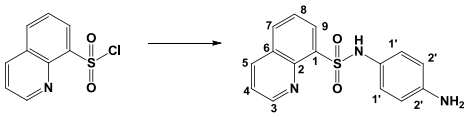
Yield: 1.25 g (84%)

^{1}H-NMR (CD$_3$OD), δ: 6.43 (d, J= 8.7 Hz, 2H, H-aromatic), 6.64 (d, J= 8.7 Hz, 2H, H-aromatic), 7.43 (t, J= 7.8 Hz, 1H, H-aromatic), 7.59-7.63 (m, 1H, H-aromatic), 7.65-7.70 (m, 1H, H-aromatic), 7.97 (d, J= 8.1 Hz, 1H, H-aromatic), 8.02-8.06 (m, 2H, H-aromatic), 8.75 (d, J= 8.4 Hz, 1H, H-aromatic).

^{13}C-NMR (CD$_3$OD), δ: 116.55, 125.11, 125.98, 126.39, 127.83 (CH, C-aromatic), 128.29 (C, C-aromatic), 128.97 (CH, C-aromatic), 129.68 (C, C-aromatic), 130.06, 131.30, 135.21 (CH, C-aromatic), 135.62, 136.04, 147.03 (C, C-aromatic).

***N*-(4-Aminophenyl)quinoline-8-sulfonamide (181)[28]**

($C_{15}H_{13}N_3O_2S$; M.W.= 299.3)



87 **181**

General procedure 12;

Yellow solid;

T.L.C. System: *n*hexane -EtOAc 6:4 v/v, Rf: 0.39.

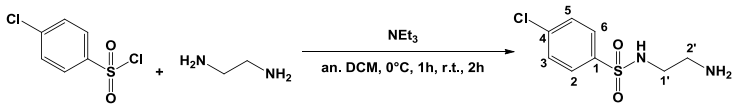
Yield: 79 g (53%)

^{1}H-NMR (DMSO-d_6), δ: 4.83 (s, 2H, N$\underline{H}_2$), 6.25 (d, J= 8.7 Hz, 2H, H-aromatic), 6.57 (d, J= 8.7 Hz, 2H, H-aromatic), 7.65 (t, J= 7.7 Hz, 1H, H-aromatic), 7.74 (dd, J_1= 8.4 Hz, J_2= 4.2 Hz, 1H, H-aromatic), 8.18 (, J_1= 7.3 Hz, J_2= 1.3 Hz, 1H, H-aromatic), 8.24 (dd, J_1= 8.2 Hz, J_2= 1.3 Hz, 1H, H-aromatic), 8.53 (dd, J_1= 8.4 Hz, J_2= 1.7 Hz, 1H, H-aromatic), 9.13 (bs, 1H, N$\underline{H}$), 9.17 (dd, J_1= 4.2 Hz, J_2= 1.7 Hz, 1H, H-aromatic).

^{13}C-NMR (DMSO-d_6), δ: 113.73, 122.54, 124.12 (CH, C-aromatic), 125.45 (C, C-aromatic), 125.59 (CH, C-aromatic), 128.27 (C, C-aromatic), 131.63, 133.74 (CH, C-aromatic), 135.49 (C, C-aromatic), 137.01 (CH, C-aromatic), 142.74, 146.33 (C, C-aromatic), 151.32 (CH, C-aromatic).

6.3.3 *N*-(2-Aminoethyl)-4-chlorobenzenesulfonamide (176)[29]

($C_8H_{11}ClN_2O_2S$; M.W.= 234.7)



13 **175** **176**

Ethylenediamine (**175**) (0.63 mL, 9.5 mmol) and triethylamine (3.27 mL, 23.7 mmol) were dissolved in 15 mL of anhydrous DCM under nitrogen atmosphere. A solution of 4-chlorobenzenesulfonyl chloride (**13**) (1g, 4.7 mmol) in 7 mL of anhydrous DCM was added dropwise under ice-cooling over a period of 10 min. The reaction mixture was stirred at 0°C for 1 h and then at r.t. for further 2 h. The reaction system was diluted with DCM (70 mL) and washed with water (2 x 100 mL) and brine (100 mL). The organic solvent was removed under vacuum after drying over $MgSO_4$ to give the title compound as a yellow solid.

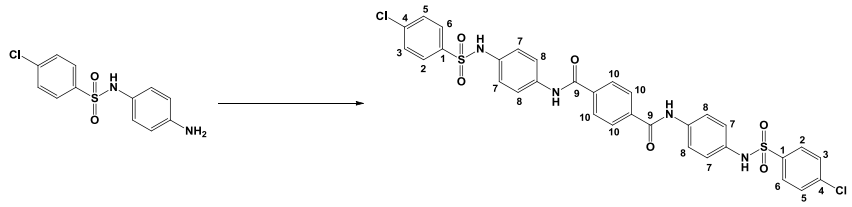
Yield: 0.65 g (59%)

^{1}H-NMR (CD_3OD), δ: 2.29 (d, J= 6.2 HZ, 2H, C<u>H</u>$_2$), 2.93 (d, J= 6.2 HZ, 2H, C<u>H</u>$_2$), 7.61 (d, J= 8.7 Hz, 2H, H-aromatic), 7.85 (d, J= 8.7 Hz, 2H, H-aromatic).

^{13}C-NMR (CD_3OD), δ: 42.23, 46.48 (CH_2, C-1', 2'), 129.74, 130.48 (CH, C-aromatic), 139.84, 140.73 (C, C-1, 4) .

6.3.4 *N,N'*-Bis-(4-arylsulfonylamino-phenyl)-terephthalamides (158, 170)

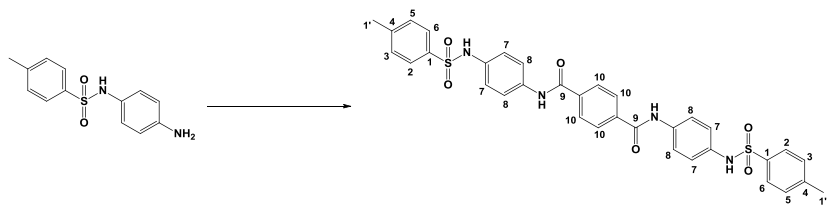
***N,N'*-Bis-[4-(4-chloro-benzenesulfonylamino)-phenyl]-terephthalamide (158)**
($C_{32}H_{24}Cl_2N_4O_6S_2$; M.W.= 695.5)



164 **158**

General procedure 13;
White solid;
Yield: 0.13 g (36%)
Melting Point: >360°C
MS $(ESI)^+$: 695.0, 697.0 $[M+H]^+$
^{1}H-NMR (DMSO-d_6), δ: 7.07 (d, J= 8.9 Hz, 4H, H-aromatic), 7.62-7.67 (m, 8H, H-aromatic), 7.73 (d, J= 8.6 Hz, 4H, H-aromatic), 8.03 (s, 4H, H-9), 10.23 (bs, 2H, NH), 10.37 (bs, 2H, NH).
^{13}C-NMR (DMSO-d_6), δ: 121.23, 121.54, 127.62, 128.62, 129.36 (CH, C-aromatic), 132.84, 135.83, 137.28, 137.70, 138.18 (C, C-aromatic), 164.58 (C, C-9).

***N,N'*-Bis-[4-(4-methylbenzenesulfonylamino)-phenyl]-terephthalamide (170)**
($C_{34}H_{30}N_4O_6S_2$; M.W.= 654.7)



165 **170**

General procedure 12;
Pale yellow solid;
Yield: 0.21 g (69%)
Melting Point: >360°C

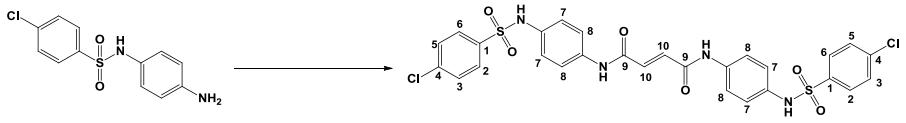
MS (ESI)$^{+}$: 677.1 $[M+Na]^{+}$

^{1}H-NMR (DMSO-d$_6$), δ: 2.34 (s, 6H, H-1'), 7.07 (d, J= 8.9 Hz, 4H, H-aromatic), 7.34 (d, J= 7.5 Hz, 4H, H-aromatic), 7.61-7.65 (m, 8H, H-aromatic), 8.03 (s, 4H, H-9), 10.10 (bs, 2H, NH), 10.30 (bs, 2H, NH).

^{13}C-NMR (DMSO-d$_6$), δ: 20-92 (CH_3, C-1'), 120.98, 121.23, 126.71, 127.58, 129.59 (CH, C-aromatic), 133.43, 135.37, 136.59, 137.27, 143.10 (C, C-aromatic), 164.52 (C, C-9).

6.3.5 *N,N'*-Bis-(4-arylsulfonylamino-phenyl)-fumaramides (160, 171-172)

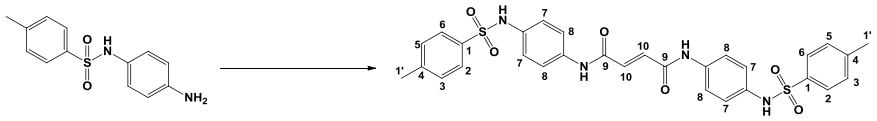
***N,N'*-Bis-[4-(4-chloro-benzenesulfonylamino)-phenyl]-fumaramide (160)**
($C_{28}H_{22}Cl_2N_4O_6S_2$; M.W.= 645.5)



164 **160**

General procedure 14;
Yellow solid;
Yield: 0.19 g (61%)
Melting Point: >360°C
MS $(ESI)^+$: 667.0, 669.0 $[M+Na]^+$
^{1}H-NMR (DMSO-d$_6$), δ: 7.05 (d, J= 8.7 Hz, 4H, H-aromatic), 7.11 (s, 2H, H-10), 7.56 (d, J= 8.7 Hz, 4H, H-aromatic), 7.62 (d, J= 8.5 Hz, 4H, H-aromatic), 7.71 (d, J= 8.5 Hz, 4H, H-aromatic), 10.19 (bs, 2H, NH), 10.42 (bs, 2H, NH).
^{13}C-NMR (DMSO-d$_6$), δ: 120.16, 121.75, 128.58, 129.36 (CH, C-aromatic, C-10), 132.88 (C, C-aromatic), 133.83 (CH, C-aromatic), 135.62, 137.69, 138.22 (C, C-aromatic), 161.82 (C, C-9).

***N,N'*-Bis-[4-(4-methylbenzenesulfonylamino)-phenyl]-fumaramide (171)**
($C_{30}H_{28}N_4O_6S_2$; M.W.= 604.6)



165 **171**

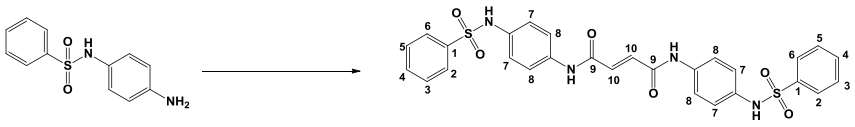
General procedure 14;
Light brown solid;
Yield: 0.14 g (47%)
Melting Point: >360°C
MS $(ESI)^+$: 605.3 $[M+H]^+$
^{1}H-NMR (DMSO-d$_6$), δ: 2.33 (s, 6H, H-1'), 7.05 (d, J= 8.3 Hz, 4H, H-aromatic), 7.10

(s, 2H, H-10), 7.33 (d, J= 7.7 Hz, 4H, H-aromatic), 7.53 (d, J= 8.3 Hz, 4H, H-aromatic), 7.61 (d, J= 7.7 Hz, 4H, H-aromatic), 10.08 (bs, 2H, NH), 10.42 (bs, 2H, NH).

^{13}C-NMR (DMSO-d$_6$), δ: 20.91 (CH_3, C-1'), 120.13, 121.18, 126.69, 129.59 (CH, C-aromatic, C-10), 133.44 (C, C-aromatic), 133.79 (CH, C-aromatic), 135.19, 136.57, 143.12 (C, C-aromatic), 161.78 (C, C-9).

***N,N'*-Bis-(4-phenylsulfonylamido)-phenyl)-fumaramide (172)**

($C_{28}H_{24}N_4O_6S_2$; M.W.= 576.6)



166 **172**

General procedure 10;

Pale yellow solid;

Yield: 0.11 g (39%)

Melting Point: 294-296°C

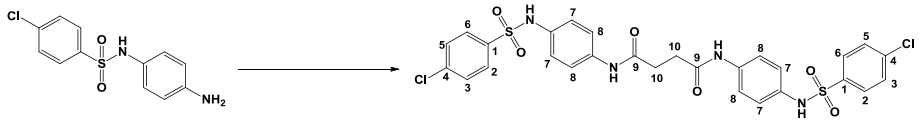
MS $(ESI)^+$: 599.0 $[M+Na]^+$

^{1}H-NMR (DMSO-d$_6$), δ: 7.05 (d, J= 8.9 Hz, 4H, H-aromatic), 7.09 (s, 2H, H-10), 7.52-7.56 (m, 8H, H-aromatic), 7.60 (tt, J_1= 7.3 Hz, J_2= 2.1 Hz, 2H, H-4), 7.73 (d, J= 8.0 Hz, 4H, H-aromatic), 10.15 (bs, 2H, NH), 10.43 (bs, 2H, NH).

^{13}C-NMR (DMSO-d$_6$), δ: 120.11, 121.37, 126.62, 129.16, 132.79 (CH, C-aromatic, C-10), 133.28 (C, C-aromatic), 133.80 (CH, C-aromatic), 135.32, 139.42 (C, C-aromatic), 161.78 (C, C-9).

6.3.6 *N,N'*-Bis-(4-(arylsulfonamido)phenyl)-succinamides (162, 173-174, 182-186)

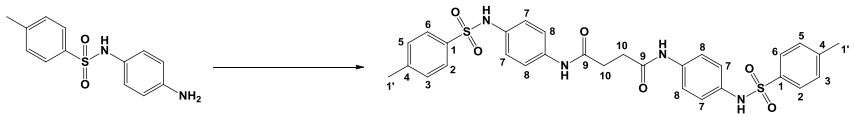
***N,N'*-Bis-(4-(4-chlorophenylsulfonamido)phenyl)succinamide (162)**
($C_{28}H_{24}Cl_2N_4O_6S_2$; M.W.= 647.5)



164 **162**

General procedure 15;
Brown solid;
Yield: 0.16 g (48%)
Melting Point: 284-286°C
MS $(ESI)^+$: 669.0, 671.0 $[M+Na]^+$
^{1}H-NMR (DMSO-d$_6$), δ: 2.54 (s, 4H, H-10), 6.97 (d, J= 8.8 Hz, 4H, H-aromatic), 7.44 (d, J= 8.8 Hz, 4H, H-aromatic), 7.61 (d, J= 8.6 Hz, 4H, H-aromatic), 7.68 (d, J= 8.6 Hz, 4H, H-aromatic), 9.92 (bs, 2H, NH), 10.08 (bs, 2H, NH).
^{13}C-NMR (DMSO-d$_6$), δ: 31.00 (CH_2, C-10), 119.59, 122.04, 128.56, 129.30 (CH, C-aromatic), 131.02, 131.86, 137.62, 138.21 (C, C-aromatic), 170.11 (C, C-9).

***N,N'*-Bis-(4-(4-methylphenylsulfonamido)phenyl)succinamide (173)**
($C_{30}H_{30}N_4O_6S_2$; M.W.= 606.7)



165 **173**

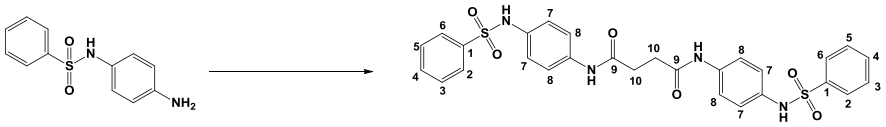
General procedure 15;
White solid;
Yield: 0.11 g (36%)
Melting Point: 284-286°C
MS $(ESI)^+$: 629.1 $[M+Na]^+$
^{1}H-NMR (DMSO-d$_6$), δ: 2.33 (6H, H-1'), 2.56 (s, 4H, H-10), 6.97 (d, J= 8.3 Hz, 4H, H-aromatic), 7.32 (d, J= 7.7 Hz, 4H, H-aromatic), 7.41 (d, J= 8.3 Hz, 4H, H-aromatic),

7.58 (d, J= 8.6 Hz, 4H, H-aromatic), 9.88 (bs, 2H, NH), 9.95 (bs, 2H, NH).

^{13}C-NMR (DMSO-d$_6$), δ: 20.91 (CH_3, C-1'), 30.99 (CH_2, C-10), 119.56, 121.47, 126.67, 129.53 (CH, C-aromatic), 132.43, 135.94, 136.57, 143.01 (C, C-aromatic), 170.07 (C, C-9).

N,N'-Bis-(4-(phenylsulfonamido)phenyl)succinamide (174)

($C_{28}H_{26}N_4O_6S_2$; M.W.= 578.6)



166 **174**

General procedure 15;

White solid;

Yield: 0.18 g (64%)

Melting Point: 270-272°C

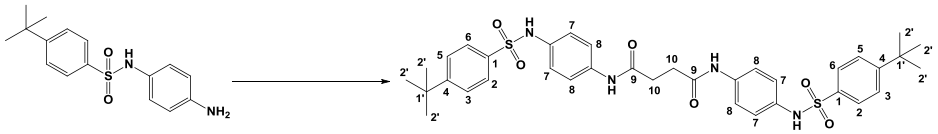
MS $(ESI)^+$: 601.1 $[M+Na]^+$

^{1}H-NMR (DMSO-d$_6$), δ: 2.56 (s, 4H, H-10), 6.97 (d, J= 8.8 Hz, 4H, H-aromatic), 7.41 (d, J= 8.8 Hz, 4H, H-aromatic), 7.50-7.55 (m, 4H, H-aromatic), 7.57-7.61 (m, 2H, H-4), 7.71-7.78 (m, 4H, H-aromatic), 9.89 (bs, 2H, NH), 10.02 (bs, 2H, NH).

^{13}C-NMR (DMSO-d$_6$), δ: 31.00 (CH_2, C-10), 119.54, 121.67, 126.61, 129.11 (CH, C-aromatic), 132.24 (C, C-aromatic), 132.71 (CH, C-aromatic), 136.08, 139.40 (C, C-aromatic), 170.08 (C, C-9).

N,N'-Bis-(4-(4-*tert*-butylphenylsulfonamido)phenyl)succinamide (182)

($C_{36}H_{42}N_4O_6S_2$; M.W.= 690.8)



177 **182**

General procedure 15;

Yellow solid;

Yield: 0.13 g (39%)

Melting Point: 260-262°C

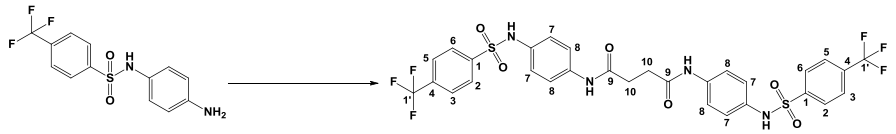
MS (ESI)$^+$: 713.2 $[M+Na]^+$

^{1}H-NMR (DMSO-d$_6$), δ: 1.26 (18H, H-2'), 2.56 (s, 4H, H-10), 7.01 (d, J= 8.6 Hz, 4H, H-aromatic), 7.42 (d, J= 8.6 Hz, 4H, H-aromatic), 7.55 (d, J= 8.4 Hz, 4H, H-aromatic), 7.65 (d, J= 8.4 Hz, 4H, H-aromatic), 9.89 (bs, 2H, NH), 10.03 (bs, 2H, NH).

^{13}C-NMR (DMSO-d$_6$), δ: 30.71 (CH_3, C-2'), 30.99 (CH_2, C-10), 34.80 (C, C-1'), 119.60, 121.06, 125.98, 126.49 (CH, C-aromatic), 132.51, 135.81, 136.81, 155.68 (C, C-aromatic), 170.07 (C, C-9).

N,N'-Bis-(4-(4-trifluoromethylphenylsulfonamido)phenyl)succinamide (183)

($C_{30}H_{24}F_6N_4O_6S_2$; M.W.= 714.1)



178 **183**

General procedure 15;

Grey solid;

Yield: 0.15 g (42%)

Melting Point: 306-308°C

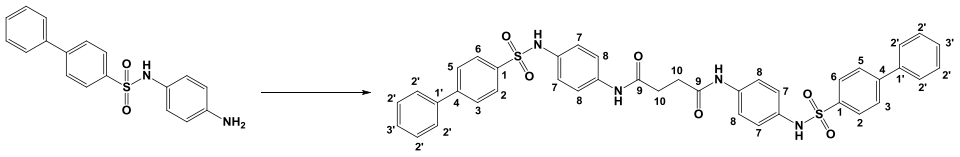
MS (ESI)$^+$: 737.1 $[M+Na]^+$

^{1}H-NMR (DMSO-d$_6$), δ: 2.57 (s, 4H, H-10), 6.99 (d, J= 8.8 Hz, 4H, H-aromatic), 7.45 (d, J= 8.8 Hz, 4H, H-aromatic), 7.89 (d, J= 8.3 Hz, 4H, H-aromatic), 7.94 (d, J= 8.3 Hz, 4H, H-aromatic), 9.94 (bs, 2H, NH), 10.26 (bs, 2H, NH).

^{13}C-NMR (DMSO-d$_6$), δ: 30.98 (CH_2, C-10),119.62, 122.18 (CH, C-aromatic), 124.43 (C, C-aromatic), 126.40, 126.43 (CH, C-3, 6), 127.62 (CH, C-aromatic), 131.57 (C, C-aromatic), 132.03, 132.29, 132.55, 132.80 (C, C-1'), 136.54, 143.28 (C, C-aromatic), 170.17 (C, C-9).

***N,N'*-Bis-(4-(4-biphenyl-4-ylsulfonamido)phenyl)succinamide (184)**

($C_{40}H_{34}N_4O_6S_2$; M.W.= 730.8)



179 **184**

General procedure 15;

Brown solid;

Yield: 0.16 g (44%)

Melting Point: 290-292°C

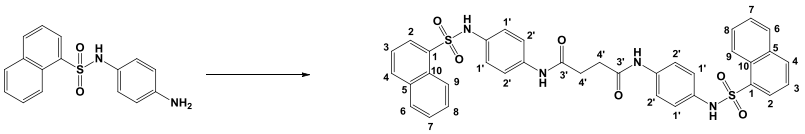
MS $(ESI)^+$: 753.2 $[M+Na]^+$

^{1}H-NMR (DMSO-d_6), δ: 2.56 (s, 4H, H-10), 7.03 (d, J= 8.8 Hz, 4H, H-aromatic), 7.41-7.49 (m, 10H, H-aromatic), 7.70 (d, J= 7.3 Hz, 4H, H-aromatic), 7.77 (d, J= 8.5 Hz, 4H, H-aromatic), 7.83 (d, J= 8.5 Hz, 4H, H-aromatic), 9.90 (bs, 2H, NH), 10.10 (bs, 2H, NH).

^{13}C-NMR (DMSO-d_6), δ: 31.01 (CH_2, C-10), 119.61, 121.54, 127.02, 127.27, 127. 30, 128.50, 129.05 (CH, C-aromatic), 132.29, 136.07, 138.27, 144.11 (C, C-aromatic), 170.10 (C, C-9).

***N,N'*-Bis-(4-(4-naphtalene-1-sulfonamido)phenyl)succinamide (185)**

($C_{36}H_{30}N_4O_6S_2$; M.W.= 678.7)



180 **185**

General procedure 15;

Brown solid;

Yield: 0.18 g (53%)

Melting Point: 270-272°C

MS $(ESI)^+$: 701.1 $[M+Na]^+$

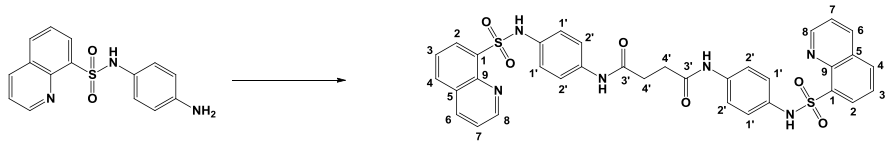
^{1}H-NMR (DMSO-d_6), δ: 2.48 (s, 4H, H-4'), 6.90 (d, J= 8.4 Hz, 4H, H-aromatic), 7.32

(d, J= 8.4 Hz, 4H, H-aromatic), 7.57 (t, J= 7.8 Hz, 2H, H-aromatic), 7.66 (t, J= 7.3 Hz, 2H, H-aromatic), 7.71-7.75 (m, 2H, H-aromatic), 8.06 (d, J= 7.8 Hz, 2H, H-aromatic), 8.13 (d, J= 7.0 Hz, 2H, H-aromatic), 8.18 (d, J= 7.9 Hz, 2H, H-aromatic), 8.73 (d, J= 8.5 Hz, 2H, H-aromatic), 9.81 (bs, 2H, NH), 10.43 (bs, 2H, NH).

^{13}C-NMR (DMSO-d$_6$), δ: 30.99 (CH_2, C-10), 119.55, 120.66, 124.35, 124.38 (CH, C-aromatic), 126.88 (C, C-aromatic), 127.46, 128.00, 129.00 (CH, C-aromatic), 129.79, 132.13 (C, C-aromatic), 133.68 (CH, C-aromatic), 134.37, 135.66 (C, C-aromatic), 169.99 (C, C-9).

N,*N*'-Bis-(4-(4-quinoline-8-sulfonamido)phenyl)succinamide (186)

($C_{34}H_{28}N_4O_6S_2$; M.W.= 680.7)



181 **186**

General procedure 15;

Brown solid;

Yield: 0.12 g (35%)

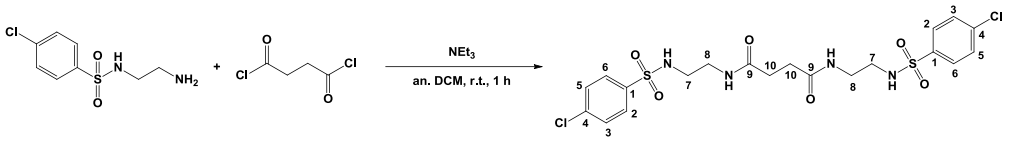
Melting Point: 296-298°C

MS $(ESI)^+$: 681.2 $[M+Na]^+$

^{1}H-NMR (DMSO-d$_6$), δ: 2.45 (s, 4H, H-4'), 6.90 (d, J= 8.9 Hz, 4H, H-aromatic), 7.23 (d, J= 8.9 Hz, 4H, H-aromatic), 7.67 (t, J= 7.8 Hz, 2H, H-aromatic), 7.72 (dd, J_1= 8.3 Hz, J_2= 4.3 Hz, 2H, H-aromatic), 8.24 (d, J= 8.3 Hz, 2H, H-aromatic), 8.28 (d, J= 7.2 Hz, 2H, H-aromatic), 8.51 (dd, J_1= 8.4 Hz, J_2= 1.4 Hz, 2H, H-aromatic), 9.15 (dd, J_1= 4.3 Hz, J_2= 1.4 Hz, 2H, H-aromatic), 9.74 (bs, 2H, NH), 9.79 (bs, 2H, NH).

^{13}C-NMR (DMSO-d$_6$), δ: 30.95 (CH_2, C-10), 119.32, 121.10, 122.58, 125.57 (CH, C-134.08 (CH, C-aromatic), 135.11, 135.65 (C, C-aromatic), 136.96 (CH, C-aromatic), 142.65 (C, C-aromatic), 151.39 (CH, C-aromatic), 169.93 (C, C-9).

6.3.7 *N,N'*-Bis-(2-(4-chlorophenylsulfonamido)ethyl)-succinamide (155) ($C_{20}H_{24}Cl_2N_4O_6S_2$; M.W.= 551.4)



176 **169** **155**

N-(2-Aminoethyl)-4-chlorobenzenesulfonamide (**176**) (0.35 g, 1.5 mmol) was dissolved in 9 mL of anhydrous DCM under N_2 atmosphere. Triethylamine (0.2 mL, 1.5 mmol) was added dropwise to the reaction mixture followed by succinyl chloride (**169**) (0.07 mL, 0.5 mmol). The reaction was stirred at r.t. for 1 h, then diluted with DCM (20 mL), washed with 2M aqueous HCl solution (2 x 30 mL), saturated $NaHCO_3$ solution (2 x 30 mL), and finally with brine (30 mL). The organic phase was concentrated at reduced pressure after drying over $MgSO_4$ and the crude residue was purified by re-crystallisation from MeOH to afford the title compound as a brown solid.

Yield: 0.16 g (48%)

Melting Point: 182-184°C

MS $(ESI)^+$: 573.0, 575.0 $[M+Na]^+$

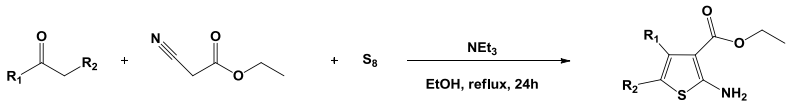
^{1}H-NMR (DMSO-d$_6$), δ: 2.23 (s, 4H, H-10), 2.74-2.79 (m, 4H, C<u>H</u>$_2$), 3.03-3.08 (m, 4H, C<u>H</u>$_2$), 7.68 (d, J= 8.7 Hz, 4H, H-aromatic), 7.75 (t, J= 5.9 Hz, 2H, N<u>H</u>), 7.79 (d, J= 8.7 Hz, 4H, H-aromatic), 7.83 (t, J= 5.6 Hz, 2H, N<u>H</u>).

^{13}C-NMR (DMSO-d$_6$), δ: 31.00 (CH_2, C-10), 38.42, 41.95 (CH_2, C-7, 8), 128.40, 129.35 (CH, C-aromatic), 137.25, 139.24 (C, C-aromatic), 171.54 (C, C-9).

6.4 Synthesis of thienopyrimidine structures

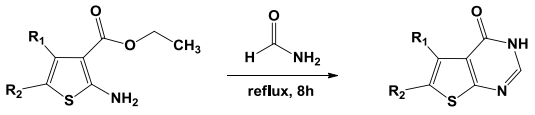
6.4.1 General procedures 16-23

General procedure 16: synthesis of ethyl 2-amino-thiophene-3-carboxylates



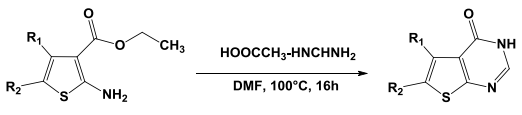
To a mixture of ketone (1 eq.), ethyl cyanoacetate (**189**) (1 eq.), and elemental sulfur (1 eq.) in absolute ethanol (1.7 mL/mmol eq.) was added triethylamine (1.5 eq.), and the mixture was refluxed for 24 h. The reaction mixture was then concentrated and the residue was partitioned between water (10 mL/mmol eq.) and ethyl acetate (10 mL/mmol eq.). The organic layer was separated, dried over $MgSO_4$, and concentrated, and the crude product was purified by recrystallization or flash column chromatography.

General procedure 17: synthesis of thieno[2,3-*d*]pyrimidin-4-ones



Intermediate ethyl 2-amino-thiophene-3-carboxylate (1 eq.) and formamide (3.2mL/mmol eq.) were refluxed for 8 h. The reaction mixture was then cooled in an ice-bath and added of cold water. The precipitate formed was collected by filtration, washed thoroughly with cold water and purified by re-crystallisation.

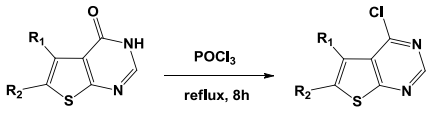
General procedure 17a: synthesis of thieno[2,3-*d*]pyrimidin-4-ones



A mixture of intermediate ethyl 2-amino-thiophene-3-carboxylate (1 eq.) and formamidine acetate salt (1.5 eq.) in DMF (2 mL/mmol eq.) was heated at 100°C for 16

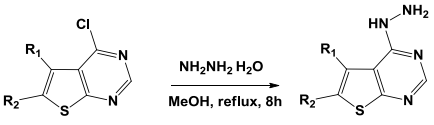
h. The reaction mixture was then cooled to r.t., DMF removed under vacuum, and the solid residue obtained washed thoroughly with water and *n*hexane.

General procedure 18: synthesis of 4-chloro-thieno[2,3-*d*]pyrimidines



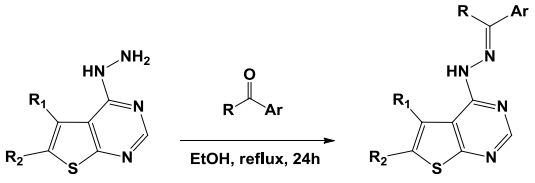
Intermediate thieno[2,3-d]pyrimidin-4-one (1 eq.) and phosphoryl chloride (2.9 mL/mmol eq.) were refluxed for 8 h. The reaction mixture was then cooled in an ice-bath and carefully neutralised by the addition of aqueous saturated $NaHCO_3$ solution. The resulting mixture was extracted with ethyl acetate (15 mL/mmol eq.), the organic layer was separated, dried over $MgSO_4$ and concentrated. The crude residue was purified by flash column chromatography.

General procedure 19: synthesis of (thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazines



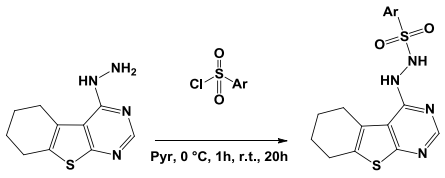
Intermediate 4-chloro-thieno[2,3-d]pyrimidine (1 eq.) was suspended in MeOH (5.1 mL/mmol eq.) and hydrazine hydrate 80% in water (2 eq.) and the mixture was refluxed for 5 h. Immediately after, hydrazine hydrate (2 eq.) was added to complete the reaction and the mixture was allowed to reflux for further 3 h. The reaction mixture was then cooled to r.t and placed in a fridge o.n. The resulting precipitate was filtered and recrystallised from a solution of 40% EtOH in water.

General procedure 20: synthesis of *N*-(1-aryl-ethylidene)-*N*'-(thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazines



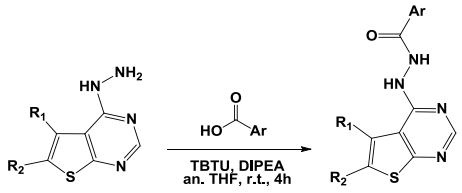
Intermediate (thieno[2,3-d]pyrimidin-4-yl)-hydrazine (1 eq.) and arylketone or aldheyde (1.2) were dissolved in EtOH (12 mL/mmol eq.), and the mixture was refluxed for 24 h. The reaction mixture was then cooled to r.t and placed in a fridge o.n. The resulting precipitate was filtered, washed with a cold solution of 80% EtOH in water and re-crystallised from EtOH.

General procedure 21: synthesis of arylsulfonyl acid *N*'-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazides



Compound **193** (0.20 g, 0.9 mmol) was suspended in pyridine (7 mL) under N_2 atmosphere. Arylsulfonyl chloride (0.7 mmol) dissolved in pyridine (3 mL) was then added dropwise to the reaction mixture under ice-cooling. The reaction mixture was stirred at 0°C for 1 h and then at r.t. for 20 h. The reaction mixture was then diluted with EtOAc (40 mL) and washed with 0.5 M HCl solution (2 x 50 mL). The water phase was then extracted with EtOAc (2 x 50 mL) and the organic layers were collected, dried over $MgSO_4$ and concentrated under vacuum. The crude residue was purified by flash column chromatography to give pure sulfonyl acid *N*'-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazides.

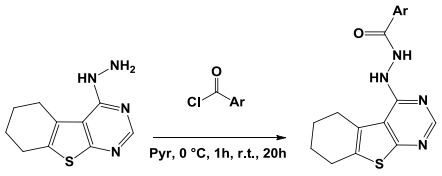
General procedure 22: synthesis of *N*'-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-arylcarbohydrazides



Aryl carboxylic acid (1.1 eq.) and TBTU (1.2) were suspended in anhydrous THF (11 mL/mmol eq.) at r.t. under N_2 atmosphere. DIPEA (2.4 eq.) was then added dropwise to the reaction mixture, followed by intermediate (thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine

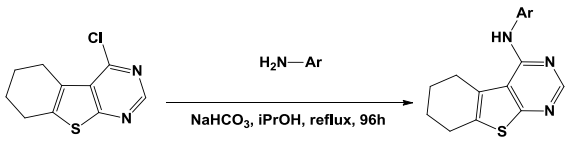
(1 eq.) dissolved in anhydrous THF (11 mL/mmol eq.). The mixture was stirred at r.t. for 4 h, then concentrated under vacuum. The residue was dissolved in EtOAc (30 mL/mmol eq.), washed with water (30 mL/mmol eq.), saturated $NaHCO_3$ solution (30 mL/mmol eq.), and finally with brine (30 mL/mmol eq.). The organic phase was concentrated under vacuum after drying over $MgSO_4$. The crude residue was purified by re-crystallisation or flash column chromatography.

General procedure 22a: synthesis of *N*'-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-arylcarbohydrazides



Compound **193** (0.20 g, 0.9 mmol) was suspended in pyridine (7 mL) under N_2 atmosphere. Acyl chloride (0.7 mmol) dissolved in pyridine (3 mL) was then added dropwise to the reaction mixture under ice-cooling. The reaction mixture was stirred at 0°C for 1 h and then at r.t. for 20 h. The reaction mixture was diluted with EtOAc (40 mL) and washed with 0.5 M HCl solution (2 x 50 mL). The water phase was then extracted with EtOAc (2 x 50 mL) and the organic layers were collected, dried over $MgSO_4$ and concentrated under vacuum. The crude residue was purified by flash column chromatography to give pure *N*'-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-arylcarbohydrazides.

General procedure 23: synthesis of *N*-aryl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amines

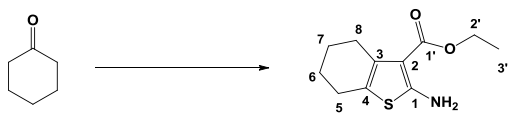


Compound **192** (0.20 g, 0.9 mmol), arylamine (1.8 mmol) and $NaHCO_3$ (0.15 g, 1.8 mmol) were heated under reflux in *i*PrOH (8 mL) for 96 h. The reaction mixture was then cooled to r.t. and concentrated under vacuum. The crude residue was purified by

flash column chromatography to give pure *N*-aryl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amines.

6.4.2 Tetrahydrobenzo[*b*]thienopyrimidines

Ethyl 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (190)[30]
($C_{11}H_{15}NO_2S$; M.W.= 225.3)



188 **190**

General procedure 16;
Reagent: cyclohexanone **188** (1g, 10.2 mmol);
Purification: re-crystallisation from 60% EtOH/H_2O;
Light brown solid;
Yield: 1.95 g (85%)
^{1}H-NMR ($CDCl_3$), δ: 1.35 (t, J= 7.1 Hz, 3H, H-3'), 1.77 (m, 4H, H-6, 7), 2.51 (m, 2H, C$\underline{H}_2$), 2.72 (m, 2H, C$\underline{H}_2$), 4.27 (q, J= 7.1 Hz, 2H, H-2'), 5.98 (bs, 2H, N$\underline{H}_2$).
^{13}C-NMR ($CDCl_3$), δ: 14.47 (CH_3, C-3'), 22.85, 23.27, 24.54, 26.96 (CH_2, C-5, 6, 7, 8), 59.36 (CH_2, C-2'), 105.73, 117.61, 132.46, 161.77 (C, C-1, 2, 3, 4), 166.14 (C, C-1').

5,6,7,8-Tetrahydro-3*H*-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-one (191)[30]
($C_{10}H_{10}N_2OS$; M.W.= 206.3)

190 **191**

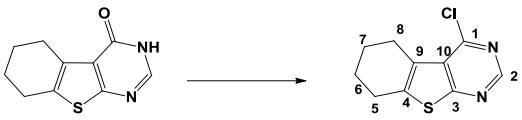
General procedure 17;
Reagent: ethyl 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate **190** (2g, 8.8 mmol);
Purification: re-crystallisation from 40% EtOH/H_2O;
Light brown solid;
Yield: 1.69 g (93%)
^{1}H-NMR (DMSO-d_6), δ: 1.78 (m, 4H, H-6, 7), 2.73 (t, J= 6.1 Hz, 2H, C$\underline{H}_2$), 2.87 (t, J=

6.1 Hz, 2H, C<u>H</u>$_2$), 7.98 (s, 1H, H-2), 12.28 (bs, 1H, N<u>H</u>).

^{13}C-NMR (DMSO-d$_6$), δ: 21.73, 22.42, 24.40, 25.30 (CH_2, C-5, 6, 7, 8), 122.67, 130.79, 132.08 (C, C-aromatic), 144.81 (CH, C-2), 157.64 (C, C-aromatic), 162.39 (C, C-1).

4-Chloro-5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidine (192)[30]

($C_{10}H_9ClN_2S$; M.W.= 224.7)



191 **192**

General procedure 18;

Reagent: 5,6,7,8-tetrahydro-3*H*-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-one **191** (1.5 g, 7.4 mmol);

T.L.C. System: *n*hexane-EtOAc 1:1 v/v, Rf: 0.85;

Purification: flash column chromatography (*n*hexane:EtOAc 100:0 v/v increasing to *n*hexane-EtOAc 85:15 v/v);

White solid;

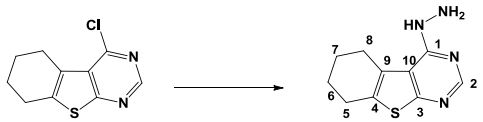
Yield: 1.31 g (79%)

^{1}H-NMR (CDCl$_3$), δ: 1.85 (m, 4H, H-6, 7), 2.89 (m, 2H, C<u>H</u>$_2$), 3.00 (m, 2H, C<u>H</u>$_2$), 8.79 (s, 1H, H-2).

^{13}C-NMR (CDCl$_3$), δ: 21.59, 21.85, 25.39, 25.78 (CH_2, C-5, 6, 7, 8), 126.60, 127.92, 139.62 (C, C-aromatic), 151.77 (CH, C-2), 152.25, 168.02 (C, C-aromatic).

(5,6,7,8-Tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (193)[31]

($C_{10}H_{12}N_4S$; M.W.= 220.3)



192 **193**

General procedure 19;

Reagent: 4-chloro-5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidine **192** (1.30 g,

5.8 mmol);

Purification: recrystallization from 40 % EtOH/H_2O;

Yellow solid;

Yield: 1.14 g (89%)

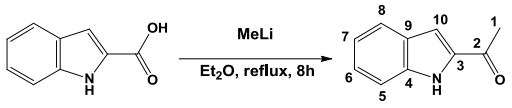
^{1}H-NMR (DMSO-d$_6$), δ: 1.79 (m, 4H, H-6, 7), 2.75 (m, 2H, C$\underline{H}_2$), 2.92 (m, 2H, C$\underline{H}_2$), 4.56 (bs, 2H, N$\underline{H}_2$), 7.87 (bs, 1H, N$\underline{H}$), 8.33 (s, 1H, H-2).

^{13}C-NMR (DMSO-d$_6$), δ: 22.00, 22.13, 24.84, 25.38 (CH_2, C-5, 6, 7, 8), 114.79, 126.77, 131.44 (C, C-aromatic), 152.46 (CH, C-2), 158.27, 163.90 (C, C-aromatic).

6.4.1.1 Aryl-ethanones(235, 236)

1-(1*H*-Indol-2-*yl*)-ethanone (235)[32]

($C_{10}H_9NO$; M.W.= 159.1)



238 **235**

A methyllithium 1.6 M solution in Et_2O (15 mL, 24 mmol) was slowly added to a solution of indole-2-carboxylic acid (**238**) (2g, 12.4 mmol) in 100 mL of anhydrous Et_2O cooled at 0°C and the mixture was refluxed for 1 h. Additional methyllithium 1.6 M in Et_2O (15 mL, 24 mmol) was added at r.t., and the reaction mixture was refluxed for additional 7 h. The reaction was then quenched by the addition of saturated aqueous NH_4Cl solution (50 mL), diluted with Et_2O (30 mL), and extracted with Et_2O (2 x 50 mL). The organic extracts were washed with water, dried over $MgSO_4$, and evaporated under vacuum. The crude residue was purified by flash column chromatography (100 % DCM) to give pure 1-(1*H*-indol-2-*yl*)-ethanone (**235**) as a pink solid.

T.L.C. System: 100 % DCM, Rf: 0.71.

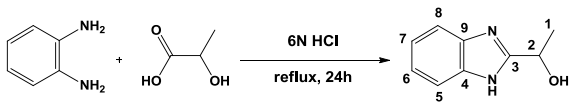
Yield: 1.37 g (69%)

^{1}H-NMR ($CDCl_3$), δ: 2.65 (s, 3H, H-1), 7.17-7.21 (m, 1H, H-aromatic), 7.24 (dd, J_1= 2.2 Hz, J_2= 0.9 Hz, 1H, H-aromatic), 7.37-7.40 (m, 1H, H-aromatic), 7.49 (dd, J_1= 8.2 Hz, J_2= 0.9 Hz, 1H, H-aromatic), 7.75 (dd, J_1= 8.2 Hz, J_2= 0.9 Hz, 1H, H-aromatic), (9.62 (bs, 1H, N<u>H</u>).

^{13}C-NMR ($CDCl_3$), δ: 25.88 (CH_3, C-1), 109.99, 112.35, 120.93, 123.04, 126.36 (CH, C-aromatic), 127.58, 135.43, 137.57 (C, C-aromatic), 190.71 (C, C-2).

1-(1*H*-Benzoimidazol-2-*yl*)-ethanol (240)[33]

($C_9H_{10}N_2O$; M.W.= 162.1)



239 **240**

A mixture of *o*-phenylendiamine (**239**) (2g, 18.5 mmol) and DL-lactic acid (2.1 mL, 27.7 mmol) in 6N HCl (18.5 mL) was refluxed for 24 h. The reaction mixture was then cooled down in an ice-bath and neutralised with a 4N NaOH solution. The precipitate formed was collected, washed with water and dried under vacuum. The crude residue was purified by re-crystallisation from EtOH to give pure 1-(1*H*-benzoimidazol-2-*yl*)-ethanol (**240**) as a white solid.

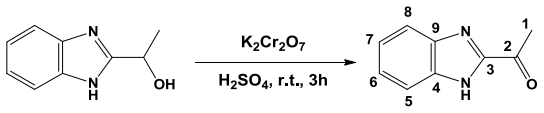
Yield: 1.46 g (49%)

^{1}H-NMR (DMSO-d$_6$), δ: 1.52 (d, J= 6.6 Hz, 3H, H-1), 4.92-4.98 (m, 1H, H-2), 5.75 (d, J= 4.8 Hz, 1H, OH), 7.10-7.16 (m, 2H, H-aromatic), 7.41-7.58 (m, 2H, H-aromatic), 12.22 (bs, 1H, NH).

^{13}C-NMR (DMSO-d$_6$), δ: 22.94 (CH_3, C-1), 63.66 (CH, C-2), 111.20, 118.39, 120.83, 121.53 (CH, C-aromatic), 158.83 (C, C-3, 4, 9).

1-(1*H*-Benzoimidazol-2-*yl*)-ethanone (236)[33]

($C_9H_8N_2O$; M.W.= 160.1)



240 **236**

A mixture of 1-(1*H*-benzoimidazol-2-*yl*)-ethanol (**240**) (0.9 g, 5.4 mmol) in 5% HSO_4 (11 mL) was treated dropwise with a $K_2Cr_2O_7$ solution (2.2 g, 7.5 mmol) in water (8 mL) and concentrated H_2SO_4 (5 mL) and stirred for 3 h. The formed solid was filtered, washed thoroughly with water and re-crystallised from EtOH to give pure 1-(1*H*-benzoimidazol-2-*yl*)-ethanone (**236**) as a light brown solid.

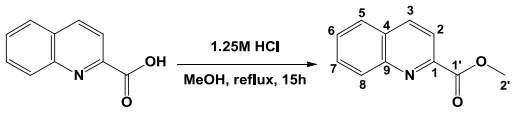
Yield: 0.31 g (36%)

^{1}H-NMR (DMSO-d$_6$), δ: 2.71 (s, 3H, H-1), 7.26-7.43 (m, 2H, H-aromatic), 7.53-7.59 (m, 1H, H-aromatic), 7.80-7.87 (m, 1H, H-aromatic), 13.28 (bs, 1H, NH).

^{13}C-NMR (DMSO-d$_6$), δ: 26.00 (CH_3, C-1), 112.83, 121.06, 122.96, 125.54 (CH, C-aromatic), 148.17 (C, C-3, 4, 9), 191.50 (C, C-2).

Methyl quinaldate (283)[34]

($C_{11}H_9NO_2$; M.W.= 187.19)



282 **283**

To a suspension of quinaldic acid (**282**) (2.0 g, 11.6 mmol) in MeOH (21 mL) was added a 1.25M HCl solution in MeOH (21 mL). The resulting mixture was refluxed for 15 h, then cooled to r.t. and concentrated in vacuo. The residue was partitioned between saturated aqueous $NaHCO_3$ solution (30 mL) and EtOAC (3 x 25 mL). The combined organic extracts were washed with saturated aqueous $NaHCO_3$ (10 mL) and water (10 mL), dried over $MgSO_4$ and dried under vacuum to give methyl quinaldate (**283**) as a white solid.

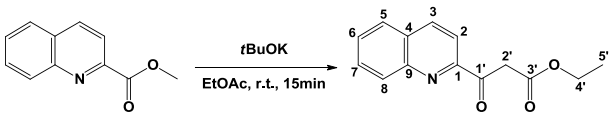
Yield: 2.16 g (76%)

^{1}H-NMR ($CDCl_3$), δ: 4.10 (s, 3H, H-2'), 7.64-7.68 (m, 1H, H-aromatic), 7.78-7.82 (m, 1H, H-aromatic), 7.89 (dd, J_1= 8.1 Hz, J_2= 1.1 Hz, 1H, H-aromatic), 8.21 (d, J= 8.5 Hz, 1H, H-aromatic), 8.30-8.33 (m, 2H, H-aromatic).

^{13}C-NMR ($CDCl_3$), δ: 53.18 (CH_3, C-2'), 121.03, 127.55, 128.63 (CH, C-aromatic), 129.36 (C, C-aromatic), 130.30, 130.71, 137.31 (CH, C-aromatic), 147.55, 147.92 (C, C-aromatic), 165.96 (C, C-1').

Ethyl 3-oxo-3-(quinolin-2-*yl*)propanoate (284)[34]

($C_{14}H_{13}NO_3$; M.W.= 243.26)



283 **284**

Solid *t*BuOK (1.32 g, 11.8 mmol) was slowly added to a solution of methyl quinaldate (**283**) (1.64 g, 8.8 mmol) in EtOAc (33 mL). The resulting mixture was stirred for 15 min. at r.t., then quenched with water (10 mL). The organic layer was separated and the aqueous phase was extracted with EtOAc (3 x 25 mL). The combined organic extracts were washed with water (25 mL) and brine (25 mL), dried over $MgSO_4$ and

concentrated under vacuum. The crude residue was purified by flash column chromatography (*n*hexane:EtOAc 100:0 v/v increasing to *n*hexane-EtOAc 90:10 v/v) to give pure ethyl 3-oxo-3-(quinolin-2-*yl*)propanoate (**284**) as an off-white paste.

T.L.C. System: *n*hexane-EtOAc 8:2 v/v, Rf: 0.59.

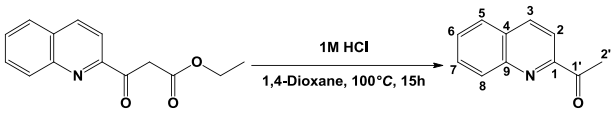
Yield: 0.68 g (29%)

^{1}H-NMR ($CDCl_3$), δ: 1.25 (t, J= 7.1 Hz, 3H, H-5'), 4.23 (q, J= 7.1 Hz, 2H, H-4'), 4.37 (s, 2H, H-2'), 7.66-7.70 (m, 1H, H-aromatic), 7.79-7.82 (m, 1H, H-aromatic), 7.90 (d, J= 8.2 Hz, 1H, H-aromatic), 8.16 (d, J= 8.4 Hz, 1H, H-aromatic), 8.17-8.20 (m, 1H, H-aromatic), 8.30 (d, J= 8.4 Hz, 1H, H-aromatic).

^{13}C-NMR ($CDCl_3$), δ: 14.12 (CH_3, C-5'), 44.74 (CH_2, C-4'), 61.13 (CH_2, C-2'), 118.08, 127.68, 128.88 (CH, C-aromatic), 129.77 (C, C-aromatic), 130.15, 130.59, 137.13 (CH, C-aromatic), 147.10, 151.95 (C, C-aromatic), 168.38, 194.96 (C, C-1', 3').

2-Acetylquinoline (285)[34]

($C_{11}H_9NO$; M.W.= 171.20)



284 **285**

A solution of ethyl 3-oxo-3-(quinolin-2-*yl*)propanoate (**284**) (0.62 g, 2.5 mmol) in 1,4-dioxane (7 mL) containing 1M aqueous HCl solution (7 mL) was stirred at 100°C for 15 h, then cooled to r.t. and concentrated in vacuo. The residue was diluted with water (20 mL) and extracted with EtOAc (3 x 25 mL). The combined organic extracts were washed with saturated aqueous $NaHCO_3$ solution (20 mL) and brine (20 mL), dried over $MgSO_4$ and concentrated under vacuum. The crude residue was filtered on a bed of silica gel (*n*hexane:DCM 1:4 v/v) to give pure 2-acetylquinoline (**285**) as a white solid.

T.L.C. System: *n*hexane-DCM 1:4 v/v, Rf: 0.69.

Yield: 0.27 g (62%)

^{1}H-NMR ($CDCl_3$), δ: 2.89 (s, 3H, H-2'), 7.65-7.68 (m, 1H, H-aromatic), 7.78-7.82 (m, 1H, H-aromatic), 7.88-7.90 (m, 1H, H-aromatic), 8.15 (d, J= 8.5 Hz, 1H, H-aromatic), 8.21-8.23 (m, 1H, H-aromatic), 8.28 (d, J= 8.5 Hz, 1H, H-aromatic).

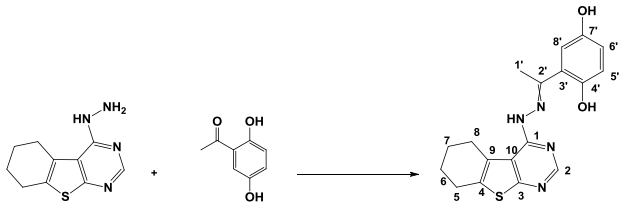
^{13}C-NMR ($CDCl_3$), δ: 25.52 (CH_3, C-2'), 117.96, 127.63, 128.53 (CH, C-aromatic),

129.58 (C, C-aromatic), 129.96, 130.59, 136.83 (CH, C-aromatic), 147.26, 153.28 (C, C-aromatic), 200.62 (C, C-1').

6.4.1.2 *N*-(1-Aryl-ethylidene)-*N*'-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*] pyrimidin-4-*yl*)-hydrazines (187, 215-223, 252, 254-265) and *N*-arylidene-*N*'-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazines (225-234, 253, 457)

2-{1-[(5,6,7,8-Tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazono]-ethyl}-benzene-1,4-diol (187)

($C_{18}H_{18}N_4O_2S$; M.W.= 354.4)



193 **194** **187**

General procedure 20;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.10 g, 0.4 mmol);

Yellow solid;

T.L.C. System: *n*hexane -EtOAc 1:1 v/v, Rf: 0.48.

Yield: 0.08 g (66%)

Melting Point: > 300 °C

MS $(ESI)^+$: 377.0 $[M+Na]^+$

Two species observed. Major/minor species ratio: 2/1.

^{1}H-NMR (DMSO-d_6), δ: (major species) 1.79 (bs, 4H, H-6, 7), 2.44 (s, 3H, H-2'), 2.74 (bs, 2H, C$\underline{H}_2$), 3.04 (bs, 2H, C$\underline{H}_2$), 6.75 (bs, 2H, H-aromatic), 6.99 (bs, 1H, H-aromatic), 7.73 (bs, 1H, N$\underline{H}$), 8.88 (bs, 1H, H-2), 11.35 (bs, 1H, O$\underline{H}$), 11.79 (bs, 1H, O$\underline{H}$).

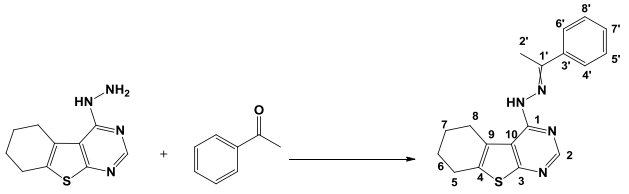
^{1}H-NMR (DMSO-d_6), δ: (minor species) 1.79 (bs, 4H, H-6, 7), 2.44 (s, 3H, H-2'), 2.74 (bs, 2H, C$\underline{H}_2$), 3.07 (bs, 2H, C$\underline{H}_2$), 6.75 (bs, 2H, H-aromatic), 6.99 (bs, 1H, H-aromatic), 8.51 (bs, 1H, N$\underline{H}$), 8.88 (bs, 1H, H-2), 9.27 (bs, 1H, O$\underline{H}$), 12.56 (bs, 1H, O$\underline{H}$).

^{13}C-NMR (DMSO-d_6), δ: (major and minor species) 12.95, 13.33 (CH_3, C-2'), 22.06, 22.28, 24.71, 26.48 (CH_2, C-5, 6, 7, 8), 113.42, 114.18, 116.92, 117.64, 118.11 (CH, C-3', 4', 6'), 119.42, 121.00, 126.23, 130.75, 132.52, 133.84 (C, C-aromatic), 143.96 (CH, C-2), 145.28, 149.23, 150.97, 152.58 (CH, C-2), 153.82, 154.47, 156.57, 162.96, 165.74

(C, C-aromatic).

***N*-(1-Phenyl-ethylidene)-*N*'-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (215)[31]**

($C_{18}H_{18}N_4S$; M.W.= 322.4)



193 **195** **215**

General procedure 20;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.10 g, 0.4 mmol);

Yellow crystals;

Yield: 0.09 g (62%)

Melting Point: 190-192°C

MS (ESI^+): 345.1 $[M+Na]^+$

Two species observed. Major/minor species ratio: 2/1.

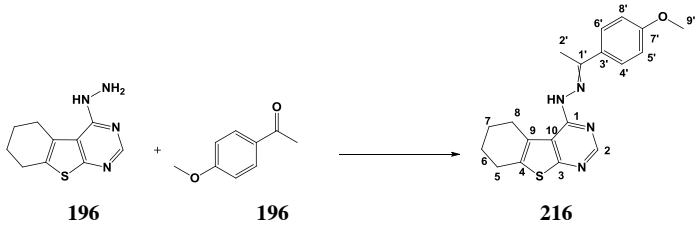
^{1}H-NMR ($CDCl_3$), δ: (major species) 1.85-1.91 (m, 4H, C<u>H</u>$_2$), 2.55 (s, 3H, H-2'), 2.79-2.82 (m, 2H, C<u>H</u>$_2$), 3.10-3.14 (m, 2H, C<u>H</u>$_2$), 7.38-7.43 (m, 5H, H-aromatic), 7.66 (s, 1H, H-2), 10.36 (bs, 1H, N<u>H</u>).

^{1}H-NMR ($CDCl_3$), δ: (minor species) 1.94-2.06 (m, 4H, C<u>H</u>$_2$), 2.38 (s, 3H, H-2'), 2.86-2.90 (m, 2H, C<u>H</u>$_2$), 3.04-3.07 (m, 2H, C<u>H</u>$_2$), 7.83-7.87 (m, 5H, H-aromatic), 8.50 (bs, 1H, N<u>H</u>), 8.64 (s, 1H, H-2).

^{13}C-NMR ($CDCl_3$), δ: (major and minor species) 13.52, 15.12 (CH_3, C-2'), 22.22, 22.38, 22.68, 22.76, 25.35, 25.47, 26.62, 26.72 (CH_2), 120.56, 124.58 (C, C-aromatic), 126.50, 126.74, 128.44, 129.11, 129.28, 129.54 (CH, C-aromatic), 129.87, 131.43, 133.71, 134.41, 135.18, 135.31, 137.94 (C, C-aromatic), 139.34 (CH, C-aromatic), 141.35, 148.05, 149.29 (C, C-aromatic), 153.45 (CH, C-aromatic), 156.79, 159.50 (C, C-aromatic).

***N*-[1-(4-Methoxy-phenyl)-ethylidene]-*N*'-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (216)**

($C_{19}H_{20}N_4OS$; M.W.= 352.4)



196 **196** **216**

General procedure 20;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.10 g, 0.4 mmol);

Yellow crystals;

Yield: 0.04 g (28%)

Melting Point: 178-180°C

MS (ESI^+): 353.1 $[M+H]^+$

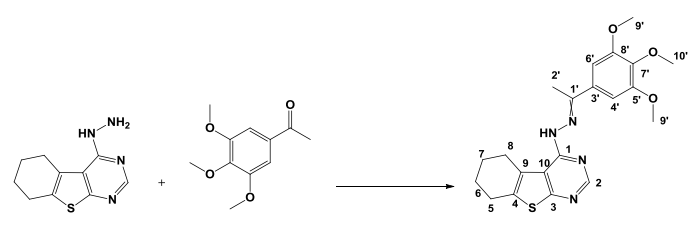
Single species observed.

^{1}H-NMR (DMSO-d_6), δ: 1.75-1.84 (m, 4H, C<u>H</u>$_2$), 2.41 (s, 3H, H-2'), 2.71-2.78 (m, 2H, C<u>H</u>$_2$), 3.00-3.04 (m, 2H, C<u>H</u>$_2$), 3.81 (s, 3H, H-9'), 6.96 (d, J= 8.5 Hz, 2H, H-aromatic), 7.75 (s, 1H, H-2), 8.00 (d, J= 8.5 Hz, 2H, H-aromatic), 11.53 (bs, 1H, N<u>H</u>).

^{13}C-NMR (DMSO-d_6), δ: 14.08 (CH_3, C-2'), 22.15, 22.39, 24.65, 26.38 (CH_2), 55.17 (CH_3, C-7'), 113.42 (CH, C-aromatic), 119.66 (C, C-aromatic), 128.03 (CH, C-aromatic), 130.84, 131.26, 132.05 (C, C-aromatic), 144.00 (CH, C-aromatic), 146.94, 156.47, 157.14, 160.09 (C, C-aromatic).

***N*-(5,6,7,8-Tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-*N*'-[1-(3,4,5-trimethoxy-phenyl)-ethylidene]-hydrazine (217)**

($C_{21}H_{24}N_4O_3S$; M.W.= 412.5)



193 **197** **217**

General procedure 20;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.10 g, 0.4 mmol);

Yellow solid;

Yield: 0.14 g (74%)

Melting Point: 224-226°C

MS (ESI^+): 413.1 $[M+H]^+$

Two species observed. Major/minor species ratio: 5/2.

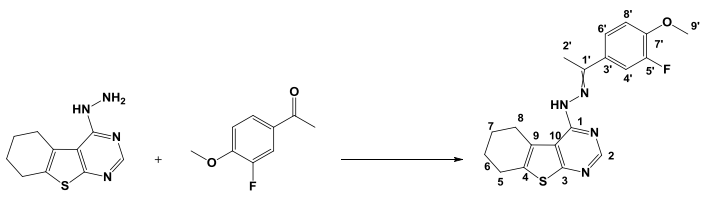
^{1}H-NMR ($CDCl_3$), δ: (major species) 1.84-1.91 (m, 4H, C<u>H</u>$_2$), 2.51 (s, 3H, H-2'), 2.79-2.83 (m, 2H, C<u>H</u>$_2$), 3.09-3.13 (m, 2H, C<u>H</u>$_2$), 3.94 (s, 3H, OC<u>H</u>$_3$), 3.96 (s, 3H, OC<u>H</u>$_3$), 3.98 (s, 3H, OC<u>H</u>$_3$), 7.07 (s, 2H, H-4', 6'), 7.67 (s, 1H, H-2), 10.25 (bs, 1H, N<u>H</u>).

^{1}H-NMR ($CDCl_3$), δ: (minor species) 1.95-2.05 (m, 4H, C<u>H</u>$_2$), 2.35 (s, 3H, H-2'), 2.87-2.91 (m, 2H, C<u>H</u>$_2$), 3.04-3.08 (m, 2H, C<u>H</u>$_2$), 3.93 (s, 3H, OC<u>H</u>$_3$), 3.97 (s, 3H, OC<u>H</u>$_3$), 3.99 (s, 3H, OC<u>H</u>$_3$), 7.07 (s, 2H, H-4', 6'), 8.42 (bs, 1H, N<u>H</u>), 8.61 (s, 1H, H-2).

^{13}C-NMR ($CDCl_3$), δ: (major and minor species) 13.91, 15.57 (CH_3, C-2'), 22.42, 22.68, 22.77, 22.93, 25.35, 25.60, 26.64, 26.78 (CH_2), 56.38, 60.94 (CH_3, C-9', 10'), 104.24 (CH, C-aromatic), 131.42, 133.80, 135.08, 135.20 (C, C-aromatic), 141.27 (CH, C-aromatic), 147.63, 153.07 (C, C-aromatic), 153.23 (CH, C-aromatic), 156.71, 159.61 (C, C-aromatic).

***N*-[1-(3-Fluoro-4-methoxy-phenyl)-ethylidene]-*N*'-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (218)**

($C_{19}H_{19}FN_4OS$; M.W.= 370.4)



193 **198** **218**

General procedure 20;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.10 g, 0.4 mmol);

Yellow solid;

Yield: 0.13 g (75%)

Melting Point: 192-194°C

MS (ESI^+): 371.1 $[M+H]^+$

Two species observed. Major/minor species ratio: 5/2.

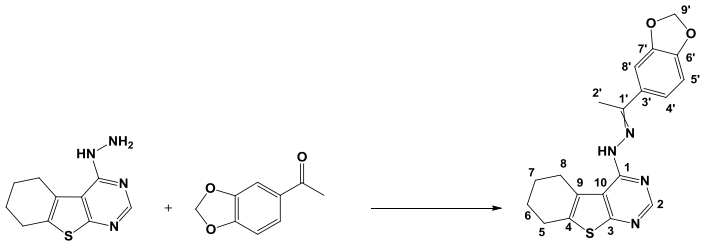
^{1}H-NMR ($CDCl_3$), δ: (major species) 1.85-1.92 (m, 4H, C<u>H</u>$_2$), 2.50 (s, 3H, H-2'), 2.78-2.82 (m, 2H, C<u>H</u>$_2$), 3.08-3.13 (m, 2H, C<u>H</u>$_2$), 3.94 (s, 3H, H-9'), 6.92-6.99 (m, 1H, H-aromatic), 7.49-7.53 (m, 1H, H-aromatic), 7.63-7.70 (m, 2H, H-aromatic), 10.33 (bs, 1H, N<u>H</u>).

^{1}H-NMR ($CDCl_3$), δ: (minor species) 1.93-2.03 (m, 4H, C<u>H</u>$_2$), 2.32 (s, 3H, H-2'), 2.86-2.90 (m, 2H, C<u>H</u>$_2$), 3.02-3.06 (m, 2H, C<u>H</u>$_2$), 3.93 (s, 3H, H-9'), 6.92-6.99 (m, 1H, H-aromatic), 7.54-7.58 (m, 1H, H-aromatic), 7.63-7.70 (m, 1H, H-aromatic), 8.45 (bs, 1H, N<u>H</u>), 8.63 (s, 1H, H-aromatic).

^{13}C-NMR ($CDCl_3$), δ: (major and minor species) 13.24, 14.75 (CH_3, C-2'), 22.36, 22.67, 22.93, 25.34, 25.57, 26.62 (CH_2), 56.27 (CH_3, C-9', 10'), 112.63, 112.78, 113.79, 113.94, 114.34, 122.64, 122.66 (CH, C-aromatic), 131.40, 132.62, 133.74, 135.74 (C, C-aromatic), 141.35 (CH, C-aromatic), 147.38, 151.23, 153.18 (C, C-aromatic), 153.28 (CH, C-aromatic), 157.03, 159.41 (C, C-aromatic).

***N*-(1-Benzo[1,3]dioxol-5-yl-ethylidene)-*N*'-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (219)**

($C_{19}H_{18}N_4O_2S$; M.W.= 366.4)



193 + **199** → **219**

General procedure 20;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.10 g, 0.4 mmol);

Yellow solid;

Yield: 0.04 g (27%)

Melting Point: 215-217°C

MS (ESI^+): 389.0 $[M+Na]^+$

Two species observed. Major/minor species ratio: 2/1.

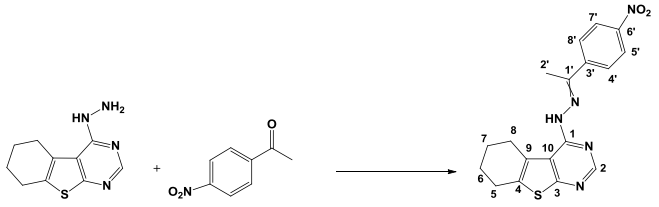
^{1}H-NMR ($CDCl_3$), δ: (major species) 1.84-1.92 (m, 4H, C<u>H</u>$_2$), 2.50 (s, 3H, H-2'), 2.78-2.82 (m, 2H, C<u>H</u>$_2$), 3.09-3.12 (m, 2H, C<u>H</u>$_2$), 6.01 (s, 2H, H-9'), 6.82-6.85 (m, 1H, H-aromatic), 7.29-7.32 (m, 1H, H-aromatic), 7.45 (d, J= 1.6 Hz, 1H, H-8'), 7.67 (s, 1H, H-2), 10.30 (bs, 1H, N<u>H</u>).

^{1}H-NMR ($CDCl_3$), δ: (minor species) 1.94-2.04 (m, 4H, C<u>H</u>$_2$), 2.33 (s, 3H, H-2'), 2.86-2.90 (m, 2H, C<u>H</u>$_2$), 3.03-3.07 (m, 2H, C<u>H</u>$_2$), 6.02 (s, 2H, H-9'), 6.82-6.85 (m, 1H, H-aromatic), 7.29-7.32 (m, 1H, H-aromatic), 7.47 (d, J= 1.6 Hz, 1H, H-8'), 8.45 (bs, 1H, N<u>H</u>), 8.63 (s, 1H, H-2).

^{13}C-NMR ($CDCl_3$), δ: (major and minor species) 13.64, 15.10 (CH_3, C-2'), 22.38, 22.68, 22.75, 22.94, 25.35, 25.57, 26.62, 26.69 (CH_2), 106.42, 106.89, 107.86, 107.90 (CH, C-aromatic), 119.99 (C, C-aromatic), 120.88, 121.06 (CH, C-aromatic), 125.40, 131.40, 133.67, 134.25, 135.08 (C, C-aromatic), 141.36 (CH, C-aromatic), 147.69, 147.81, 148.61, 150.75 (C, C-aromatic), 153.39 (CH, C-aromatic), 153.95, 157.44, 158.81 (C, C-aromatic).

***N*-[1-(4-Nitro-phenyl)-ethylidene]-*N*'-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (220)**

($C_{18}H_{17}N_5O_2S$; M.W.= 367.4)



193 **200** **220**

General procedure 20;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.10 g, 0.4 mmol);

Orange solid;

Yield: 0.13 g (76%)

Melting Point: 289-291°C

MS (ESI$^+$): 390.0 $[M+Na]^+$

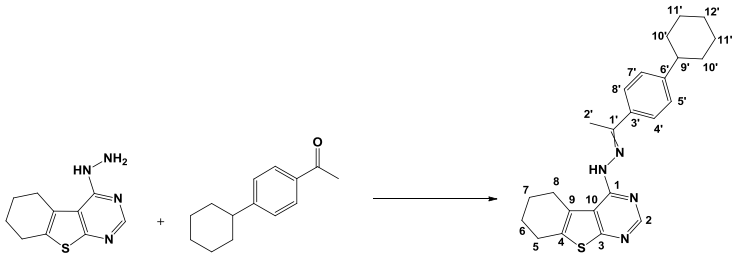
Single species observed.

^{1}H-NMR (DMSO-d$_6$), δ: 1.76-1.86 (m, 4H, C<u>H</u>$_2$), 2.38 (s, 3H, H-2'), 2.74-2.82 (m, 2H, C<u>H</u>$_2$), 3.00-3.08 (m, 2H, C<u>H</u>$_2$), 7.87 (s, 1H, H-2), 8.25 (d, J= 7.8 Hz, 2H, -aromatic), 8.32 (d, J= 7.8 Hz, 2H, H-aromatic), 11.87 (bs, 1H, N<u>H</u>).

^{13}C-NMR (DMSO-d$_6$), δ: 14.11 (CH_3, C-2'), 22.11, 22.34, 24.71, 26.41 (CH_2), 119.63 (C, C-aromatic), 123.20, 127.41 (CH, C-aromatic), 130.81, 132.82 (C, C-aromatic), 143.91 (CH, C-aromatic), 144.76, 147.17, 148.25, 155.43, 157.83 (C, C-aromatic).

***N*-[1-(4-Cyclohexyl-phenyl)-ethylidene]-N'-(5,6,7,8-tetrahydro-benzo[4,5]thieno [2,3-*d*]pyrimidin-4-*yl*)-hydrazine (221)**

($C_{24}H_{28}N_4S$; M.W.= 404.6)



193 **201** **221**

General procedure 20;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.10 g, 0.4 mmol);

Yellow solid;

Yield: 0.14 g (76%)

Melting Point: 258-260°C

MS (ESI$^+$): 405.1 $[M+Na]^+$

Two species observed. Major/minor species ratio: 2/1.

^{1}H-NMR ($CDCl_3$), δ: major specis- 1.38-1.52 (m, 6H, CH_2), 1.76-1.82 (m, 2H, CH_2), 1.85-1.98(m, 6H, C<u>H</u>$_2$), 2.52-2.59 (m, 4H, H-2', 9'), 2.79-2.82 (m, 2H, C<u>H</u>$_2$), 3.11-3.14 (m, 2H, C<u>H</u>$_2$), 7.24-7.27 (m, 2H, H-aromatic), 7.65 (s, 1H, H-2), 7.76-7.80 (m, 2H, H-aromatic), 10.34 (bs, 1H, N<u>H</u>).

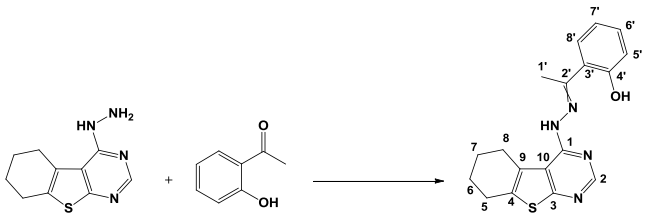
^{1}H-NMR ($CDCl_3$), δ: (minor species) 1.38-1.52 (m, 14H, CH_2), 2.37 (s, 3H, H-2'), 2.73-2.76 (m, 1H, H-9'), 2.87-2.90 (m, 2H, C<u>H</u>$_2$), 3.06-3.09 (m, 2H, C<u>H</u>$_2$), 7.24-7.27

(m, 2H, H-aromatic), 7.76-7.80 (m, 2H, H-aromatic), 8.49 (bs, 1H, NH), 8.64(s, 1H, H-2).

^{13}C-NMR ($CDCl_3$), δ: (major and minor species) 13.52, 15.11 (CH_3, C-2'), 22.41, 22.69, 22.77, 22.95, 25.35, 26.13, 26.62, 26.74, 26.78, 26.86, 34.30, 34.35, 34.49 (CH_2), 44.43 (CH, C-9'), 119.78, 126.51 (C, C-aromatic), 126.72, 126.76, 126.91, 128.21 (CH, C-aromatic), 131.42, 133.63, 134.30, 135.24, 136.98 (C, C-aromatic), 141.36 (CH, C-aromatic), 147.81, 149.43, 149.57 (C, C-aromatic), 153.44 (CH, C-aromatic), 153.93, 156.65, 159.63 (C, C-aromatic).

2-{1-[(5,6,7,8-Tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazono]-ethyl}-phenol (222)

($C_{18}H_{18}N_4OS$; M.W.= 338.4)



193 **202** **222**

General procedure 20;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.10 g, 0.4 mmol);

Pale yellow solid;

Yield: 0.15 g (98%)

Melting Point: 250-252°C

MS (ESI^+): 339.1 $[M+H]^+$

Two species observed. Major/minor species ratio: 2/1.

^{1}H-NMR (DMSO-d_6), δ: (major species) 1.76-1.91 (m, 4H, C<u>H</u>$_2$), 2.52 (s, 3H, H-1'), 2.73-2.85 (m, 2H, C<u>H</u>$_2$), 2.97-3.19 (m, 2H, C<u>H</u>$_2$), 6.89-6.93 (m, 2H, H-aromatic), 7.27-7.31 (m, 1H, H-aromatic), 7.60-7.63 (m, 1H, H-aromatic), 7.75 (s, 1H, H-2), 11.40 (bs, 1H, N<u>H</u>), 12.52 (bs, 1H, O<u>H</u>).

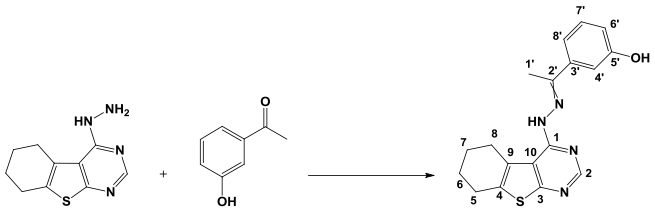
^{1}H-NMR (DMSO-d_6), δ: (minor species) 1.76-1.91(m, 4H, C<u>H</u>$_2$), 2.52 (s, 3H, H-1'), 2.73-2.85 (m, 2H, C<u>H</u>$_2$), 2.97-3.19 (m, 2H, C<u>H</u>$_2$), 6.89-6.93 (m, 2H, H-aromatic), 7.27-7.31 (m, 1H, H-aromatic), 7.64-7.67 (m, 1H, H-aromatic), 8.54 (s, 1H, H-2), 9.38 (bs,

1H, N<u>H</u>), 13.33 (bs, 1H, O<u>H</u>).

^{13}C-NMR (DMSO-d$_6$), δ: (major and minor species) 12.97, 14.83 (CH_3, C-1'), 22.08, 22.32, 24.69, 25.00, 26.50 (CH_2), 116.51, 117.28, 118.64 (CH, C-aromatic), 119.40, 120.96, 126.34 (C, C-aromatic), 128.03, 128.71, 130.69 (CH, C-aromatic), 132.61, 133.96 (C, C-aromatic), 144.02 (CH, C-aromatic), 145.33 (C, C-aromatic), 152.65 (CH, C-2), 153.91, 156.62, 158.22, 163.29 (C, C-aromatic).

3-{1-[(5,6,7,8-Tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazono]-ethyl}-phenol (223)

($C_{18}H_{18}N_4OS$; M.W.= 338.4)



General procedure 20;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.10 g, 0.4 mmol);

Pale orange solid;

Yield: 0.15 g (99%)

Melting Point: 307-309°C

MS (ESI$^+$): 339.1 $[M+H]^+$

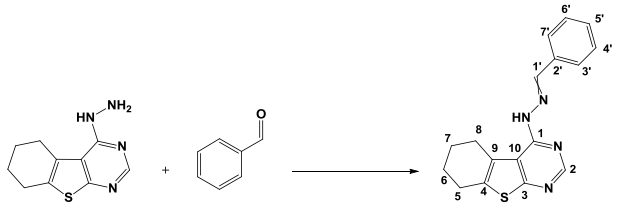
Single species observed.

^{1}H-NMR (DMSO-d$_6$), δ: 1.76-1.84 (m, 4H, C<u>H</u>$_2$), 2.40 (s, 3H, H-1'), 2.73-2.76 (m, 2H, C<u>H</u>$_2$), 3.01-3.05 (m, 2H, C<u>H</u>$_2$), 6.79-6.83 (m, 1H, H-aromatic), 7.19-7.22(m, 1H, H-aromatic), 7.37-7.39 (m, 1H, H-aromatic), 7.49 (d, J= 7.9 Hz, 1H, H-aromatic), 7.76 (s, 1H, H-2), 9.39 (bs, 1H, O<u>H</u>), 11.54 (bs, 1H, N<u>H</u>),

^{13}C-NMR (DMSO-d$_6$), δ: 14.47 (CH_3, C-1'), 22.14, 22.39, 24.66, 26.40 (CH_2), 113.54, 115.92, 117.51 (CH, C-aromatic), 119.61 (C, C-aromatic), 128.87 (CH, C-aromatic), 130.84, 132.20, 140.15 (C, C-aromatic), 144.01 (CH, C-aromatic), 147.25, 156.71, 157.16, 157.71 (C, C-aromatic).

_N_-Benzylidene-_N_'-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-_d_]pyrimidin-4-_yl_)-hydrazine (224)[31]

($C_{17}H_{16}N_4S$; M.W.= 308.4)



193 **204** **224**

General procedure 20;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-_d_]pyrimidin-4-_yl_)-hydrazine (**193**) (0.10 g, 0.4 mmol);

Pale yellow solid;

Yield: 0.06 g (46%)

Melting Point: 162-164°C (lit. 163°C)[31]

MS (ESI^+): 309.1 $[M+H]^+$

Two species observed. Major/minor species ratio: 3/2.

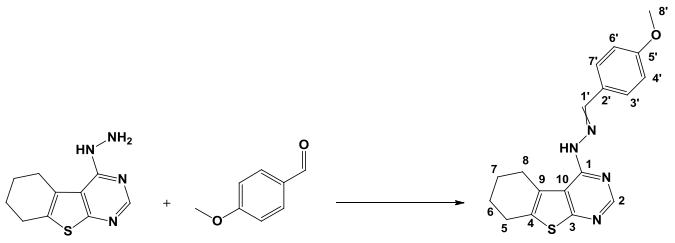
^{1}H-NMR ($CDCl_3$), δ: (major species) 1.85-1.91 (m, 4H, C<u>H</u>$_2$), 2.78-2.83 (m, 2H, C<u>H</u>$_2$), 3.04-3.12 (m, 2H, C<u>H</u>$_2$), 7.38-7.44 (m, 5H, H-aromatic), 7.70 (s, 1H, H-2), 8.50 (s, 1H, H-1'), 10.44 (bs, 1H, N<u>H</u>).

^{1}H-NMR ($CDCl_3$), δ: (minor species) 1.94-2.02 (m, 4H, C<u>H</u>$_2$), 2.86-2.89 (m, 2H, C<u>H</u>$_2$), 3.04-3.12 (m, 2H, C<u>H</u>$_2$), 7.75-7.82 (m, 5H, H-aromatic), 8.02 (s, 1H, H-1'), 8.61 (bs, 1H, N<u>H</u>), 8.65 (s, 1H, H-2).

^{13}C-NMR ($CDCl_3$), δ: (major and minor species) 22.42, 22.54, 22.91, 25.35, 25.60, 26.73, 26.93 (CH_2), 119.79, 124.25 (C, C-aromatic), 127.50, 127.64, 128.67, 129.93, 130.13 (CH, C-aromatic), 131.43, 132.55, 133.27, 134.18, 135.69 (C, C-aromatic), 141.17, 145.15, (CH, C-aromatic), 149.57 (C, C-aromatic), 153.17 (CH, C-aromatic), 153.79 (C, C-aromatic), 154.27 (CH, C-aromatic), 157.48 (C, C-aromatic).

***N*-(4-Methoxy-benzylidene)-*N*'-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (225)[31]**

($C_{18}H_{18}N_4OS$; M.W.= 338.4)



193 **205** **225**

General procedure 20;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.10 g, 0.4 mmol);

Yellow solid;

Yield: 0.11 g (75%)

Melting Point: 193-195°C (lit. 184-185 °C)[31]

MS (ESI^+): 361.1 $[M+Na]^+$

Two species observed. Major/minor species ratio: 5/4.

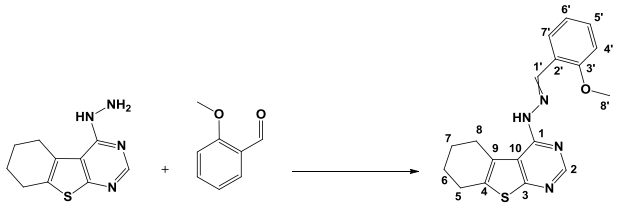
^{1}H-NMR ($CDCl_3$), δ: (major species) 1.82-1.91 (m, 4H, C<u>H</u>$_2$), 2.78-2.83 (m, 2H, C<u>H</u>$_2$), 3.04-3.11 (m, 2H, C<u>H</u>$_2$), 3.86 (s, 3H, H-8'), 6.94 (d, J= 8.8 Hz, 2H, H-aromatic), 7.66-7.75 (m, 3H, H-aromatic), 8.44 (s, 1H, C<u>H</u>), 10.40 (bs, 1H, N<u>H</u>).

^{1}H-NMR ($CDCl_3$), δ: (minor species) 1.93-2.03 (m, 4H, C<u>H</u>$_2$), 2.84-2.88 (m, 2H, C<u>H</u>$_2$), 3.04-3.11 (m, 2H, C<u>H</u>$_2$), 3.86 (s, 3H, H-8'), 6.94 (d, J= 8.8 Hz, 2H, H-aromatic), 7.66-7.75 (m, 2H, H-aromatic), 7.96 (s, 1H, C<u>H</u>), 8.53 (s, 1H, C<u>H</u>), 8.63 (s, 1H, N<u>H</u>).

^{13}C-NMR ($CDCl_3$), δ: (major and minor species) 22.57, 22.90, 25.44, 26.79 (CH_2), 55.37 (CH_3, C-8'), 114.50 (CH, C-aromatic), 119.82 (C, C-aromatic), 129.47 (CH, C-aromatic), 124.15 (C, C-aromatic), 131.55, 135.92 (C, C-aromatic), 141.20 (CH, C-aromatic), 149.51 (C, C-aromatic), 154.00 (CH, C-aromatic), 157.34, 166.56 (C, C-aromatic).

***N*-(2-Methoxy-benzylidene)-*N*'-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (226)**

($C_{18}H_{18}N_4OS$; M.W.= 338.4)



193 **206** **226**

General procedure 16;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.10 g, 0.4 mmol);

Yellow solid;

Yield: 0.11 g (75%)

Melting Point: 216-218°C

MS (ESI^+): 361.1 $[M+Na]^+$

Two species observed. Major/minor species ratio: 3/2.

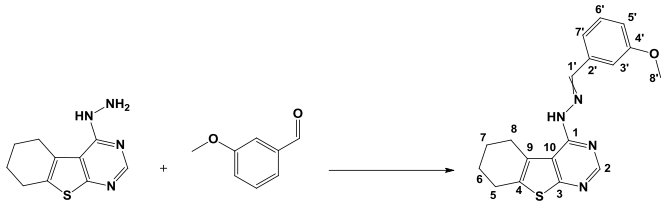
^{1}H-NMR ($CDCl_3$), δ: (major species) 1.92-2.02 (m, 4H, C<u>H</u>$_2$), 2.84-2.89 (m, 2H, C<u>H</u>$_2$), 3.04-3.12 (m, 2H, C<u>H</u>$_2$), 3.90 (s, 3H, H-8'), 6.90-6.94 (m, 1H, H-aromatic), 6.95-7.03 (m, 1H, H-aromatic), 7.34-7.39 (m, 1H, H-aromatic, H-2), 8.16 (d, J= 7.6 Hz, 1H, H-aromatic), 8.41 (s, 1H, H-2), 8.62-8.66 (m, 2H, C<u>H</u>, N<u>H</u>).

^{1}H-NMR ($CDCl_3$), δ: (minor species) 1.82-21.91 (m, 4H, C<u>H</u>$_2$), 2.77-2.81 (m, 2H, C<u>H</u>$_2$), 3.04-3.12 (m, 2H, C<u>H</u>$_2$), 3.90 (s, 3H, H-8'), 6.90-6.94 (m, 1H, H-aromatic), 6.95-7.03 (m, 1H, H-aromatic), 7.34-7.39 (m, 1H, H-aromatic, H-2), 7.67 (s, 1H, H-1'), 8.00 (d, J= 7.6 Hz, 1H, H-aromatic), 8.88 (s, 1H, H-2), 10.47 (bs, 1H, N<u>H</u>).

^{13}C-NMR ($CDCl_3$), δ: (major and minor species) 22.42, 22.59, 22.93, 25.35, 25.58, 26.73, 26.86 (CH_2), 55.54 (CH_3, C-8'), 110.80, 111.21 (CH, C-aromatic), 119.79 (C, C-aromatic), 120.60, 120.96 (CH, C-aromatic), 124.10, 124.25, 124.79 (C, C-aromatic), 126.59, 127.21, 131.16, 131.38 (CH, C-aromatic), 131.47, 132.59, 133.34, 134.89, 135.39 (C, C-aromatic), 141.10, 141.32 (CH, C-aromatic), 149.37 (C, C-aromatic), 150.20, 153.30 (CH, C-aromatic), 154.04, 157.65, 166.83 (C, C-aromatic).

***N*-(3-Methoxy-benzylidene)-*N*'-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (227)[31]**

($C_{18}H_{18}N_4OS$; M.W.= 338.4)



193 **207** **227**

General procedure 20;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.10 g, 0.4 mmol);

Yellow solid;

Yield: 0.09 g (57%)

Melting Point: 199-201°C

MS (ESI^+): 361.0 $[M+Na]^+$

Two species observed. Major/minor species ratio: 4/3.

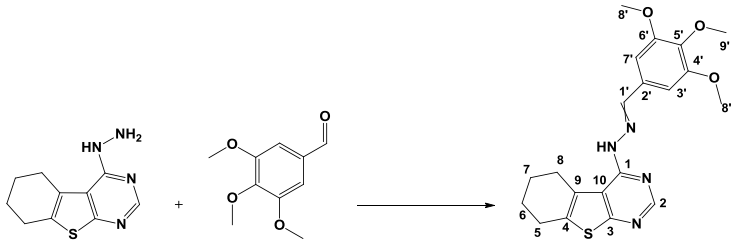
^{1}H-NMR ($CDCl_3$), δ: (major species) 1.84-1.92 (m, 4H, C<u>H</u>$_2$), 2.79-2.83 (m, 2H, C<u>H</u>$_2$), 3.06-3.11 (m, 2H, C<u>H</u>$_2$), 3.88 (s, 3H, H-8'), 6.95-6.99 (m, 1H, H-aromatic), 7.32-7.35 (m, 3H, H-aromatic, H-2), 7.71 (d, J= 2.8 Hz, 1H, H-aromatic), 8.47 (s, 1H, H-1'), 10.40 (bs, 1H, N<u>H</u>).

^{1}H-NMR ($CDCl_3$), δ: (minor species) 1.94-2.04 (m, 4H, C<u>H</u>$_2$), 2.87-2.91 (m, 2H, C<u>H</u>$_2$), 3.06-3.11 (m, 2H, C<u>H</u>$_2$), 3.89 (s, 3H, H-8'), 6.95-6.99 (m, 1H, H-aromatic), 7.32-7.35 (m, 2H, H-aromatic), 7.37-7.39 (m, 1H, H-aromatic), 8.01 (s, 1H, H-1'), 8.62 (bs, 1H, N<u>H</u>), 8.64 (s, 1H, H-2).

^{13}C-NMR ($CDCl_3$), δ: (major and minor species) 22.43, 22.52, 22.61, 22.90, 25.35, 25.62, 26.72, 26.96 (CH_2), 55.38, 55.47 (CH_3, C-8'), 111.45, 112.20, 115.82, 116.77 (CH, C-aromatic), 119.28 (C, C-aromatic), 120.67, 120.76 (CH, C-aromatic), 124.33, 124.44, 124.99 (C, C-aromatic), 129.70 (CH, C-aromatic), 131.43, 132.39, 133.74, 134.89, 136.60 (C, C-aromatic), 141.14, 145.22 (CH, C-aromatic), 149.73 (C, C-aromatic), 153.14, 154.21 (CH, C-aromatic), 154.00, 157.46, 166.23 (C, C-aromatic).

_N_-(5,6,7,8-Tetrahydro-benzo[4,5]thieno[2,3-_d_]pyrimidin-4-_yl_)-_N_'-(3,4,5-trimethoxy-benzylidene)-hydrazine (228)[31]

($C_{20}H_{22}N_4O_3S$; M.W.= 398.4)



193 **208** **228**

General procedure 20;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.10 g, 0.4 mmol);

Yellow solid;

Yield: 0.16 g (91%)

Melting Point: 224-226°C (lit. 200°C)[31]

MS (ESI^+): 399.1 $[M+H]^+$

Two species observed. Major/minor species ratio: 4/3.

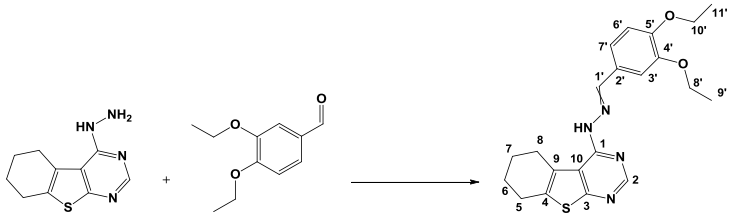
^{1}H-NMR ($CDCl_3$), δ: (major species) 1.83-1.92 (m, 4H, C<u>H</u>$_2$), 2.78-2.82 (m, 2H, C<u>H</u>$_2$), 3.06-3.11 (m, 2H, C<u>H</u>$_2$), 3.91 (s, 3H, OC<u>H</u>$_3$), 3.92 (s, 3H, OC<u>H</u>$_3$), 3.95 (s, 3H, OC<u>H</u>$_3$), 7.00-7.02 (m, 2H, H-aromatic), 7.68 (s, 1H, H-2), 8.44 (s, 1H, H-1'), 10.43 (bs, 1H, N<u>H</u>).

^{1}H-NMR ($CDCl_3$), δ: (minor species) 1.93-2.03 (m, 4H, C<u>H</u>$_2$), 2.86-2.90 (m, 2H, C<u>H</u>$_2$), 3.06-3.11 (m, 2H, C<u>H</u>$_2$), 3.90 (s, 3H, OC<u>H</u>$_3$), 3.93 (s, 3H, OC<u>H</u>$_3$), 3.94 (s, 3H, OC<u>H</u>$_3$), 7.00-7.02 (m, 2H, H-aromatic), 7.98 (s, 1H, H-1'), 8.59 (bs, 1H, NH), 8.62 (s, 1H, H-2).

^{13}C-NMR ($CDCl_3$), δ: (major and minor species) 22.43, 22.53, 22.62, 22.90, 25.35, 25.61, 26.75, 26.97 (CH_2), 56.22, 56.32, 60.99 (CH_3, C-8', 9'), 104.66, 104.75 (CH, C-aromatic), 119.40, 124.37, 124.54, 124.89, 128.99, 130.57, 131.41, 133.91, 135.27, 136.75 (C, C-aromatic), 141.17, 145.39 (CH, C-aromatic), 148.99 (C, C-aromatic), 153.06 (CH, C-aromatic), 153.50 (C, C-aromatic), 154.55 (CH, C-aromatic), 154.13, 157.65, 166.12 (C, C-aromatic).

***N*-(3,4-Diethoxy-benzylidene)-*N*'-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-d]pyrimidin-4-*yl*)-hydrazine (229)**

($C_{21}H_{24}N_4O_2S$; M.W.= 396.5)



193 **209** **229**

General procedure 20;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.10 g, 0.4 mmol);

Yellow solid;

Yield: 0.13 g (77%)

Melting Point: 237-239°C

MS (ESI^+): 397.1 $[M+H]^+$

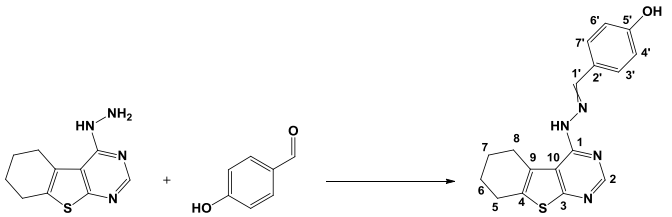
Single species observed.

^{1}H-NMR (DMSO-d$_6$), δ: 1.33-1.39 (m, 6H, H-9', 11'), 1.74-1.85 (m, 4H, C<u>H</u>$_2$), 2.73-2.78 (m, 2H, C<u>H</u>$_2$), 2.96-3.01 (m, 2H, C<u>H</u>$_2$), 4.06-4.15 (m, 4H, H-8', 10'), 7.00 (d, J= 8.3 Hz, 1H, H-aromatic), 7.35 (d, J= 8.3 Hz, 1H, H-aromatic), 7.62 (s, 1H, H-aromatic), 7.78 (s, 1H, H-aromatic), 8.31 (s, 1H, H-2), 8.44 (s, 1H, H-1'), 11.69 (bs, 1H, N<u>H</u>).

^{13}C-NMR (DMSO-d$_6$), δ: 14.66, 14.78 (CH_3, C-9', 11'), 22.00, 22.39, 24.64, 26.55 (CH_2), 63.76, 64.05 (CH_2, C-8', 11'), 111.76, 112.73 (CH, C-aromatic), 118.89 (C, C-aromatic), 122.27 (CH, C-aromatic), 128.15, 130.85, 132.05 (C, C-aromatic), 143.90 (CH, C-aromatic), 148.25, 150.08 (C, C-aromatic), 153.15 (CH, C-aromatic), 156.80 (C, C-aromatic).

4-[(5,6,7,8-Tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazonomethyl]-phenol (230)[31]

($C_{17}H_{16}N_4OS$; M.W.= 324.4)



193 **210** **230**

General procedure 20;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.10 g, 0.4 mmol);

Pale yellow solid;

Yield: 0.05 g (34%)

Melting Point: 255-257°C

MS (ESI^+): 325.1 $[M+H]^+$

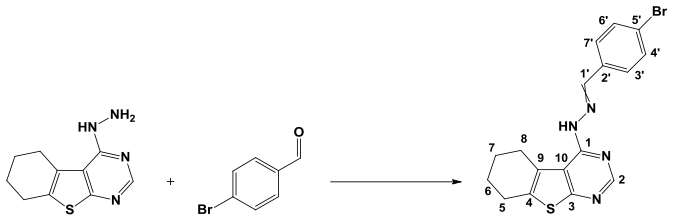
Single species observed.

^{1}H-NMR (DMSO-d_6), δ: 1.74-1.84 (m, 4H, C<u>H</u>$_2$), 2.70-2.77 (m, 2H, C<u>H</u>$_2$), 2.95-3.02 (m, 2H, C<u>H</u>$_2$), 6.83 (d, J= 8.3 Hz, 1H, H-aromatic), 7.72-7.80 (m, 3H, H-aromatic, 1'), 8.29 (s, 1H, H-2), 9.83 (bs, 1H, O<u>H</u>), 11.67 (bs, 1H, N<u>H</u>).

^{13}C-NMR (DMSO-d_6), δ: 22.00, 22.37, 24.64, 26.51 (CH_2), 115.39 (CH, C-aromatic), 118.89, 126.53 (C, C-aromatic), 129.45 (CH, C-aromatic), 131.90, 132.25 (C, C-aromatic), 143.93 (CH, C-aromatic), 148.99 (C, C-aromatic), 152.95 (CH, C-aromatic), 156.87, 159.16 (C, C-aromatic).

***N*-(4-Bromo-benzylidene)-*N*'-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (231)[31]**

($C_{17}H_{15}BrN_4S$; M.W.= 387.3)



General procedure 20;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.10 g, 0.4 mmol);

Yellow solid;

Yield: 0.06 g (36%)

Melting Point: 180-182°C

MS (ESI^+): 387.0, 389.0 $[M+H]^+$

Two species observed. Major/minor species ratio: 3/2.

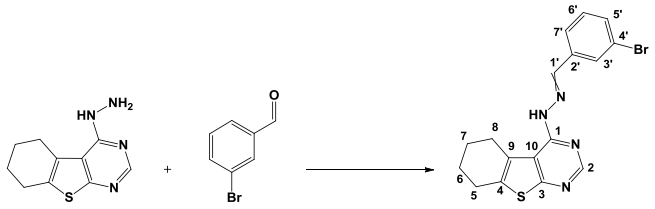
^{1}H-NMR ($CDCl_3$), δ: major speices- 1.84-1.92 (m, 4H, C<u>H</u>$_2$), 2.79-2.84 (m, 2H, C<u>H</u>$_2$), 3.04-3.10 (m, 2H, C<u>H</u>$_2$), 7.53-7.57 (m, 2H, H-aromatic), 7.63 (d, J= 8.3 Hz, 2H, H-aromatic), 7.73 (s, 1H, H-2), 8.43 (s, 1H, H-1'), 10.39 (bs, 1H, N<u>H</u>).

^{1}H-NMR ($CDCl_3$), δ: (minor species) 1.94-2.03 (m, 4H, C<u>H</u>$_2$), 2.87-2.91 (m, 2H, C<u>H</u>$_2$), 3.04-3.10 (m, 2H, C<u>H</u>$_2$), 7.53-7.57 (m, 2H, H-aromatic), 7.66 (d, J= 8.3 Hz, 2H, H-aromatic), 7.98 (s, 1H, H-1'), 8.64 (s, 1H, H-2), 8.65 (bs, 1H, N<u>H</u>).

^{13}C-NMR ($CDCl_3$), δ: (major and minor species) 22.30, 22.53, 22.88, 25.37, 25.57, 26.74 (CH_2), 119.80, 124.02, 124.30, 124.59 (C, C-aromatic), 128.87, 129.39 (CH, C-aromatic), 131.39 (C, C-aromatic), 132.03, 132.11 (CH, C-aromatic), 132.49, 133.05, 134.02, 134.18, 135.49 (C, C-aromatic), 141.14, 143.78 (CH, C-aromatic), 149.57 (C, C-aromatic), 153.03 (CH, C-aromatic), 153.86, 157.53 (C, C-aromatic), 161.14 (CH, C-aromatic).

***N*-(3-Bromo-benzylidene)-*N*'-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (232)**

($C_{17}H_{15}BrN_4S$; M.W.= 387.3)



193 **212** **232**

General procedure 20;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.10 g, 0.4 mmol);

Yellow solid;

Yield: 0.11 g (65%)

Melting Point: 222-224°C

MS (ESI^+): 387.0, 389.0 $[M+H]^+$

Two species observed. Major/minor species ratio: 2/1.

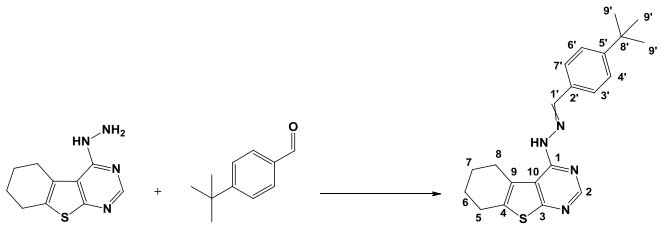
^{1}H-NMR ($CDCl_3$), δ: (major species) 1.83-1.92 (m, 4H, C<u>H</u>$_2$), 2.77-2.83 (m, 2H, C<u>H</u>$_2$), 3.04-3.12 (m, 2H, C<u>H</u>$_2$), 7.26-7.30 (m, 1H, H-aromatic), 7.49-7.54 (m, 1H, H-aromatic), 7.75 (d, J= 2.6 Hz, 1H, H-3'), 7.97-7.99 (m, 2H, H-aromatic), 8.41 (s, 1H, H-aromatic), 10.40 (bs, 1H, N<u>H</u>).

^{1}H-NMR ($CDCl_3$), δ: (minor species) 1.94-2.05 (m, 4H, C<u>H</u>$_2$), 2.86-2.96 (m, 2H, C<u>H</u>$_2$), 3.04-3.12 (m, 2H, C<u>H</u>$_2$), 7.26-7.30 (m, 1H, H-aromatic), 7.49-7.54 (m, 1H, H-aromatic), 7.58-7.61 (m, 2H, H-aromatic), 7.66-7.69 (m, 1H, H-aromatic), 8.65-8.67 (m, 2H, H-aromatic, N<u>H</u>).

^{13}C-NMR ($CDCl_3$), δ: (major and minor species) 22.30, 22.53, 22.88, 25.37, 25.57, 26.74 (CH_2), 119.87, 124.07, 124.41, 124.79 (C, C-aromatic), 126.08, 126.69, 129.68, 129.94, 130.19, 132.60 (CH, C-aromatic), 132.77 (C, C-aromatic), 132.95 (CH, C-aromatic), 133.11, 134.23, 134.38, 135.09 (C, C-aromatic), 137.39, 141.06, 143.23 (CH, C-aromatic), 149.66 (C, C-aromatic), 152.34 (CH, C-aromatic), 153.75, 157.41 (C, C-aromatic), 161.07 (CH, C-aromatic).

***N*-(4-*tert*-Butyl-benzylidene)-*N*'-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine 233)[31]**

($C_{21}H_{24}N_4S$; M.W.= 364.5)



193 **213** **233**

General procedure 20;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.10 g, 0.4 mmol);

Yellow solid;

Yield: 0.10 g (61%)

Melting Point: 221-223°C

MS (ESI^+): 365.1 $[M+H]^+$

Two species observed. Major/minor species ratio: 3/2.

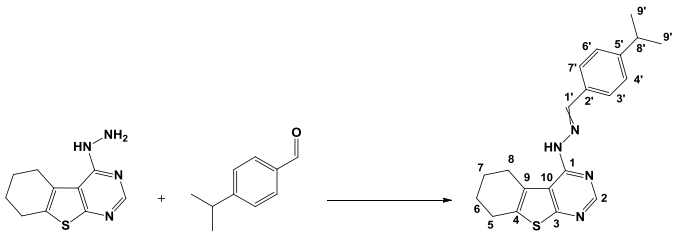
^{1}H-NMR ($CDCl_3$), δ: (major species) 1.36 (s, 9 H, H-9'), 1.85-1.90 (m, 4H, C$\underline{H}_2$), 2.78-2.81 (m, 2H, C$\underline{H}_2$), 3.05-3.12 (m, 2H, C$\underline{H}_2$), 7.42-7.45 (m, 2H, H-aromatic), 7.63 (s, 1H, C$\underline{H}$), 7.69 (d, J= 8.4 Hz, 2H, H-aromatic), 8.48 (s, 1H, C$\underline{H}$), 10.48 (bs, 1H, N$\underline{H}$).

^{1}H-NMR ($CDCl_3$), δ: (minor species) 1.36 (s, 9 H, H-9'), 1.93-2.02 (m, 4H, C$\underline{H}_2$), 2.85-2.89 (m, 2H, C$\underline{H}_2$), 3.05-3.12 (m, 2H, C$\underline{H}_2$), 7.42-7.45 (m, 2H, H-aromatic), 7.71 (d, J= 8.4 Hz, 2H, H-aromatic), 7.99 (s, 1H, C$\underline{H}$), 8.58 (bs, 1H, N$\underline{H}$), 8.64 (s, 1H, C$\underline{H}$).

^{13}C-NMR ($CDCl_3$), δ: (major and minor species) 22.43, 22.54, 22.62, 22.92, 25.46, 25.60, 26.73, 26.95 (CH_2), 31.22 (CH_3, C-9'), 34.89, 34.91 (C, C-8'), 115.29, 119.83, 124.83 (C, C-aromatic), 125.63, 126.45, 127.34, 127.57 (CH, C-aromatic), 130.75, 131.42, 132.49, 133.69, 135.01 (C, C-aromatic), 141.28, 145.31 (CH, C-aromatic), 149.18 (C, C-aromatic), 152.19 (CH, C-aromatic), 153.38, 153.63, 154.14 (C, C-aromatic), 154.28 (CH, C-aromatic), 157.13, 166.98 (C, C-aromatic).

***N*-(4-Isopropyl-benzylidene)-*N*'-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (234)[31]**

($C_{20}H_{22}N_4S$; M.W.= 350.4)



193 **214** **234**

General procedure 20;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.10 g, 0.4 mmol);

Yellow solid;

Yield: 0.06 g (38%)

Melting Point: 195-197°C

MS (ESI^+): 373.1 $[M+Na]^+$

Two species observed. Major/minor species ratio: 3/2.

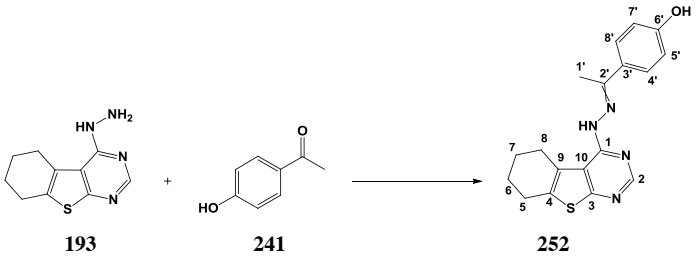
^{1}H-NMR ($CDCl_3$), δ: (major species) 1.29 (d, J= 5.4 Hz, 6 H, H-9'), 1.84-1.92 (m, 4H, C<u>H</u>$_2$), 2.78-2.83 (m, 2H, C<u>H</u>$_2$), 2.93-2.99 (m, 1H, H-8'), 3.06-3.12 (m, 2H, C<u>H</u>$_2$), 7.27-7.30 (m, 2H, H-aromatic), 7.68-7.74 (m, 3H, H-aromatic), 8.48 (s, 1H, C<u>H</u>), 10.41 (bs, 1H, N<u>H</u>).

^{1}H-NMR ($CDCl_3$), δ: (minor species) 1.29 (d, J= 5.4 Hz,\ 6 H, H-9'), 1.94-2.03 (m, 4H, C<u>H</u>$_2$), 2.86-2.89 (m, 2H, C<u>H</u>$_2$), 2.93-2.99 (m, 1H, H-8'), 3.06-3.12 (m, 2H, C<u>H</u>$_2$), 7.27-7.30 (m, 2H, H-aromatic), 7.68-7.74 (m, 2H, H-aromatic), 8.00 (s, 1H, C<u>H</u>), 8.57 (bs, 1H, N<u>H</u>), 8.64 (s, 1H, C<u>H</u>).

^{13}C-NMR ($CDCl_3$), δ: (major and minor species) 22.44, 22.54, 22.72, 22.92 (CH_2), 23.81 (CH_3, C-9'), 25.35, 25.60, 26.72, 26.93 (CH_2), 34.13 (CH, C-8'), 115.29, 119.72, 124.81 (C, C-aromatic), 125.99, 126.80, 127.61, 127.72 (CH, C-aromatic), 130.65, 131.32, 132.60, 133.75, 135.06 (C, C-aromatic), 141.20, 145.37 (CH, C-aromatic), 149.10 (C, C-aromatic), 153.21 (CH, C-aromatic), 153.47, 153.60, 154.23 (C, C-aromatic), 154.37 (CH, C-aromatic), 157.02, 166.96 (C, C-aromatic).

4-{1-[(5,6,7,8-Tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazono]-ethyl}-phenol (252)

($C_{18}H_{18}N_4OS$; M.W.= 338.4)



General procedure 20;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.10 g, 0.4 mmol);

Orange solid;

Yield: 0.10 g (66%)

Melting Point: 263-265°C

MS (ESI^+): 339.1 $[M+H]^+$

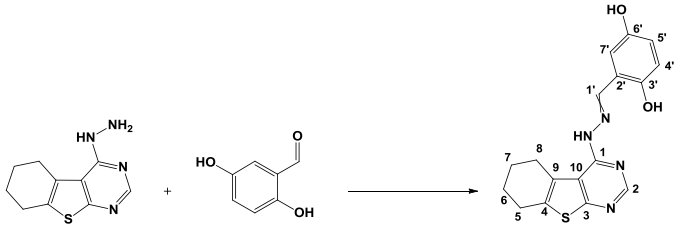
Single species observed.

^{1}H-NMR (DMSO-d_6), δ: 1.74-1.83 (m, 4H, C<u>H</u>$_2$), 2.39 (s, 3H, H-1'), 2.71-2.75 (m, 2H, C<u>H</u>$_2$), 2.99-3-03 (m, 2H, C<u>H</u>$_2$), 6.80 (d, J= 8.7 Hz, 2H, H-aromatic), 7.74 (s, 1H, H-2), 7.90 (d, J= 8.7 Hz, 2H, H-aromatic), 9.68 (bs, 1H, O<u>H</u>), 11.48 (bs, 1H, N<u>H</u>),

^{13}C-NMR (DMSO-d_6), δ: 14.03 (CH_3, C-1'), 22.15, 22.40, 24.65, 26.38 (CH_2), 114.84 (CH, C-aromatic), 119.69 (C, C-aromatic), 128.11 (CH, C-aromatic), 129.71, 130.83, 131.97 (C, C-aromatic), 144.02 (CH, C-aromatic), 146.70, 156.31, 157.41, 158.51 (C, C-aromatic).

2-[(5,6,7,8-Tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazonomethyl]-benzene-1,4-diol (253)

($C_{17}H_{16}N_4O_2S$; M.W.= 340.4)



General procedure 20;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.10 g, 0.4 mmol);

Yellow solid;

Yield: 0.09 g (61%)

Melting Point: 235-237°C

MS (ESI^+): 341.1 $[M+H]^+$

Two species observed. Major/minor species ratio: 2/1.

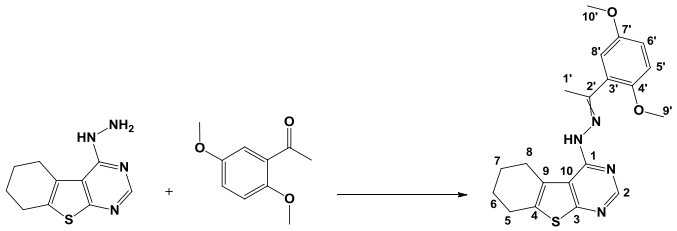
^{1}H-NMR (DMSO-d_6), δ: (major species) 1.75-1.88 (m, 4H, C$\underline{H}_2$), 2.71-2.86 (m, 2H, C$\underline{H}_2$), 2.97-3.14 (m, 2H, C$\underline{H}_2$), 6.71-6.76 (m, 2H, H-aromatic), 7.19 (s, 1H, H-aromatic), 7.76 (s, 1H, C$\underline{H}$), 8.54 (s, 1H, H-aromatic), 8.91 (bs, 1H, O$\underline{H}$), 9.60 (bs, 1H, N$\underline{H}$), 11.48 (bs, 1H, O$\underline{H}$).

^{1}H-NMR (DMSO-d_6), δ: (minor species) 1.75-1.88 (m, 4H, C$\underline{H}_2$), 2.71-2.86 (m, 2H, C$\underline{H}_2$), 2.97-3.14 (m, 2H, C$\underline{H}_2$), 6.71-6.76 (m, 2H, H-aromatic), 6.92 (s, 1H, aromatic), 8.48 (s, 1H, H-aromatic), 8.54 (s, 1H, H-aromatic), 8.91 (bs, 1H, O$\underline{H}$), 10.09 (bs, 1H, N$\underline{H}$), 10.75 (bs, 1H, O$\underline{H}$).

^{13}C-NMR (DMSO-d_6), δ: (major and minor species) 21.98, 22.13, 22.35, 24.67, 25.02, 25.51, 26.59 (CH_2), 114.15, 114.47, 116.61, 116.97, 118.30, 118.60 (CH, C-aromatic), 118.81, 119.42, 120.45, 126.46, 130.86, 132.24, 133.13 (C, C-aromatic), 143.93, 146.48 (CH, C-aromatic), 147.54, 149.81, 149.94 (C, C-aromatic), 152.55, 153.36 (CH, C-aromatic), 156.97 (C, C-aromatic).

4-(2-(1-(2,5-dimethoxyphenyl)ethylidene)hydrazinyl)-5,6,7,8-tetrahydrobenzo [4,5]thieno[2,3-*d*]pyrimidine (254)

($C_{20}H_{22}N_4O_2S$; M.W.= 382.4)



193 **242** **254**

General procedure 20;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.10 g, 0.4 mmol);

Purification: re-crystallisation from DCM/ *n*hexane;

Grey solid;

Yield: 0.07 g (41%)

Melting Point: 143-145°C

MS (ESI^+): 383.1 $[M+H]^+$

Two species observed. Major/minor species ratio: 3/2.

^{1}H-NMR (DMSO-d_6), δ: (major species) 1.57-1.63 (m, 2H, C$\underline{H}_2$), 1.68-1.77 (m, 2H, C$\underline{H}_2$), 2.10-2.15 (m, 2H, C$\underline{H}_2$), 2.29 (s, 3H, H-1'), 2.69-2.75 (m, 2H, C$\underline{H}_2$), 3.75 (s, 3H, OC$\underline{H}_3$), 3.76 (s, 3H, OC$\underline{H}_3$), 6.91-6.95 (m, 1H, H-aromatic), 7.06-7.12 (m, 1H, H-aromatic), 7.18 (d, J= 9.0 Hz, 1H, H-aromatic), 8.38 (s, 1H, H-2), 8.40 (bs, 1H, N$\underline{H}$).

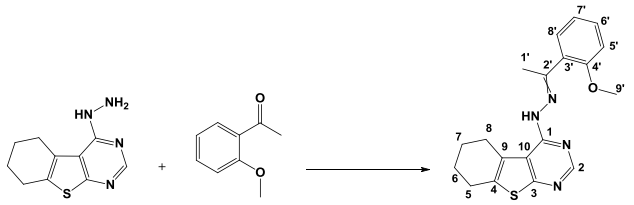
^{1}H-NMR (DMSO-d_6), δ: (minor species) 1.45-1.51 (m, 2H, C$\underline{H}_2$), 1.68-1.77 (m, 2H, C$\underline{H}_2$), 2.29 (s, 3H, H-1'), 2.32-2.39 (m, 2H, C$\underline{H}_2$), 2.61-2.65 (m, 2H, C$\underline{H}_2$), 3.65 (s, 3H, OC$\underline{H}_3$), 3.69 (s, 3H, OC$\underline{H}_3$), 6.70-6.73 (m, 1H, H-aromatic), 6.83-6.87 (m, 1H, H-aromatic), 6.91-6.95 (m, 1H, H-aromatic), 7.63 (s, 1H, H-2), 11.43 (bs, 1H, N$\underline{H}$).

^{13}C-NMR (DMSO-d_6), δ: (major and minor species) 21.71, 21.88, 22.34 (CH_2), 23.86, 23.98 (CH_3, C-1'), 24.48, 24.74, 24.81, 25.82 (CH_2), 55.44, 55.68, 55.86, 56.21 (CH_3, C-9', 10'), 112.11, 113.48, 113.57 (CH, C-aromatic), 114.30 (C, C-aromatic), 114.37, 114.64, 115.97 (CH, C-aromatic), 119.22, 123.49, 125.00, 129.34, 130.84, 131.56, 133.37 (C, C-aromatic), 143.82 (CH, C-aromatic), 145.63, 148.89, 149.49, 150.40 (C, C-aromatic), 152.58 (CH, C-aromatic), 152.79, 153.28, 153.89, 155.88, 157.89, 165.21

(C, C-aromatic).

***N*-[1-(2-Methoxy-phenyl)-ethylidene]-*N*'-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (255)**

($C_{19}H_{20}N_4OS$; M.W.= 352.4)



193 **243** **255**

General procedure 20;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.10 g, 0.4 mmol);

T.L.C. System: *n*hexane -EtOAc 5:5v/v, Rf: 0.61.

Purification: flash column chromatography (*n*hexane:EtOAc 100:0 v/v increasing to 70:30 v/v).

Yellow solid;

Yield: 0.08 g (51%)

Melting Point: 61-63°C

MS (ESI^+): 353.1 $[M+H]^+$

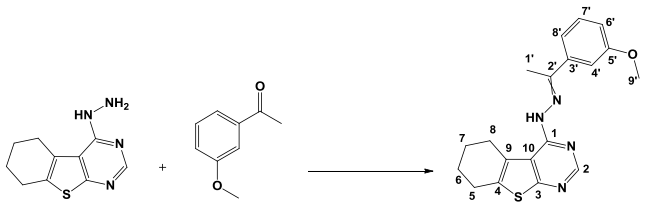
Single species observed.

^{1}H-NMR (DMSO-d_6), δ: 1.76-1.83 (m, 4H, C<u>H</u>$_2$), 2.35 (s, 3H, H-1'), 2.71-2.76 (m, 2H, C<u>H</u>$_2$), 2.99-3.04 (m, 2H, C<u>H</u>$_2$), 3.82 (s, 3H, H-9'), 6.96-7.02 (m, 1H, H-aromatic), 7.04-7.10 (m, 1H, aromatic), 7.34-7.41 (m, 1H, H-aromatic), 7.46-7.52 (m, 1H, H-aromatic), 7.67 (s, 1H, H-2), 11.46 (bs, 1H, N<u>H</u>).

^{13}C-NMR (DMSO-d_6), δ: 18.60 (CH_3, C-1'), 22.15, 22.42, 25.70, 26.35 (CH_2), 55.52 (CH_3, C-9'), 111.47 (CH, C-aromatic), 119.52 (C, C-aromatic), 120.25, 129.69, 129.78 (CH, C-aromatic), 129.84, 130.88, 132.01 (C, C-aromatic), 143.95 (CH, C-aromatic), 147.22, 156.58, 157.29, 159.88 (C, C-aromatic).

***N*-[1-(3-Methoxy-phenyl)-ethylidene]-*N*'-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (256)**

($C_{19}H_{20}N_4OS$; M.W.= 352.4)



193 **244** **256**

General procedure 20;

Reagent(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.10 g, 0.4 mmol);

Purification: re-crystallisation from EtOH/H_2O;

Yellow solid;

Yield: 0.05g (35%)

Melting Point: 137-139°C

MS (ESI^+): 353.1 $[M+H]^+$

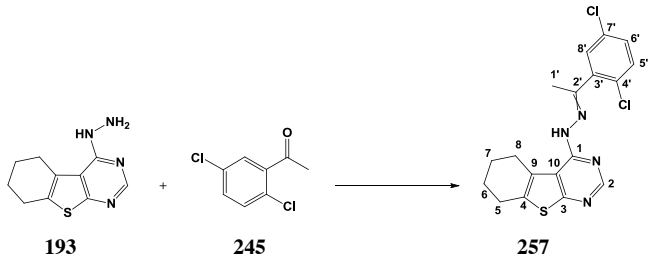
Single species observed.

^{1}H-NMR (DMSO-d_6), δ: 1.76-1.83 (m, 4H, C<u>H</u>$_2$), 2.43 (s, 3H, H-1'), 2.73-2.77 (m, 2H, C<u>H</u>$_2$), 3.01-3.05 (m, 2H, C<u>H</u>$_2$), 3.83 (s, 3H, H-9'), 6.97-7.00 (m, 1H, H-aromatic), 7.32-7.36 (m, 1H, aromatic), 7.56-7.60 (m, 2H, H-aromatic), 7.79 (s, 1H, H-2), 11.58 (bs, 1H, N<u>H</u>).

^{13}C-NMR (DMSO-d_6), δ: 14.41 (CH_3, C-1'), 22.14, 22.37, 24.68, 26.36 (CH_2), 55.13 (CH_3, C-9'), 112.35, 114.19, 119.11 (CH, C-aromatic), 119.57 (C, C-aromatic), 128.81 (CH, C-aromatic), 130.79, 132.29, 140.13 (C, C-aromatic), 143.98 (CH, C-aromatic), 147.46, 156.89, 157.41, 159.19 (C, C-aromatic).

***N*-[1-(2,5-Dichloro-phenyl)-ethylidene]-*N*'-(5,6,7,8-tetrahydro-benzo[4,5]thieno [2,3-*d*]pyrimidin-4-*yl*)-hydrazine (257)**

($C_{18}H_{16}Cl_2N_4S$; M.W.= 391.3)



193 **245** **257**

General procedure 20;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.10 g, 0.4 mmol);

T.L.C. System: *n*hexane -EtOAc 7:3 v/v, Rf: 0.57

Purification: flash column chromatography (*n*hexane:EtOAc 100:0 v/v increasing to 70:30 v/v).

Yellow solid;

Yield: 0.08 g (46%)

Melting Point: 82-84°C

MS (ESI^+): 391.0, 393.0 $[M+H]^+$

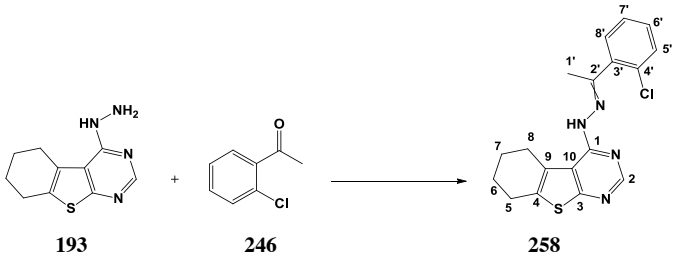
Single species observed.

^{1}H-NMR (DMSO-d_6), δ: 1.76-1.84 (m, 4H, C<u>H</u>$_2$), 2.38 (s, 3H, H-1'), 2.74-2.77 (m, 2H, C<u>H</u>$_2$), 3.01-3.04 (m, 2H, C<u>H</u>$_2$), 7.49 (dd, J_1= 8.6 Hz, J_2= 2.5 Hz, 1H, H-6'), 7.55 (d, J= 8.6 Hz, 1H, H-5'), 7.61 (d, J= 2.5 Hz, 1H, H-8'), 7.71(s, 1H, H-2), 11.58 (bs, 1H, N<u>H</u>).

^{13}C-NMR (DMSO-d_6), δ: 18.48 (CH_3, C-1'), 22.13, 22.38, 24.67, 26.35 (CH_2), 119.27 (C, C-aromatic), 129.48 (CH, C-aromatic), 130.07 (C, C-aromatic), 130.30 (CH, C-aromatic), 130.87 (C, C-aromatic), 131.31 (CH, C-aromatic), 131.69, 132.45, 141.30 (C, C-aromatic), 143.77 (CH, C-aromatic), 148.26, 157.29, 157.65 (C, C-aromatic).

***N*-[1-(2-Chloro-phenyl)-ethylidene]-*N*'-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (258)**

($C_{18}H_{17}ClN_4S$; M.W.= 356.8)



193 **246** **258**

General procedure 20;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.10 g, 0.4 mmol);

T.L.C. System: *n*hexane -EtOAc 7:3 v/v, Rf: 0.52

Purification: flash column chromatography (*n*hexane:EtOAc 100:0 v/v increasing to 50:50 v/v).

Yellow solid;

Yield: 0.08 g (51%)

Melting Point: 77-79°C

MS (ESI^+): 357.1, 359.1 $[M+H]^+$

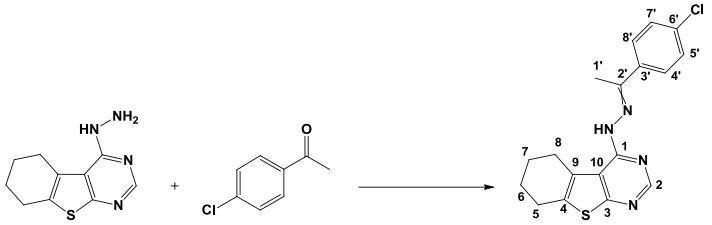
Single species observed.

^{1}H-NMR (DMSO-d_6), δ: 1.76-1.85 (m, 4H, C<u>H</u>$_2$), 2.39 (s, 3H, H-1'), 2.73-2.77 (m, 2H, C<u>H</u>$_2$), 3.02-3.05 (m, 2H, C<u>H</u>$_2$), 7.38-7.44 (m, 2H, H-aromatic), 7.48-7.56 (m, 2H, H-aromatic), 7.68 (s, 1H, H-2), 11.50 (bs, 1H, N<u>H</u>).

^{13}C-NMR (DMSO-d_6), δ: 18.78 (CH_3, C-1'), 22.14, 22.40, 24.66, 26.36 (CH_2), 119.30 (C, C-aromatic), 127.04, 129.55, 129.76 (CH, C-aromatic), 130.72 (C, C-aromatic), 130.88 (CH, C-aromatic), 131.26, 132.29, 139.82 (C, C-aromatic), 143.81 (CH, C-aromatic), 147.93, 157.03, 159.00 (C, C-aromatic).

***N*-[1-(4-Chloro-phenyl)-ethylidene]-*N*'-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (259)**

($C_{18}H_{16}ClN_4S$; M.W.= 356.8)



193 **247** **259**

General procedure 20;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.10 g, 0.4 mmol);

Yellow solid;

Yield: 0.12 g (77%)

Melting Point: 199-201 °C

MS (ESI^+): 357.1, 359.1 $[M+H]^+$

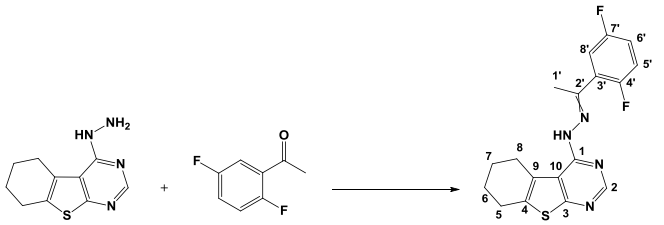
Single species observed.

^{1}H-NMR (DMSO-d_6), δ: 1.74-1.84 (m, 4H, C<u>H</u>$_2$), 2.42 (s, 3H, H-1'), 2.72-2.76 (m, 2H, C<u>H</u>$_2$), 2.99-3.04 (m, 2H, C<u>H</u>$_2$), 7.46 (d, J= 8.3 Hz, 2H, H-aromatic), 7.79 (s, 1H, H-2), 8.06 (d, J= 8.3 Hz, 2H, H-aromatic), 11.65 (bs, 1H, N<u>H</u>).

^{13}C-NMR (DMSO-d_6), δ: 13.99 (CH_3, C-1'), 22.12, 22.36, 24.67, 26.37 (CH_2), 119.29 (C, C-aromatic), 128.02, 128.21 (CH, C-aromatic), 130.82, 132.34, 133.55, 137.44 (C, C-aromatic), 143.90 (CH, C-aromatic), 147.74, 156.23, 157.02 (C, C-aromatic).

***N*-[1-(2,5-Difluoro-phenyl)-ethylidene]-*N*'-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (260)**

($C_{18}H_{17}F_2N_4S$; M.W.= 358.4)



193 **248** **260**

General procedure 20;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.10 g, 0.4 mmol);

Purification: re-crystallisation from EtOH/H_2O;

Yellow solid;

Yield: 0.07 g (45%)

Melting Point: 131-133°C

MS (ESI^+): 359.1 $[M+H]^+$

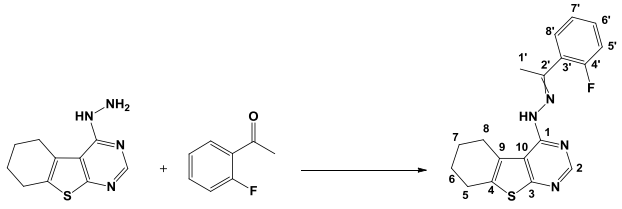
Single species observed.

^{1}H-NMR (DMSO-d$_6$), δ: 1.74-1.83 (m, 4H, C<u>H</u>$_2$), 2.42 (d, J= 4.1 Hz, 3H, H-1'), 2.72-2.76 (m, 2H, C<u>H</u>$_2$), 2.99-3.03 (m, 2H, C<u>H</u>$_2$), 7.24-7.32 (m, 2H, H-aromatic), 7.77 (s, 1H, H-2), 7.82-7.87 (m, 1H, H-aromatic), 11.64 (bs, 1H, N<u>H</u>).

^{13}C-NMR (DMSO-d$_6$), δ: 17.36, 17.42 (CH_3, C-1'), 22.11, 22.35, 24.66, 26.34 (CH_2), 115.71, 115.74, 115.90, 115.94, 116.81, 116.88, 117.01, 117.08, 117.61, 117.68, 117.82, 117.89 (CH, C-5', 6', 8'), 119.43, 128.83, 128.89, 130.83, 132.49 (C, C-aromatic), 143.77 (CH, C-2), 148.21, 154.29, 155.62, 157.06, 157.32, 157.55, 158.95 (C, C-aromatic).

***N*-[1-(2-Fluoro-phenyl)-ethylidene]-*N*'-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (261)**

($C_{18}H_{17}FN_4S$; M.W.= 340.4)



193 **249** **261**

General procedure 20;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.10 g, 0.4 mmol);

Yellow solid;

Yield: 0.06 g (42%)

Melting Point: 65-67°C

MS (ESI^+): 341.1 $[M+H]^+$

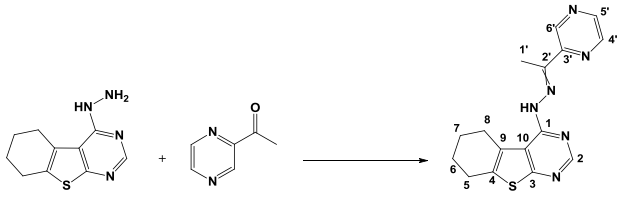
Single species observed.

^{1}H-NMR (DMSO-d$_6$), δ: 1.74-1.85 (m, 4H, C<u>H</u>$_2$), 2.42 (d, J= 3.4 Hz, 3H, H-1'), 2.74-2.77 (m, 2H, C<u>H</u>$_2$), 3.01-3.04 (m, 2H, C<u>H</u>$_2$), 7.23-7.28 (m, 2H, H-aromatic), 7.42-7.47 (m, 1H, H-aromatic), 7.42 (s, 1H, H-2), 7.88-7.92 (m, 1H, H-aromatic), 11.54 (bs, 1H, N<u>H</u>).

^{13}C-NMR (DMSO-d$_6$), δ: 17.84, 17.88 (CH_3, C-1'), 22.13, 22.38, 24.66, 26.36 (CH_2), 115.92, 116.10 (CH, C-aromatic), 119.42 (C, C-aromatic), 124.16, 124.18 (CH, C-aromatic), 127.62, 127.71 (C, C-aromatic), 130.18 (CH, C-aromatic), 130.21 (C, C-aromatic), 130.54, 130.61, 130.86 (CH, C-aromatic), 132.36 (C, C-aromatic), 143.87 (CH, C-2), 147.83, 155.68, 157.03, 159.31, 161.28 (C, C-aromatic).

***N*-(1-Pyrazin-2-*yl*-ethylidene)-*N*'-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (262)**

($C_{16}H_{16}N_6S$; M.W.= 324.4)



193 **250** **262**

General procedure 20;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.10 g, 0.4 mmol);

Yellow solid;

Yield: 0.08 g (55%)

Melting Point: 201-203°C

MS (ESI^+): 325.1 $[M+H]^+$

Microanalysis: Calculated for $C_{16}H_{16}N_6S$ (324.4); Theoretical: %C = 59.24, %H = 4.97, %N = 25.89; Found: %C = 59.14, %H = 4.79, %N = 25.95.

Two species observed. Major/minor species ratio: 3/2.

^{1}H-NMR (DMSO-d$_6$), δ: (major species) 1.86-191 (m, 4H, C<u>H</u>$_2$), 2.59 (s, 3H, H-1'), 2.78-2.82 (m, 2H, C<u>H</u>$_2$), 3.10-3.13 (m, 2H, C<u>H</u>$_2$), 7.73 (d, J= 1.6 Hz, 1H, H-6'), 8.46 (d,

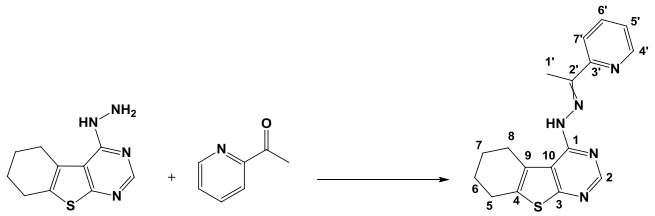
J= 2.7 Hz, 1H, H-5'), 8.53 (dd, J_1= 2.7 Hz, J_2= 1.6 Hz, 1H, H-5'), 9.32 (s, 1H, H-aromatic), 10.56 (bs, 1H, NH).

^{1}H-NMR (DMSO-d$_6$), δ: (minor species) 1.93-1.98 (m, 2H, CH$_2$), 1.99-2.05 (m, 2H, CH$_2$), 2.49 (s, 3H, H-1'), 2.86-2.90 (m, 2H, CH$_2$), 3.05-3.08 (m, 2H, CH$_2$), 8.50-8.52 (m, 2H, H-aromatic), 8.66 (s, 1H, H-aromatic), 8.73 (bs, 1H, NH), 9.51 (s, 1H, H-aromatic).

^{13}C-NMR (DMSO-d$_6$), δ: (major and minor species) 10.83, 13.43 (CH$_3$, C-1'), 22.33, 22.63, 22.70, 22.87, 25.38, 25.60, 26.63, 26.67 (CH$_2$), 116.22, 120.55, 124.38, 131.48, 134.35, 136.00 (C, C-aromatic), 141.18, 142.82, 142.95, 143.22, 143.34, 143.42, 143.82 (CH, C-aromatic), 147.68, 149.07, 150.40, 152.02 (C, C-aromatic), 153.08 (CH, C-aromatic), 153.74, 157.90, 158.35, 167.34 (C, C-aromatic).

***N*-(1-Pyridin-2-*yl*-ethylidene)-*N*'-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (263)**

(C$_{17}$H$_{17}$N$_5$S; M.W.= 323.4)



193 **251** **263**

General procedure 20;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.10 g, 0.4 mmol);

Purification: re-crystallisation from EtOH/H$_2$O;

Orange solid;

Yield: 0.11 g (81%)

Melting Point: 195-197°C

MS (ESI$^+$): 324.1 $[M+H]^+$

Two species observed. Major/minor species ratio: 5/2.

^{1}H-NMR (DMSO-d$_6$), δ: (major species) 1.75-1.81 (m, 4H, CH$_2$), 2.54 (s, 3H, H-1'), 2.71-2.74 (m, 2H, CH$_2$), 2.99-3.03 (m, 2H, CH$_2$), 7.36 (dd, J_1= 7.5 Hz, J_2= 5.1 Hz, 1H, H-aromatic), 7.79-7.83 (m, 2H, H-aromatic), 8.53 (d, J= 8.0 Hz, 1H, H-aromatic), 8.57-

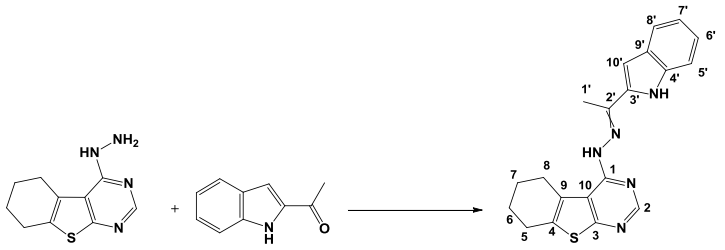
8.61 (m, 1H, H-aromatic), 11.74 (bs, 1H, NH).

^{1}H-NMR (DMSO-d$_6$), δ: (minor species) 1.82-1.86 (m, 2H, CH$_2$), 1.91-1.97 (m, 2H, CH$_2$), 2.42 (s, 3H, H-1'), 2.75-2.78 (m, 2H, CH$_2$), 3.06-3.09 (m, 2H, CH$_2$), 7.52 (dd, J_1= 7.8 Hz, J_2= 5.1 Hz, 1H, H-aromatic), 7.74 (d, J= 8.0 Hz, 1H, H-aromatic), 8.01-8.03 (m, 1H, H-aromatic), 8.41 (s, 1H, H-aromatic), 8.57-8.61 (m, 1H, H-aromatic), 9.73 (bs, 1H, NH).

^{13}C-NMR (DMSO-d$_6$), δ: (major and minor species) 13.17 (CH_3, C-1'), 21.87, 22.10, 22.25, 22.35, 24.67, 24.95, 26.13, 26.36 (CH_2), 119.62 (C, C-aromatic), 120.86, 123.51, 124.06, 124.37 (CH, C-aromatic), 130.86, 132.47, 132.78 (C, C-aromatic), 135.84, 143.82, 147.15 (CH, C-aromatic), 148.08 (C, C-aromatic), 148.40 (CH, C-aromatic), 156.06, 157.36, 158.65 (C, C-aromatic).

***N*-[1-(1*H*-Indol-2-*yl*)-ethylidene]-*N*'-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (264)**

($C_{20}H_{19}N_5S$; M.W.= 361.4)



General procedure 20;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.10 g, 0.4 mmol);

Yellow solid;

Yield: 0.08 g (53%)

Melting Point: 213-215°C

MS (ESI$^+$): 362.1 $[M+H]^+$

Single species observed.

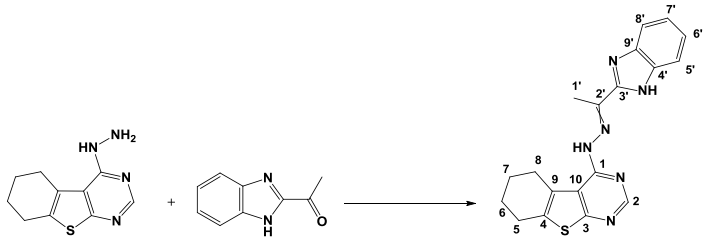
^{1}H-NMR (DMSO-d$_6$), δ: 1.77-1.85 (m, 4H, CH$_2$), 2.41 (s, 3H, H-1'), 2.74-2.78 (m, 2H, CH$_2$), 3.00-3.06 (m, 2H, CH$_2$), 6.87 (s, 1H, H-10'), 7.01-7.03 (m, 1H, H-aromatic), 7.18-7.20 (m, 1H, H-aromatic), 7.44 (d, J= 7.9 Hz, 1H, H-aromatic), 7.96 (d, J= 7.9 Hz,

1H, H-aromatic), 7.96 (s, 1H, H-2), 11.35 (bs, 1H, NH), 11.98 (bs, 1H, NH).

^{13}C-NMR (DMSO-d$_6$), δ: 13.58 (CH_3, C-1'), 22.13, 22.39, 24.67, 26.30 (CH_2), 103.33, 110.90, 119.15 (CH, C-aromatic), 119.67 (C, C-aromatic), 120.79, 123.03 (CH, C-aromatic), 128.15, 130.87, 132.41, 136.83, 137.78 (C, C-aromatic), 143.59 (CH, C-aromatic), 147.98, 150.37, 156.95 (C, C-aromatic).

***N*-[1-(1*H*-Benzoimidazol-2-*yl*)-ethylidene]-*N*'-(5,6,7,8-tetrahydro-benzo[4,5]thieno [2,3-*d*]pyrimidin-4-*yl*)-hydrazine (265)**

($C_{19}H_{18}N_6S$; M.W.= 362.4)



General procedure 20;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.10 g, 0.4 mmol);

Light brown solid;

Yield: 0.06 g (42%)

Melting Point: 287-289°C

MS (ESI^+): 363.1 $[M+H]^+$

Two species observed. Major/minor species ratio: 5/1.

^{1}H-NMR (DMSO-d$_6$), δ: 1.74-1.84 (m, 4H, C$\underline{H}_2$), 2.72-2.81 (m, 2H, C$\underline{H}_2$), 3.01-3.08 (m, 2H, C$\underline{H}_2$), 3.18 (s, 3H, H-1'), 7.17-7.23 (m, 1H, H-aromatic), 7.25-7.31 (m, 1H, H-aromatic), 7.54-7.61 (m, 1H, H-aromatic), 7.63-7.73 (m, 1H, H-aromatic), 8.04 (s, 1H, H-2), 12.10 (bs, 1H, N$\underline{H}$), 12.57 (bs, 1H, N$\underline{H}$).

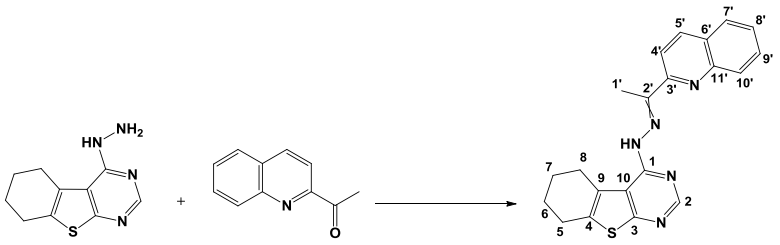
^{1}H-NMR (DMSO-d$_6$), δ: 1.91-1.96 (m, 2H, C$\underline{H}_2$), 2.02-2.10 (m, 2H, C$\underline{H}_2$), 2.83-2.87 (m, 2H, C$\underline{H}_2$), 3.42-3.46 (m, 2H, C$\underline{H}_2$), 4.08 (s, 3H, H-1'), 7.36-7.43 (m, 2H, H-aromatic), 7.63-7.73 (m, 2H, H-aromatic), 8.50 (s, 1H, H-2), 13.03 (bs, 1H, N$\underline{H}$), 14.31 (bs, 1H, N$\underline{H}$).

^{13}C-NMR (DMSO-d$_6$), δ: 13.10 (CH_3, C-1'), 22.08, 22.35, 24.68, 26.29 (CH_2), 48.57

(CH_3, C-1'), 111.06, 119.39 (CH, C-aromatic), 119.53 (C, C-aromatic), 121.48, 123.50 (CH, C-aromatic), 130.87, 132.92, 133.52 (C, C-aromatic), 143.46 (CH, C-aromatic), 143.79, 149.20, 149.71, 151.77, 157.94 (C, C-aromatic).

4-(2-(1-(Quinolin-2-*yl*)ethylidene)hydrazinyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno [2,3-*d*]pyrimidine (290)

($C_{21}H_{19}N_5S$; M.W.= 373.4)



193 **285** **290**

General procedure 20;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.10 g, 0.4 mmol);

Purification: re-crystallisation from $EtOH/H_2O$;

Orange solid;

Yield: 0.11 g (71%)

Melting Point: 121-123°C

MS (ESI^+): 374.0 $[M+H]^+$

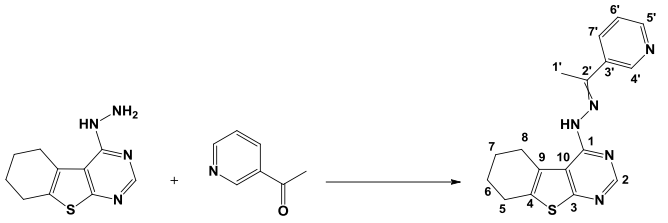
Single species observed.

^{1}H-NMR (DMSO-d$_6$), δ: 1.79-1.83 (m, 4H, C$\underline{H}_2$), 2.62 (s, 3H, H-1'), 2.74-2.78 (m, 2H, C$\underline{H}_2$), 3.05-3.09 (m, 2H, C$\underline{H}_2$), 7.58-7.61 (m, 1H, H-aromatic), 7.74-7.78 (m, 1H, H-aromatic), 7.89 (s, 1H, H-2), 7.94 (d, J= 8.3 Hz, 1H, H-aromatic), 8.04 (d, J= 8.3 HZ, 1H, H-aromatic), 8.34 (d, J= 8.7 Hz, 1H, H-aromatic), 8.77 (d, J= 8.7 Hz, 1H, H-aromatic), 11.90 (bs, 1H, N$\underline{H}$).

^{13}C-NMR (DMSO-d$_6$), δ: 12.97 (CH_3, C-1'), 22.12, 22.35, 24.70, 26.39 (CH_2), 119.25 (CH, C-aromatic), 119.57 (C, C-aromatic), 126.66, 127.67 (CH, C-aromatic), 127.76 (C, C-aromatic), 129.08, 129.46 (CH, C-aromatic), 130.91, 132.71 (C, C-aromatic), 135.23, 143.87 (CH, C-aromatic), 146.97, 148.43, 156.24, 158.04, 159.15 (C, C-aromatic).

4-(2-(1-(Pyridin-3-*yl*)ethylidene)hydrazinyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno [2,3-*d*]pyrimidine (291)

($C_{17}H_{17}N_5S$; M.W.= 323.4)



193 **286** **291**

General procedure 20;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.10 g, 0.4 mmol);

Yellow crystals;

Yield: 0.08 g (61%)

Melting Point: 188-191°C

MS (ESI^+): 324.1 $[M+H]^+$

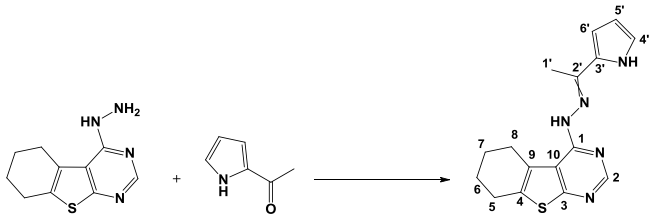
Single species observed.

^{1}H-NMR (DMSO-d$_6$), δ: 1.76-1.83 (m, 4H, C<u>H</u>$_2$), 2.46 (s, 3H, H-1'), 2.73-2.76 (m, 2H, C<u>H</u>$_2$), 3.01-3.05 (m, 2H, C<u>H</u>$_2$), 7.42-7.45 (m, 1H, H-aromatic), 7.80 (d, J= 3.6 Hz, 1H, H-aromatic), 8.33-8.36 (m, 1H, H-aromatic), 8.57 (dd, J_1= 4.7 Hz, J_2= 1.5 Hz, 1H, H-aromatic), 9.28 (s, 1H, H-2), 11.74 (bs, 1H, N<u>H</u>).

^{13}C-NMR (DMSO-d$_6$), δ: 13.84 (CH_3, C-1'), 22.12, 22.36, 24.67, 26.38 (CH_2), 119.54 (C, C-aromatic), 123.15 (CH, C-aromatic), 130.83, 132.43 (C, C-aromatic), 133.60 (CH, C-aromatic), 133.98 (C, C-aromatic), 143.90, 147.87 (CH, C-aromatic), 148.03 (C, C-aromatic), 149.44 (CH, C-aromatic), 155.41, 157.20 (C, C-aromatic).

4-(2-(1-(1*H*-Pyrrol-2-*yl*)ethylidene)hydrazinyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno [2,3-*d*]pyrimidine (292)

($C_{16}H_{17}N_5S$; M.W.= 311.4)



193 **287** **292**

General procedure 20;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.10 g, 0.4 mmol);

Yellow solid;

Yield: 0.08 g (62%)

Melting Point: 84-89°C

MS (ESI^+): 312.1 $[M+H]^+$

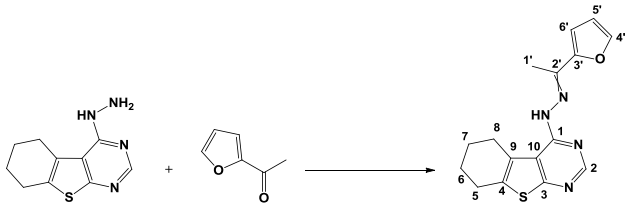
Single species observed.

^{1}H-NMR (DMSO-d_6), δ: 1.76-1.86 (m, 4H, C<u>H</u>$_2$), 2.27 (s, 3H, H-1'), 2.71-2.75 (m, 2H, C<u>H</u>$_2$), 2.97-3.01 (m, 2H, C<u>H</u>$_2$), 6.11-6.14 (m, 1H, H-aromatic), 6.47-6.49 (m, 1H, H-aromatic), 7.00-7.02 (m, 1H, H-aromatic), 7.82 (s, 1H, H-2), 11.31 (bs, 1H, N<u>H</u>), 11.80 (bs, 1H, N<u>H</u>).

^{13}C-NMR (DMSO-d_6), δ: 13.48 (CH_3, C-1'), 22.14, 22.42, 24.63, 26.26 (CH_2), 108.74, 110.21 (CH, C-aromatic), 119.70 (C, C-aromatic), 120.71 (CH, C-aromatic), 130.83, 131.80, 131.91 (C, C-aromatic), 143.61 (CH, C-aromatic), 146.88, 150.42, 156.13 (C, C-aromatic).

4-(2-(1-(Furan-2-*yl*)ethylidene)hydrazinyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidine (293)

($C_{16}H_{16}N_4OS$; M.W.= 312.3)



193 **288** **293**

General procedure 16;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.10 g, 0.4 mmol);

Yellow solid;

Yield: 0.05 g (36%)

Melting Point: 134-139°C

MS (ESI^+): 313.1 $[M+H]^+$

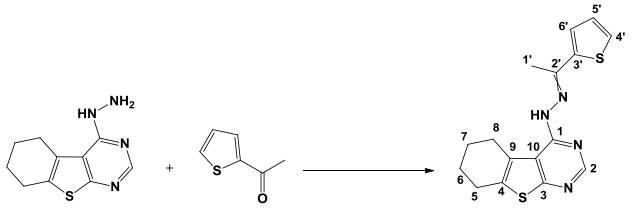
Single species observed.

^{1}H-NMR (DMSO-d_6), δ: 1.75-1.82 (m, 4H, C<u>H</u>$_2$), 2.33 (s, 3H, H-1'), 2.72-2.75 (m, 2H, C<u>H</u>$_2$), 2.98-3.01 (m, 2H, C<u>H</u>$_2$), 6.63 (dd, J_1= 3.4 Hz, J_2= 1.7 Hz, 1H, H-aromatic), 7.08 (d, J= 3.4 Hz, 1H, H-aromatic), 7.76-7.79 (m, 2H, H-aromatic), 11.47 (bs, 1H, N<u>H</u>).

^{13}C-NMR (DMSO-d_6), δ: 13.72 (CH_3, C-1'), 22.12, 22.37, 24.65, 26.32 (CH_2), 109.99, 111.96 (CH, C-aromatic), 119.59, 130.81, 132.30 (C, C-aromatic), 143.82, 143.94 (CH, C-aromatic), 147.33, 149.57, 153.31, 156.80 (C, C-aromatic).

4-(2-(1-(Thiophen-2-*yl*)ethylidene)hydrazinyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno [2,3-*d*]pyrimidine (294)

($C_{16}H_{16}N_4S_2$; M.W.= 328.4)



193 **289** **294**

General procedure 20;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.10 g, 0.4 mmol);

Yellow crystals;

Yield: 0.06 g (44%)

Melting Point: 129-132°C

MS (ESI^+): 329.1 $[M+H]^+$

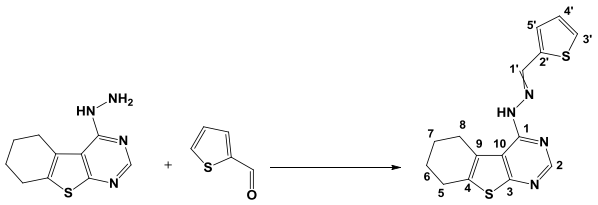
Single species observed.

^{1}H-NMR (DMSO-d$_6$), δ: 1.76-1.83 (m, 4H, C<u>H</u>$_2$), 2.42 (s, 3H, H-1'), 2.72-2.75 (m, 2H, C<u>H</u>$_2$), 2.98-3.01 (m, 2H, C<u>H</u>$_2$), 7.11 (dd, J_1= 5.2 Hz, J_2= 3.7 Hz, 1H, H-aromatic), 7.51 (dd, J_1= 3.7 Hz, J_2= 0.9 Hz, 1H, H-aromatic), 7.58 (dd, J_1= 5.2 Hz, J_2= 0.9 Hz, 1H, H-aromatic), 7.80 (s, 1H, H-2), 11.24 (bs, 1H, N<u>H</u>).

^{13}C-NMR (DMSO-d$_6$), δ: 14.96 (CH_3, C-1'), 22.13, 22.37, 24.66, 26.36 (CH_2), 119.59 (C, C-aromatic), 127.01, 127.50, 127.91 (CH, C-aromatic), 130.80, 132.33 (C, C-aromatic), 144.07 (CH, C-aromatic), 144.43, 146.80, 153.74, 156.73 (C, C-aromatic).

4-(2-(Thiophen-2-*yl*methylene)hydrazinyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidine (457)[35]

($C_{15}H_{14}N_4S_2$; M.W.= 314.4)



193 **452** **457**

General procedure 20;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.10 g, 0.4 mmol);

Yellow solid;

Yield: 0.04 g (34%)

Melting Point: 156-161°C (lit. 179-180°C)

MS (ESI^+): 314.9 $[M+H]^+$

Two species observed. Major/minor species ratio: 6/1.

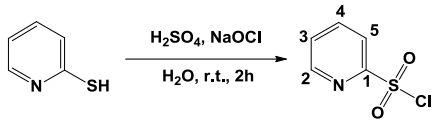
^{1}H-NMR (DMSO-d$_6$), δ: (major species) 1.73-1.86 (m, 4H, C<u>H</u>$_2$), 2.72-2.75 (m, 2H, C<u>H</u>$_2$), 2.95-2.99 (m, 2H, C<u>H</u>$_2$), 7.13-7.15 (m, 1H, H-aromatic), 7.47-7.48 (m, 1H, H-aromatic), 7.63-7.66 (m, 1H, H-aromatic), 7.78 (d, J= 3.7 Hz, 1H, H-aromatic), 8.57 (s, 1H, H-aromatic), 11.49 (bs, 1H, N<u>H</u>).

^{1}H-NMR (DMSO-d$_6$), δ: (minor species) 1.73-1.86 (m, 4H, C<u>H</u>$_2$), 2.81-2.84 (m, 2H, C<u>H</u>$_2$), 3.05-3.08 (m, 2H, C<u>H</u>$_2$), 7.13-7.15 (m, 1H, H-aromatic), 7.40 (d, J= 3.1 Hz, 1H, H-aromatic), 7.63-7.66 (m, 1H, H-aromatic), 8.45 (s, 1H, H-aromatic), 8.57 (s, 1H, H-aromatic), 10.22 (bs, 1H, N<u>H</u>).

^{13}C-NMR (DMSO-d$_6$), δ: (major and minor species) 21.98, 22.19, 22.36, 24.65, 25.19, 26.47, 26.52 (CH_2), 118.82 (C, C-aromatic), 127.74, 127.79, 128.19, 128.61, 129.80, 130.48 (CH, C-aromatic), 130.80, 132.27, 132.87, 139.19, 140.10 (C, C-aromatic), 140.58, 144.00, 147.61 (CH, C-aromatic), 148.26 (C, C-aromatic), 152.16 (CH, C-aromatic), 157.13 (C, C-aromatic).

6.4.1.3 2-Pyridinesulfonyl chloride (266)[36]

($C_5H_4ClNO_2S$; M.W.= 177.6)



270 **266**

Under ice-cooling, 2-mercaptopyridine (**270**) (1 g, 9 mmol) was added to sulphuric acid (25 mL) and the mixture was stirred at r.t. A sodium hypochlorite solution (63 mL, available chlorine 4.00-4.99%) was then added dropwise to the reaction mixture over a period of 1.5 h, and the mixture was further stirred at r.t. for 30 min.

The reaction was then diluted with water (50 mL), and extracted with DCM (2 x 50 mL). The extract was then washed with brine (100 mL), dried over $MgSO_4$, and concentrated at reduced pressure to give the title compound as a colourless oil.

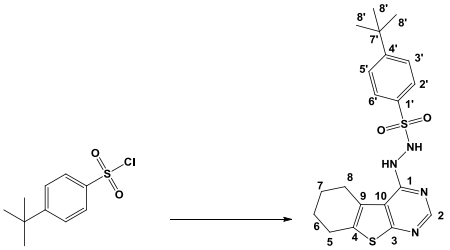
Yield: 1.23 g (77%)

^{1}H-NMR ($CDCl_3$), δ: 7.70-7.74 (m, 1H, H-aromatic), 8.07-8.10 (m, 1H, H-aromatic), 8.12-8.14 (m, 1H, H-aromatic), 8.83-8.85 (m, 1H, H-aromatic).

^{13}C-NMR ($CDCl_3$), δ: 121.94, 129.12, 139.09, 150.71 (CH, C-aromatic), 159.22 (C, C-1).

6.4.1.4 Arylsulfonyl acid *N*'-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazides (267-269)

4-*tert*-Butyl-phenylsulfonyl acid *N*'-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazide (267)
($C_{20}H_{24}N_4O_2S_2$; M.W.= 416.5)

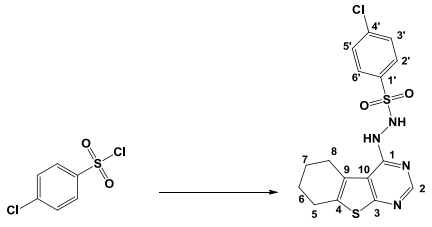


83 **267**

General procedure 21;
T.L.C. System: *n*hexane-EtOAc 4:6 v/v, Rf: 0.66.
Purification: flash column chromatography (*n*hexane:EtOAc 100:0 v/v increasing to 70:30 v/v).
White solid;
Yield: 0.11 g (38%)
Melting Point: 171-173°C
MS (ESI^+): 417.1 $[M+H]^+$
^{1}H-NMR ($CDCl_3$), δ: 1.24 (s, 9H, H-8'), 1.90-2.01 (m, 4H, C<u>H</u>$_2$), 2.83 (t, J= 5.9 Hz, 2H, C<u>H</u>$_2$), 2.96 (t, J= 5.9 Hz, 2H, C<u>H</u>$_2$), 7.16 (bs, 1H, N<u>H</u>), 7.33 (d, J= 8.5 Hz, 2H, H-aromatic), 7.80 (d, J= 8.5 Hz, 2H, H-aromatic), 8.00 (s, 1H, H-2), 8.02 (bs, 1H, N<u>H</u>),
^{13}C-NMR ($CDCl_3$), δ: 22.35, 22.43, 25.46, 26.17 (CH_2), 30.92 (CH_3, C-8'), 35.14 (C, C-7'), 125.07 (C, C-aromatic), 125.52, 128.60 (CH, C-aromatic), 133.66, 135.81 (C, C-aromatic), 151.18 (CH, C-2), 154.56, 157.62, 158.18, 166.24 (C, C-aromatic).

4-Chlorosulfonyl acid *N*'-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazide (268)

($C_{16}H_{15}ClN_4O_2S_2$; M.W.= 394.8)



13 **268**

General procedure 21;

T.L.C. System: *n*hexane -EtOAc 4:6 v/v, Rf: 0.63.

Purification: flash column chromatography (*n*hexane:EtOAc 100:0 v/v increasing to 50:50 v/v).

Pale yellow solid;

Yield: 0.12 g (46%)

Melting Point: 186-188°C

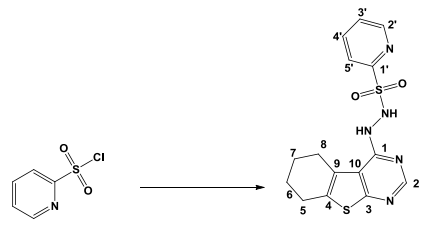
MS (ESI^+): 395.0, 397.0 $[M+H]^+$

^{1}H-NMR ($CDCl_3$), δ: 1.91-2.01 (m, 4H, C<u>H</u>$_2$), 2.84 (t, J= 5.8 Hz, 2H, C<u>H</u>$_2$), 2.95 (t, J= 5.8 Hz, 2H, C<u>H</u>$_2$), 7.14 (bs, 1H, N<u>H</u>), 7.34 (d, J= 8.5 Hz, 2H, H-aromatic), 7.85 (d, J= 8.5 Hz, 2H, H-aromatic), 8.08 (bs, 1H, N<u>H</u>), 8.12 (s, 1H, H-2).

^{13}C-NMR ($DMSO-d_6$), δ: 21.84, 22.07, 24.91, 25.15 (CH_2), 126.50 (C, C-aromatic), 128.71, 129.70 (CH, C-aromatic), 133.47, 137.70, 138.45 (C, C-aromatic), 151.31 (CH, C-2), 155.98, 161.27, 164.56 (C, C-aromatic).

2-Pyridine sulfonyl acid *N*'-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazide (269)

($C_{15}H_{15}N_5O_2S_2$; M.W.= 361.4)



266 **269**

General procedure 21;

Purification: re-crystallisation from EtOH.

White solid;

Yield: 0.08 g (34%)

Melting Point: 189-191°C

MS (ESI^+): 362.1 $[M+H]^+$

Two species observed. Major/minor species ratio: 3/1.

^{1}H-NMR (DMSO-d$_6$), δ: (major species) 1.78-1.84 (m, 4H, C$\underline{H}_2$), 2.75-2.79 (m, 2H, C$\underline{H}_2$), 2.90-2.94 (m, 2H, C$\underline{H}_2$), 7.59-7.62 (m, 1H, H-aromatic), 7.90 (d, J= 7.7 Hz, 1H, H-aromatic), 7.94-8.00 (m, 1H, H-aromatic), 8.02 (s, 1H, H-2), 8.63-8.67 (m, 1H, H-aromatic), 8.83 (bs, 1H, N$\underline{H}$), 10.10 (bs, 1H, N$\underline{H}$).

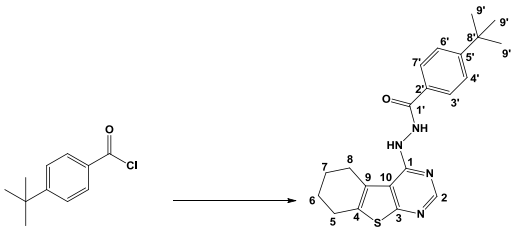
^{1}H-NMR (DMSO-d$_6$), δ: (minor species) 1.50-1.54 (m, 2H, C$\underline{H}_2$), 1.64-1.68 (m, 2H, C$\underline{H}_2$), 2.26-2.30 (m, 2H, C$\underline{H}_2$), 2.62-2.66 (m, 2H, C$\underline{H}_2$), 7.66-7.71 (m, 2H, H-aromatic), 7.94-8.00 (m, 1H, H-aromatic), 8.08-8.12 (m, 1H, H-aromatic), 8.78 (s, 1H, H-2), 8.83 (bs, 1H, N$\underline{H}$), 9.44 (bs, 1H, N$\underline{H}$), 10.10 (bs, 1H, N$\underline{H}$), 11.60 (bs, 1H, N$\underline{H}$).

^{13}C-NMR (DMSO-d$_6$), δ: (major and minor species) 21.86, 22.05, 22.20, 24.49, 24.90, 25.25, 25.98 (CH_2), 115.43 (C, C-aromatic), 122.78, 124.19 (CH, C-aromatic), 126.34 (C, C-aromatic), 126.98, 127.21 (CH, C-aromatic), 133.38 (C, C-aromatic), 137.69, 138.24, 143.27, 149.62, 149.75, 151.41 (CH, C-aromatic), 156.30, 156.89, 165.29 (C, C-aromatic).

6.4.1.5 Aryl-carboxylic acid *N*'-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazides (272-273, 278-281, 302-309)

4-*tert*-Butyl-benzoic acid *N*'-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-yl)-hydrazide (272)

($C_{21}H_{24}N_4OS$; M.W.= 380.5)



271 **272**

General procedure 22a;

T.L.C. System: *n*hexane -EtOAc 4:6 v/v, Rf: 0.63.

Purification: flash column chromatography (*n*hexane:EtOAc 100:0 v/v increasing to 40:60 v/v).

White solid;

Yield: 0.23 g (86%)

Melting Point: 137-139°C

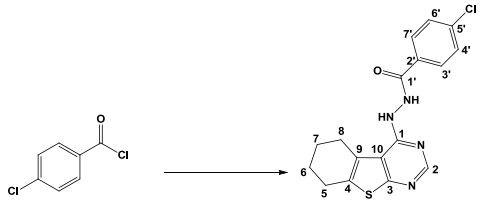
MS (ESI^+): 381.2 $[M+H]^+$

^{1}H-NMR ($CDCl_3$), δ: 1.31 (s, 9H, H-9'), 1.87-1.94 (m, 4H, C$\underline{H}_2$), 2.81-2.85 (m, 2H, C$\underline{H}_2$), 3.07-3.10 (m, 2H, C$\underline{H}_2$), 7.36 (d, J= 8.4 Hz, 2H, H-aromatic), 7.85 (d, J= 8.4 Hz, 2H, H-aromatic), 8.35 (bs, 1H, N$\underline{H}$), 8.40 (s, 1H, H-2), 10.69 (bs, 1H, N$\underline{H}$).

^{13}C-NMR ($CDCl_3$), δ: 22.37, 22.40, 25.50, 26.19 (CH_2), 31.07 (CH_3, C-9'), 34.92 (C, C-8'), 116.14 (C, C-aromatic), 125.42 (CH, C-aromatic), 125.67 (C, C-aromatic), 127.12 (CH, C-aromatic), 128.24, 135.23 (C, C-aromatic), 151.59 (CH, C-aromatic), 155.61, 156.23, 165.52 (C, C-aromatic), 166.26 (C, C-1').

4-Chloro-benzoic acid *N*'-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazide (273)

($C_{17}H_{15}ClN_4OS$; M.W.= 358.8)



119 **273**

General procedure 22a;

T.L.C. System: *n*hexane -EtOAc 4:6 v/v, Rf: 0.50.

Purification: flash column chromatography (*n*hexane:EtOAc 100:0 v/v increasing to 30:70 v/v).

White solid;

Yield: 0.09 g (37%)

Melting Point: 210-212 °C

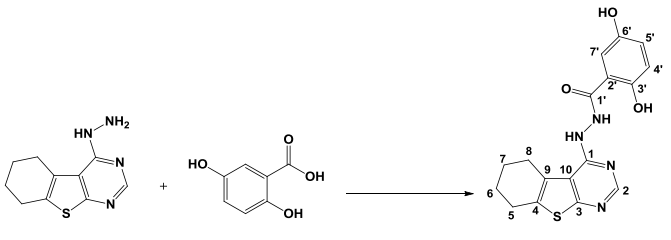
MS (ESI^+): 359.0, 361.0 $[M+H]^+$

^{1}H-NMR ($CDCl_3$), δ: 1.90-2.01 (m, 4H, C<u>H</u>$_2$), 2.85-2.88 (m, 2H, C<u>H</u>$_2$), 3.07-3.11 (m, 2H, C<u>H</u>$_2$), 7.42 (d, J= 8.5 Hz, 2H, H-aromatic), 7.68 (d, J= 8.5 Hz, 2H, H-aromatic), 8.28 (bs, 1H, N<u>H</u>), 8.44 (s, 1H, H-2), 10.14 (bs, 1H, N<u>H</u>).

^{13}C-NMR ($CDCl_3$), δ: 22.38, 22.43, 25.51, 26.14 (CH_2), 125.44 (C, C-aromatic), 128.57, 128.99 (CH, C-aromatic), 129.64, 135.71, 138.69 (C, C-aromatic), 151.62 (CH, C-2), 156.67, 161.43, 164.89 (C, C-aromatic), 166.39 (C, C-1').

2,5-Dihydroxy-benzoic acid *N*'-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazide (278)

($C_{17}H_{16}N_4O_3S$; M.W.= 356.4)



193 **274** **278**

General procedure 22;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.20 g, 0.9 mmol);

Purification: re-crystallisation from EtOH.

Light grey solid;

Yield: 0.08 g (28%)

Melting Point: 261-263°C

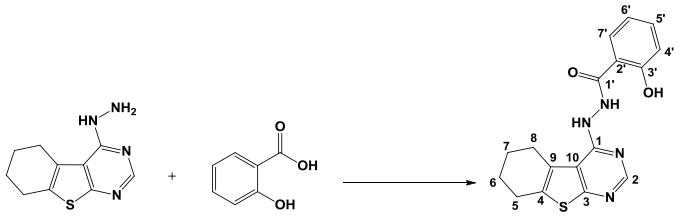
MS (ESI^+): 357.1 $[M+H]^+$

^{1}H-NMR (DMSO-d$_6$), δ: 1.81-1.91 (m, 4H, C<u>H</u>$_2$), 2.80-2.86 (m, 2H, C<u>H</u>$_2$), 2.99-3.08 (m, 2H, C<u>H</u>$_2$), 6.82 (d, J= 8.7 Hz, 1H, H-aromatic), 6.91 (dd, J_1= 8.7 Hz, J_2= 1.6 Hz, 1H, H-aromatic), 7.37 (d, J= 1.6 Hz, 1H, H-aromatic), 8.36 (s, 1H, H-2), 8.77 (bs, 1H, N<u>H</u>), 9.09 (bs, 1H, N<u>H</u>), 10.77 (bs 1H, <u>O</u>H), 11.17 (bs, 1H, <u>O</u>H).

^{13}C-NMR (DMSO-d$_6$), δ: 21.94, 22.11, 24.95, 25.62 (CH_2), 114.00 (CH, C-aromatic), 115.19, 115.40 (C, C-aromatic), 117.82, 121.48 (CH, C-aromatic), 126.42, 132.99, 149.61, 151.18 (C, C-aromatic), 152.33 (CH, C-aromatic), 156.42, 165.28 (C, C-aromatic), 166.48 (C, C-1').

2-Hydroxy-benzoic acid *N*'-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazide (279)[37]

($C_{17}H_{16}N_4O_2S$; M.W.= 340.4)



193 **275** **279**

General procedure 22;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.20 g, 0.9 mmol);

Purification: re-crystallisation from EtOH/H_2O.

Light orange solid;

Yield: 0.10 g (35%)

Melting Point: 327-329°C

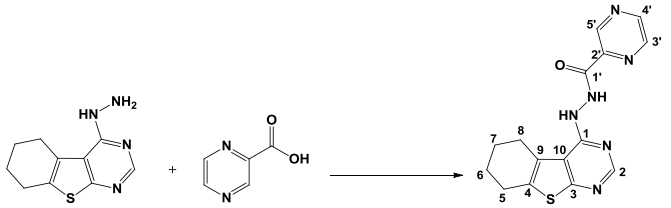
MS (ESI^+): 341.1 $[M+H]^+$

^{1}H-NMR (DMSO-d$_6$), δ: 1.84-1.88 (m, 4H, C<u>H</u>$_2$), 2.79-2.88 (m, 2H, C<u>H</u>$_2$), 2.98-3.08 (m, 2H, C<u>H</u>$_2$), 6.94-7.01 (m, 2H, H-aromatic), 7.74 (t, J= 7.7 Hz, 1H, H-aromatic), 8.00 (d, J= 7.7 Hz, 1H, H-aromatic), 8.37 (s, 1H, H-2), 8.79 (bs, 1H, N<u>H</u>), 10.85 (bs, 1H, N<u>H</u>), 10.77 (bs 1H, <u>O</u>H), 11.99 (bs, 1H, <u>O</u>H).

^{13}C-NMR (DMSO-d$_6$), δ: 21.94, 22.11, 24.95, 25.66 (CH_2), 114.73 (C, C-aromatic), 117.35, 119.03 (CH, C-aromatic), 126.40, (C, C-aromatic), 128.34 (CH, C-aromatic), 133.06 (C, C-aromatic), 134.04 (CH, C-aromatic), 149.47, 151.23 (C, C-aromatic), 152.33 (CH, C-aromatic), 156.65 (C, C-aromatic), 159.20 (C, C-1').

Pyrazine-2-carboxylic acid *N*'-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazide (280)

($C_{15}H_{14}N_6OS$; M.W.= 326.3)



General procedure 22;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.20 g, 0.9 mmol);

Purification: re-crystallisation from DCM/ *n*hexane.

Pale yellow solid;

Yield: 0.08 g (29%)

Melting Point: 200-202°C

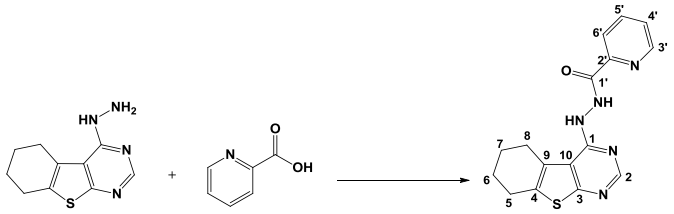
MS (ESI^+): 327.1 $[M+H]^+$

^{1}H-NMR ($CDCl_3$), δ: 1.90-2.00 (m, 4H, C<u>H</u>$_2$), 2.84-2.87 (m, 2H, C<u>H</u>$_2$), 3.06-3.10 (m, 2H, C<u>H</u>$_2$), 8.19 (bs, 1H, N<u>H</u>), 8.51 (s, 1H, H-2), 8.61-8.62 (m, 1H, H-aromatic), 8.81 (d, J= 2.4 Hz, 1H, H-aromatic), 9.39 (d, J= 1.3 Hz, 1H, H-aromatic), 10.68 (bs, 1H, N<u>H</u>).

^{13}C-NMR ($CDCl_3$), δ: 22.40, 22.46, 25.49, 26.08 (CH_2), 115.85, 125.22, 135.57 (C, C-aromatic), 143.07 (CH, C-aromatic), 143.20 (C, C-aromatic), 144.20, 147.91, 152.23 (CH, C-aromatic), 154.39, 159.25 (C, C-aromatic), 166.39 (C, C-1').

Pyridine-2-carboxylic acid *N*'-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazide (281)

($C_{16}H_{15}N_5OS$; M.W.= 325.3)



193 **277** **281**

General procedure 22;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.20 g, 0.9 mmol);

Purification: re-crystallisation from EtOH.

Light green solid;

Yield: 0.19 g (67%)

Melting Point: 232-234°C

MS (ESI^+): 326.1 $(M+H)^+$

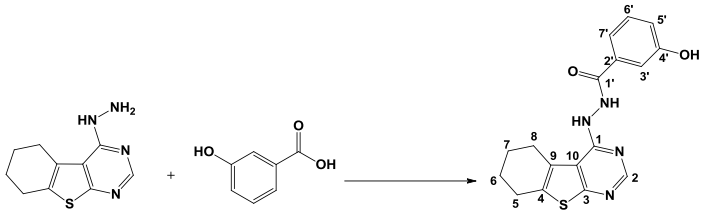
Microanalysis: Calculated for $C_{16}H_{15}N_5OS$ (325.3); Theoretical: %C = 59.06, %H = 4.65, %N = 21.51; Found: %C = 58.99, %H = 4.61, %N = 21.51.

^{1}H-NMR ($CDCl_3$), δ: 1.89-2.00 (m, 4H, C<u>H</u>$_2$), 2.83-2.87 (m, 2H, C<u>H</u>$_2$), 3.07-3.11 (m, 2H, C<u>H</u>$_2$), 7.47-7.51 (m, 1H, H-aromatic), 7.89 (td, J_1= 7.7 Hz, J_2= 1.6 Hz, 1H, H-aromatic), 8.15-8.20 (m, 2H, H-aromatic, N<u>H</u>), 8.52 (s, 1H, H-2), 8.65 (d, J= 4.7 Hz, 1H, H-aromatic), 10.77 (bs, 1H, N<u>H</u>).

^{13}C-NMR ($CDCl_3$), δ: 22.43, 22.49, 25.49, 26.11 (CH_2), 115.78 (C, C-aromatic), 122.35 (CH, C-aromatic), 125.31 (C, C-aromatic), 126.74 (CH, C-aromatic), 135.18 (C, C-aromatic), 137.41 (CH, C-aromatic), 148.33 (C, C-aromatic), 148.72, 152.48 (CH, C-aromatic), 154.56, 160.53 (C, C-aromatic), 166.23 (C, C-1').

3-Hydroxy-*N*'-(5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)benzo hydrazide (302)

($C_{17}H_{16}N_4O_2S$; M.W.= 340.4)



193 **295** **302**

General procedure 22;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.20 g, 0.9 mmol);

Purification: re-crystallisation from EtOH.

White solid;

Yield: 0.09 g (29%)

Melting Point: 241-243°C

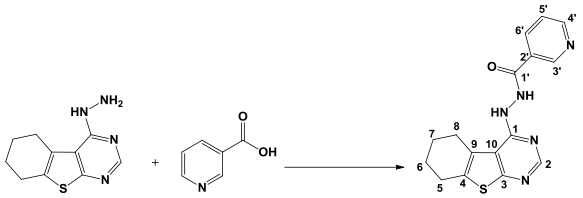
MS (ESI^+): 341.0 $[M+H]^+$

^{1}H-NMR (DMSO-d_6), δ: 1.83-1.87 (m, 4H, C<u>H</u>$_2$), 2.81-2.84 (m, 2H, C<u>H</u>$_2$), 3.01-3.04 (m, 2H, C<u>H</u>$_2$), 6.96-6.99 (m, 1H, H-aromatic), 7.29-7.33 (m, 1H, H-aromatic), 7.34-7.35 (m, 1H, H-aromatic), 7.40 (dt, J_1= 7.7 Hz, J_2= 1.2 Hz, 1H, H-aromatic), 8.34 (s, 1H, H-2), 8.58 (bs, 1H, N<u>H</u>), 9.70 (bs, 1H, O<u>H</u>), 10.48 (bs, 1H, N<u>H</u>).

^{13}C-NMR (DMSO-d_6), δ: 21.98, 22.12, 24.97, 25.79 (CH_2), 114.52 (CH, C-aromatic), 115.18 (C, C-aromatic), 117.98, 118.63 (CH, C-aromatic), 126.43 (C, C-aromatic), 129.43 (CH, C-aromatic), 132.71, 134.14 (C, C-aromatic), 152.44 (CH, C-aromatic), 157.35, 157.50, 165.30 (C, C-aromatic), 165.94 (C, C-1').

_N_'-(5,6,7,8-Tetrahydrobenzo[4,5]thieno[2,3-_d_]pyrimidin-4-_yl_)nicotinohydrazide (291)

($C_{16}H_{15}N_5OS$; M.W.= 325.3)



193 **296** **303**

General procedure 22;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.20 g, 0.9 mmol);

Purification: re-crystallisation from EtOH/H_2O.

Pale yellow solid;

Yield: 0.07 g (23%)

Melting Point: 137-140 °C

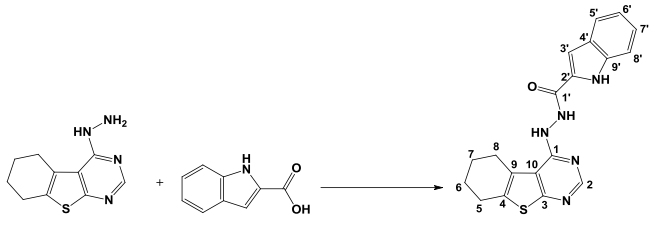
MS (ESI+): 326.0 $[M+H]^+$

^{1}H-NMR (DMSO-d$_6$), δ: 1.84-1.89 (m, 4H, C<u>H</u>$_2$), 2.81-2.85 (m, 2H, C<u>H</u>$_2$), 3.02-3.06 (m, 2H, C<u>H</u>$_2$), 7.58 (dd, J_1= 8.4 Hz, J_2= 4.8 Hz, 1H, H-aromatic), 8.28-8.31 (m, 1H, H-aromatic), 8.36 (s, 1H, H-2), 8.69- 8.71 (m, 1H, H-aromatic), 8.78-8.80 (m, 1H, H-aromatic), 9.12 (bs, 1H, N<u>H</u>), 10.80 (bs, 1H, N<u>H</u>).

^{13}C-NMR (DMSO-d$_6$), δ: 21.97, 22.11, 24.97, 25.76 (CH_2), 115.22 (C, C-aromatic), 123.67 (CH, C-aromatic), 126.40, 128.38, 132.93 (C, C-aromatic), 135.24, 148.49, 152.43 (CH, C-aromatic), 157.28, 164.56 (C, C-aromatic), 165.40 (C, C-1').

***N*'-(5,6,7,8-Tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-1*H*-indole-2-carbo hydrazide (304)**

($C_{19}H_{17}N_5OS$; M.W.= 363.4)



193 **297** **304**

General procedure 22;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.20 g, 0.9 mmol);

Purification: re-crystallisation from EtOH.

Pale yellow solid;

Yield: 0.12 g (36%)

Melting Point: 232-235°C

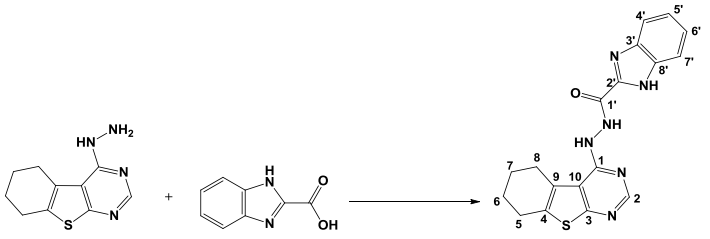
MS (ESI^+): 364.1 $[M+H]^+$

^{1}H-NMR (DMSO-d$_6$), δ: 1.83-1.89 (m, 4H, C<u>H</u>$_2$), 2.81-2.85 (m, 2H, C<u>H</u>$_2$), 3.03-3.08 (m, 2H, C<u>H</u>$_2$), 7.06-7.09 (m, 1H, H-aromatic), 7.20-7.24 (m, 1H, H-aromatic), 7.34 (s, 1H, H-3'), 7.47 (d, J= 8.2 Hz, 1H, H-aromatic), 7.68 (d, J= 8.0 Hz, 1H, H-aromatic), 8.35 (s, 1H, H-2), 8.70 (bs, 1H, N<u>H</u>), 10.58 (bs, 1H, N<u>H</u>), 11.69 (bs, 1H, N<u>H</u>).

^{13}C-NMR (DMSO-d$_6$), δ: 22.00, 22.12, 24.99, 25.80 (CH_2), 103.44, 112.34 (CH, C-aromatic), 115.15 (C, C-aromatic), 119.87, 121.68, 123.61 (CH, C-aromatic), 126.47, 127.03, 129.76, 132.79, 136.60 (C, C-aromatic), 157.50, 160.99 (C, C-aromatic), 165.37 (C, C-1').

***N*'-(5,6,7,8-Tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-1*H*-benzo[*d*] imidazole-2-carbohydrazide (305)**

($C_{18}H_{16}N_6OS$; M.W.= 364.4)



193 **298** **305**

General procedure 22;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.20 g, 0.9 mmol);

Purification: re-crystallisation from EtOH.

Yellow solid;

Yield: 0.09 g (26%)

Melting Point: >260°C

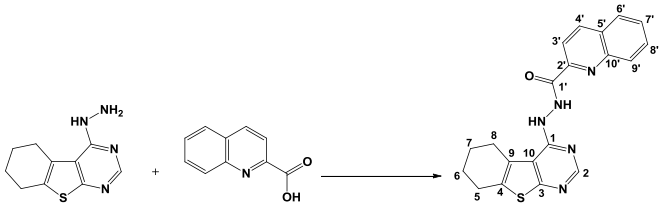
MS (ESI^+): 365.1 $[M+H]^+$

^{1}H-NMR (DMSO-d_6), δ: 1.83-1.86 (m, 4H, C<u>H</u>$_2$), 2.80-2.84 (m, 2H, C<u>H</u>$_2$), 3.00-3.04 (m, 2H, C<u>H</u>$_2$), 7.31-7.37 (m, 2H, H-aromatic), 7.55-7.61 (m, 1H, H-aromatic), 7.76-7.82 (m, 1H, H-aromatic), 8.34 (s, 1H, H-2), 8.79 (bs, 1H, N<u>H</u>), 10.89 (bs, 1H, N<u>H</u>), 13.40 (bs, 1H, N<u>H</u>).

^{13}C-NMR (DMSO-d_6), δ: 21.97, 22.13, 24.96, 25.76 (CH_2), 112.54 (CH, C-aromatic), 115.23 (C, C-aromatic), 120.06, 122.71, 124.35 (CH, C-aromatic), 126.42, 132.83, 134.34, 142.59, 144.36 (C, C-aromatic), 152.33 (CH, C-aromatic), 156.92, 158.43 (C, C-aromatic), 165.37 (C, C-1').

***N*'-(5,6,7,8-Tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)quinoline-2-carbohydrazide (306)**

($C_{20}H_{17}N_5OS$; M.W.= 375.4)



193 **282** **306**

General procedure 22;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.20 g, 0.9 mmol);

Purification: re-crystallisation from EtOH.

White solid;

Yield: 0.23 g (68%)

Melting Point: 261-263°C

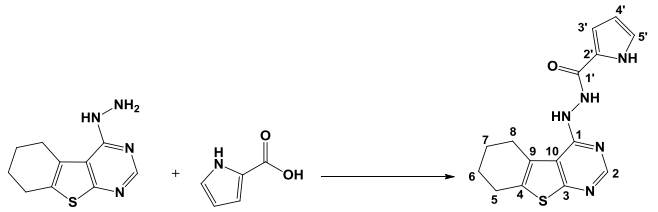
MS (ESI^+): 376.0 $[M+H]^+$

^{1}H-NMR (DMSO-d$_6$), δ: 1.85-1.89 (m, 4H, C<u>H</u>$_2$), 2.82-2.86 (m, 2H, C<u>H</u>$_2$), 3.04-3.08 (m, 2H, C<u>H</u>$_2$), 7.75-7.78 (m, 1H, H-aromatic), 7.90-7.94 (m, 1H, H-aromatic), 8.12 (d, J= 8.1 Hz, 1H, H-aromatic), 8.17 (d, J= 8.4 Hz, 1H, H-aromatic), 8.20 (d, J= 8.4 Hz, 1H, H-aromatic), 8.34 (s, 1H, H-2), 8.61 (d, J= 8.4 Hz, 1H, H-aromatic), 8.76 (bs, 1H, N<u>H</u>), 10.84 (bs, 1H, N<u>H</u>).

^{13}C-NMR (DMSO-d$_6$), δ: 21.97, 22.13, 24.96, 25.76 (CH_2), 115.27 (C, C-aromatic), 118.80 (CH, C-aromatic), 122.34, 126.52 (C, C-aromatic), 128.14, 128.32 (CH, C-aromatic), 128.93 (C, C-aromatic), 129.30, 130.65 (CH, C-aromatic), 132.86 (C, C-aromatic), 137.95 (CH, C-aromatic), 146.08, 149.76 (C, C-aromatic), 152.40 (CH, C-aromatic), 158.65 (C, C-aromatic), 164.29 (C, C-1').

***N*'-(5,6,7,8-Tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-1*H*-pyrrole-2-carbohydrazide (307)**

($C_{15}H_{15}N_5OS$; M.W.= 313.3)



General procedure 22;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.20 g, 0.9 mmol);

Purification: re-crystallisation from MeOH.

Pale yellow solid;

Yield: 0.18 g (65%)

Melting Point: charring > 300°C

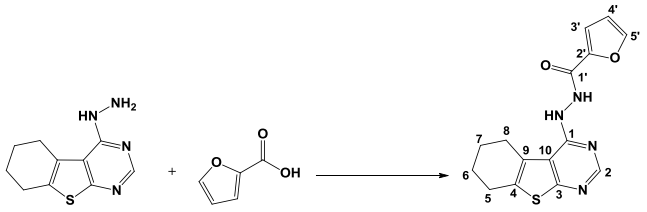
MS (ESI^+): 314.0 $[M+H]^+$

^{1}H-NMR (DMSO-d$_6$), δ: 1.83-1.87 (m, 4H, C<u>H</u>$_2$), 2.80-2.84 (m, 2H, C<u>H</u>$_2$), 3.00-3.04 (m, 2H, C<u>H</u>$_2$), 6.14-6.17 (m, 1H, H-aromatic), 6.93-6.95 (m, 1H, H-aromatic), 6.98-7.00 (m, 1H, H-aromatic), 8.32 (s, 1H, H-2), 8.52 (bs, 1H, N<u>H</u>), 10.08 (bs, 1H, N<u>H</u>), 11.56 (bs, 1H, N<u>H</u>).

^{13}C-NMR (DMSO-d$_6$), δ: 22.00, 22.11, 24.97, 25.80 (CH_2), 108.75, 110.97 (CH, C-aromatic), 115.07 (C, C-aromatic), 122.04 (CH, C-aromatic), 124.34, 126.47, 132.61 (C, C-aromatic), 152.43 (CH, C-aromatic), 157.42, 160.43 (C, C-aromatic), 165.25 (C, C-1').

***N*'-(5,6,7,8-Tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)furan-2-carbo hydrazide (308)**

($C_{15}H_{14}N_4O_2S$; M.W.= 314.3)



General procedure 22;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.20 g, 0.9 mmol);

Purification: re-crystallisation from MeOH.

White crystals;

Yield: 0.10 g (35%)

Melting Point: 216-219°C

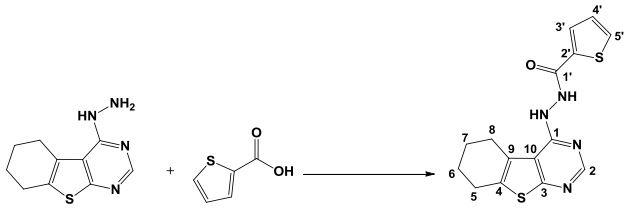
MS (ESI^+): 314.9 $[M+H]^+$

^{1}H-NMR (DMSO-d$_6$), δ: 1.83-1.86 (m, 4H, C<u>H</u>$_2$), 2.80-2.84 (m, 2H, C<u>H</u>$_2$), 2.99-3.03 (m, 2H, C<u>H</u>$_2$), 6.69 (dd, J_1= 3.5 Hz, J_2= 1.7 Hz, 1H, H-aromatic), 7.29 (dd, J_1= 3.5 Hz, J_2= 0.4 Hz, 1H, H-aromatic), 7.92-7.93 (m, 1H, H-aromatic), 8.34 (s, 1H, H-2), 8.59 (bs, 1H, N<u>H</u>), 10.45 (bs, 1H, N<u>H</u>).

^{13}C-NMR (DMSO-d$_6$), δ: 21.97, 22.11, 24.97, 25.76 (CH_2), 111.81, 114.43 (CH, C-aromatic), 115.13, 126.40, 132.81 (C, C-aromatic), 145.66 (CH, C-aromatic), 146.46 (C, C-aromatic), 152.39 (CH, C-aromatic), 157.34, 157.53 (C, C-aromatic), 165.35 (C, C-1').

***N*'-(5,6,7,8-Tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)thiophene-2-carbo hydrazide (309)**

($C_{15}H_{14}N_4OS_2$; M.W.= 330.4)



193 **301** **309**

General procedure 22;

Reagent: (5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazine (**193**) (0.20 g, 0.9 mmol);

Purification: re-crystallisation from MeOH.

White solid;

Yield: 0.04 g (15%)

Melting Point: 219-221°C

MS (ESI^+): 330.9 $[M+H]^+$

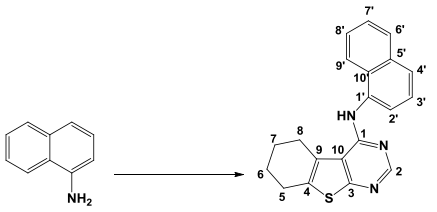
^{1}H-NMR (DMSO-d$_6$), δ: 1.82-1.89 (m, 4H, C<u>H</u>$_2$), 2.80-2.86 (m, 2H, C<u>H</u>$_2$), 2.99-3.05 (m, 2H, C<u>H</u>$_2$), 7.21-7.25 (m, 1H, H-aromatic), 7.84-7.85 (m, 1H, H-aromatic), 7.94-7.98 (m, 1H, H-aromatic), 8.35 (s, 1H, H-2), 8.64 (bs, 1H, N<u>H</u>), 10.60 (bs, 1H, N<u>H</u>).

^{13}C-NMR (DMSO-d$_6$), δ: 21.97, 22.10, 24.97, 25.76 (CH_2), 115.12, 126.41 (c, C-aromatic), 128.12, 128.93, 131.47 (CH, C-aromatic), 132.83, 137.60 (C, C-aromatic), 152.43 (CH, C-aromatic), 157.34, 160.91 (C, C-aromatic), 165.43 (C, C-1').

6.4.1.6 *N*-Aryl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine (310-312)

N-(Naphthalen-1-*yl*)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine (310)

($C_{20}H_{17}N_3S$; M.W.= 331.4)



313 **310**

General procedure 23;

T.L.C. System: *n*hexane -EtOAc 7:3 v/v, Rf: 0.46.

Purification: flash column chromatography (*n*hexane:EtOAc 100:0 v/v increasing to 70:30 v/v).

Pale yellow solid;

Yield: 0.06 g (21%)

Melting Point: 169-172°C

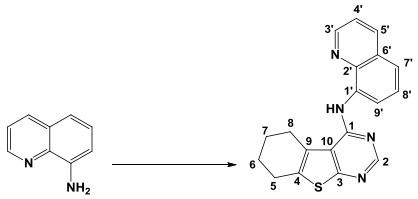
MS (ESI^+): 332.1 $[M+H]^+$

^{1}H-NMR ($CDCl_3$), δ: 1.97-2.02 (m, 2H, C<u>H</u>$_2$), 2.03-2.08 (m, 2H, C<u>H</u>$_2$), 2.91-2.94 (m, 2H, C<u>H</u>$_2$), 3.20-3.23 (m, 2H, C<u>H</u>$_2$), 7.38 (bs, 1H, N<u>H</u>), 7.52-7.56 (m, 2H, H-aromatic), 7.57-7.59 (m, 1H, H-aromatic), 7.79 (d, J= 8.1 Hz, 1H, H-aromatic), 7.91-7.95 (m, 2H, H-aromatic), 7.99 (d, J= 7.4 Hz, 1H, H-aromatic), 8.43 (s, 1H, H-2).

^{13}C-NMR ($CDCl_3$), δ: 22.50, 22.69, 25.60, 26.84 (CH_2), 116.78 (C, C-aromatic), 121.35, 122.20 (CH, C-aromatic), 125.02 (C, C-aromatic), 125.80, 126.11, 126.14, 126.45 (CH, C-aromatic), 128.70 (C, C-aromatic), 128.88 (CH, C-aromatic), 133.47, 134.49, 134.65 (C, C-aromatic), 152.94 (CH, C-aromatic), 156.36, 166.54 (C, C-aromatic).

_N_-(Quinolin-8-_yl_)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-_d_]pyrimidin-4-amine (311)

($C_{19}H_{16}N_4S$; M.W.= 332.4)



314 **311**

General procedure 23;

T.L.C. System: *n*hexane -EtOAc 7:3 v/v, Rf: 0.59.

Purification: flash column chromatography (*n*hexane:EtOAc 100:0 v/v increasing to 85:15 v/v).

Pale yellow solid;

Yield: 0.03 g (14%)

Melting Point: 193-196 °C

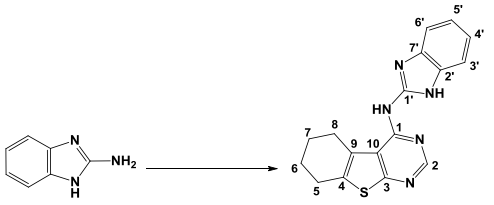
MS (ESI^+): 333.1 $[M+H]^+$

^{1}H-NMR ($CDCl_3$), δ: 1.95-1.99 (m, 2H, C<u>H</u>$_2$), 2.03-2.08 (m, 2H, C<u>H</u>$_2$), 2.86-2.90 (m, 2H, C<u>H</u>$_2$), 3.32-3.37 (m, 2H, C<u>H</u>$_2$), 7.40-7.44 (m, 2H, H-aromatic), 7.55-7.59 (m, 1H, H-aromatic), 8.14 (d, J= 7.8 Hz, 1H, H-aromatic), 8.61 (s, 1H, H-2), 8.78-8.81 (m, 1H, H-aromatic), 9.16 (d, J= 7.5 Hz, 1H, H-aromatic), 10.24 (bs, 1H, N<u>H</u>).

^{13}C-NMR ($CDCl_3$), δ: 22.53, 22.84, 25.69, 26.54 (CH_2), 116.29 (CH, C-aromatic), 117.97 (C, C-aromatic), 119.93, 121.39 (CH, C-aromatic), 125.88 (C, C-aromatic), 127.49 (CH, C-aromatic), 128.04, 134.13, 135.74 (C, C-aromatic), 136.29 (CH, C-aromatic), 138.97 (C, C-aromatic), 147.85, 152.40 (CH, C-aromatic), 154.40, 166.06 (C, C-aromatic).

***N*-(1*H*-Benzo[*d*]imidazol-2-*yl*)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine (312)**

($C_{18}H_{15}N_5S$; M.W.= 321.4)



315 **312**

General procedure 23;

T.L.C. System: EtOAc-MeOH 9:1 v/v, Rf: 0.65.

Purification: flash column chromatography (*n*hexane:EtOAc 50:50 v/v increasing to EtOAc:MeOH 95:5 v/v).

Pale yellow solid;

Yield: 0.03 g (12%)

Melting Point: 122-125°C

MS (ESI^+): 322.1 $[M+H]^+$

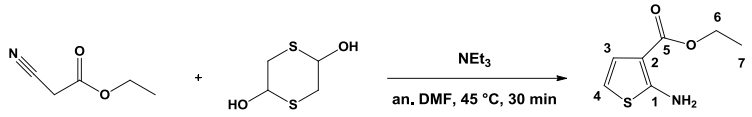
^{1}H-NMR ($CDCl_3$), δ: 1.52-1.60 (m, 1H, C<u>H</u>$_2$), 1.61-1.68 (m, 1H, C<u>H</u>$_2$), 1.76-1.84 (m, 1H, C<u>H</u>$_2$), 1.86-1.93 (m, 1H, C<u>H</u>$_2$), 2.01-2.09 (m, 1H, C<u>H</u>$_2$), 2.28-2.34 (m, 1H, C<u>H</u>$_2$), 2.89-2.93 (m, 2H, C<u>H</u>$_2$), 5.54 (bs, 2H, N<u>H</u>), 6.79 (d, J= 7.9 Hz, 1H, H-aromatic), 6.99-7.02 (m, 1H, H-aromatic), 7.16-7.19 (m, 1H, H-aromatic), 7.46 (d, J= 7.9 Hz, 1H, H-aromatic), 9.01 (s, 1H, H-2).

^{13}C-NMR ($CDCl_3$), δ: 21.98, 22.47, 24.33, 26.13 (CH_2), 108.85, 116.87, 120.64, 122.82 (CH, C-aromatic), 126.71, 127.18, 134.98, 141.03, 142.04, 148.05 (C, C-aromatic), 152.46 (CH, C-aromatic), 153.25, 171.75 (C, C-aromatic).

6.4.3 Thieno[2,3-*d*]pyrimidines

Ethyl 2-aminothienophene-3-carboxylate (318)[38]

($C_7H_9NO_2S$; M.W.= 171.2)



189 **317** **318**

Triethylamine (1.8 mL, 13.3 mmol) was added dropwise over 10 min. to a mixture of ethyl cyanoacetate (**189**) (3 g, 26.6 mmol) and 1,4-dithiane-2,5-diol (**317**) (2 g, 13.3 mmol) in anhydrous DMF (10 mL) at r.t. under N_2 atmosphere. After the addition was complete the reaction mixture was heated at 45°C for 30 min. The reaction mixture was diluted with 0.4 M acetic acid (70 mL) and extracted with EtOAc (4 x 50 mL). The combined extracts were washed with water (2 x 100mL), dried over $MgSO_4$, and concentrated in vacuo to give a crude brown oil that was purified by flash column chromatography (*n*hexane:EtOAc 100:0 v/v increasing to *n*hexane-EtOAc 95: 5 v/v) to give pure ethyl 2-aminothienophene-3-carboxylate **318** as a colourless oil.

T.L.C. System: *n*hexane-EtOAc 4:1 v/v, Rf: 0.55.

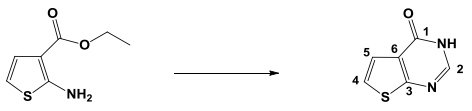
Yield: 2.93 g (64%)

^{1}H-NMR ($CDCl_3$), δ: 1.36 (t, J= 7.1 Hz, 3H, H-7), 4.29 (q, J= 7.1 Hz, 2H, H-6), 5.87 (bs, 2H, N<u>H</u>$_2$), 6.19 (d, J= 5.9 Hz, 1H, H-aromatic), 6.99 (d, J= 5.9 Hz, 1H, H-aromatic),

^{13}C-NMR ($CDCl_3$), δ: 14.50 (CH_3, C-7), 59.74 (CH_2, C-2'), 106.88 (CH, C-aromatic), 107.20 (C, C-aromatic), 125.92 (CH, C-aromatic), 162.97 (C, C-aromatic), 165.47 (C, C-5).

3*H*-Thieno[2,3-*d*]pyrimidin-4-one (319)[39]

($C_6H_{10}N_2OS$; M.W.= 206.3)



318 **319**

General procedure 17;

Reagent: ethyl 2-aminothienophene-3-carboxylate **318** (2.93 g, 17.1 mmol);

Purification: re-crystallisation from 40% EtOH/H_2O;

Yellow solid;

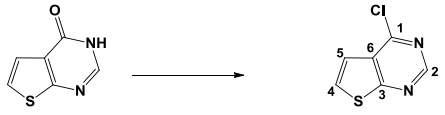
Yield: 1.15 g (44%)

^{1}H-NMR (DMSO-d$_6$), δ: 7.39 (d, J= 5.8 Hz, 1H, H-aromatic), 7.57 (d, J= 5.8 Hz, 1H, H-aromatic), 8.12 (s, 1H, H-2), 12.47 (bs, 1H, NH).

^{13}C-NMR (DMSO-d$_6$), δ: 121.58, 123.76 (CH, C-aromatic), 124.58 (C, C-aromatic), 145.56 (CH, C-2), 157.45 (C, C-aromatic), 164.19 (C, C-1).

4-Chloro-thieno[2,3-*d*]pyrimidine (320)[39]

($C_6H_3ClN_2S$; M.W.= 170.6)



319 **320**

General procedure 18;

Reagent: 3*H*-thieno[2,3-*d*]pyrimidin-4-one (**319**) (1.15 g, 7.5 mmol);

T.L.C. System: *n*hexane-EtOAc 8:2 v/v, Rf: 0.45;

Purification: flash column chromatography (*n*hexane:EtOAc 100:0 v/v increasing to *n*hexane-EtOAc 90:10 v/v);

Pale yellow solid;

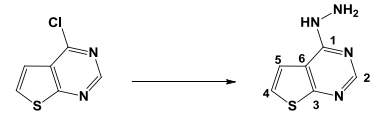
Yield: 1.01 g (79%)

^{1}H-NMR ($CDCl_3$), δ: 7.45 (d, J= 6.0 Hz, 1H, H-aromatic), 7.64 (d, J= 6.0 Hz, 1H, H-aromatic), 8.87 (s, 1H, H-2).

^{13}C-NMR ($CDCl_3$), δ: 119.90, 128.38 (CH, C-4, 5), 129.55 (C, C-aromatic), 155.09 (CH, C-2), 168.82 (C, C-aromatic).

Thieno[2,3-*d*]pyrimidin-4-*yl*-hydrazine (321)[41]

($C_6H_6N_4S$; M.W.= 166.2)



320 **321**

General procedure 19;

Reagent: 4-chloro-thieno[2,3-d]pyrimidine **320** (1.01 g, 5.9 mmol);

Purification: recrystallization from 40 % EtOH/H_2O;

White crystals;

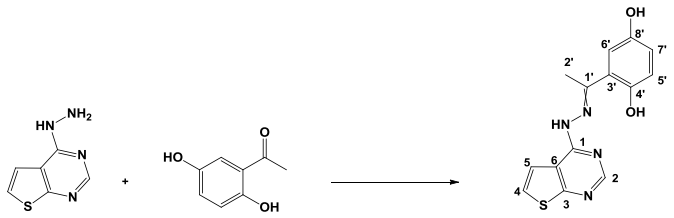
Yield: 0.63 g (63%)

^{1}H-NMR (DMSO-d_6), δ: 4.63 (bs, 2H, N$\underline{H}_2$), 7.53 (d, J= 5.7 Hz, 2H, H-4, 5), 7.61 (bs, 1H, N$\underline{H}$), 8.37 (s, 1H, H-2), 9.19 (bs, 1H, N$\underline{H}$).

^{13}C-NMR (DMSO-d_6), δ: 114.47 (C, C-aromatic), 122.51 (CH, C-4, 5), 153.61 (CH, C-2), 158.13, 163.77 (C, C-aromatic).

3.4.2.1 *N*-(1-Aryl-ethylidene)-*N*'-(thieno[2,3-*d*]pyrimidin-4-*yl*)-hydrazines (322-325)

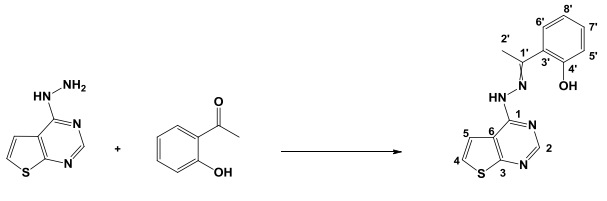
2-[1-(Thieno[2,3-*d*]pyrimidin-4-*yl*-hydrazono)-ethyl]-benzene-1,4-diol (322)
($C_{14}H_{12}N_4O_2S$; M.W.= 300.3)



321 **194** **322**

General procedure 20;
Reagent: thieno[2,3-*d*]pyrimidin-4-*yl*-hydrazine (**321**) (0.15 g, 0.9 mmol);
Yellow solid;
Yield: 0.22 g (81%)
Melting Point: 267-269°C
MS (ESI^+): 301.1 $[M+H]^+$
Single species observed.
^{1}H-NMR (DMSO-d_6), δ: 2.47 (s, 3H, H-2'), 6.75-6.76 (m, 2H, H-aromatic), 6.98-7.00 (m, 1H, H-aromatic), 7.73 (d, J= 5.8 Hz, 1H, H-aromatic), 7.93 (d, J= 5.8 Hz, 1H, H-aromatic), 7.93 (bs, 1H, OH), 8.90 (s, 1H, H-2), 10.74 (bs, 1H, NH), 12.10 (bs, 1H, OH).
^{13}C-NMR (DMSO-d_6), δ: 14.33 (CH_3, C-2'), 113.47 (CH, C-aromatic), 115.35 (C, C-aromatic), 117.53, 118.38, 120.82 (CH, C-aromatic), 121.21 (C, C-aromatic), 123.93 (CH, C-aromatic), 149.20, 150.63, 152.90 (C, C-aromatic), 153.38 (CH, C-aromatic), 154.48, 167.47 (C, C-aromatic).

2-[1-(Thieno[2,3-*d*]pyrimidin-4-*yl*-hydrazono)-ethyl]-phenol (323)
($C_{14}H_{12}N_4OS$; M.W.= 284.3)



321 **202** **323**

General procedure 20;

Reagent: thieno[2,3-*d*]pyrimidin-4-*yl*-hydrazine (**321**) (0.15 g, 0.9 mmol);

Pale yellow crystals;

Yield: 0.15 g (61%)

Melting Point: 178-180°C

MS (ESI^+): 285.1 $[M+H]^+$

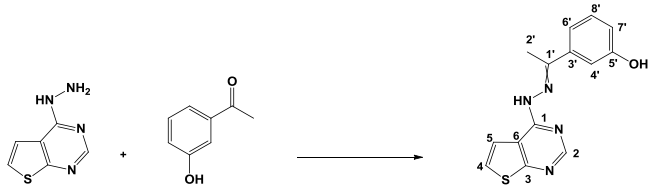
Single species observed.

^{1}H-NMR (DMSO-d$_6$), δ: 2.54 (s, 3H, H-2'), 6.89-6.94 (m, 2H, H-aromatic), 7.27-7.31 (m, 1H, H-aromatic), 7.61 (dd, J_1= 7.9 Hz, J_2= 1.3 Hz, 1H, H-aromatic), 7.72-7.76 (m, 1H, H-aromatic), 7.91-7.95 (m, 1H, H-aromatic), 8.60 (s, 1H, H-2), 10.79 (bs, 1H, NH), 12.93 (bs, 1H, OH).

^{13}C-NMR (DMSO-d$_6$), δ: 14.21 (CH_3, C-2'), 115.41 (C, C-aromatic), 117.08, 118.54, 120.17 (CH, C-aromatic), 120.58 (C, C-aromatic), 124.03, 128.23, 130.70, 153.35 (CH, C-aromatic), 154.24, 154.74, 158.04, 167.38 (C, C-aromatic).

3-[1-(Thieno[2,3-*d*]pyrimidin-4-*yl*-hydrazono)-ethyl]-phenol (324)

($C_{14}H_{12}N_4OS$; M.W.= 284.3)



321 **203** **324**

General procedure 20;

Reagent: thieno[2,3-*d*]pyrimidin-4-*yl*-hydrazine (**321**) (0.15 g, 0.9 mmol);

Yellow solid;

Yield: 0.09 g (37%)

Melting Point: 203-205°C

MS (ESI^+): 285.1 $[M+H]^+$

Single species observed.

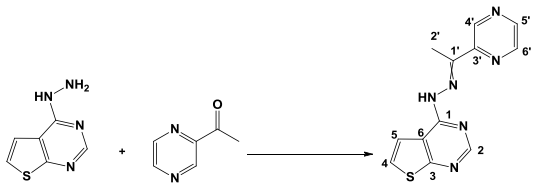
^{1}H-NMR (DMSO-d$_6$), δ: 2.39 (s, 3H, H-2'), 6 84 (d, J= 7.3 Hz, 1H, H-aromatic), 7.22-7.29 (m, 3H, H-aromatic), 7.68 (d, J= 5.9 Hz, 1H, H-aromatic), 8.08 (d, J= 5.9 Hz, 1H,

H-aromatic), 8.52 (s, 1H, H-2), 9.60 (bs, 1H, NH), 10.73 (bs, 1H, OH).

^{13}C-NMR (DMSO-d$_6$), δ: 13.96 (CH_3, C-2'), 112.57 (CH, C-aromatic), 115.01 (C, C-aromatic), 116.19, 117.01, 122.85, 123.16, 129.56 (CH, C-aromatic), 139.78, 150.21 (C, C-aromatic), 152.72 (CH, C-aromatic), 156.29, 157.48, 169.02 (C, C-aromatic).

3-[1-(Thieno[2,3-*d*]pyrimidin-4-*yl*-hydrazono)-ethyl]-phenol (325)

($C_{12}H_{10}N_6S$; M.W.= 270.3)



321 **250** **325**

General procedure 20;

Reagent: thieno[2,3-*d*]pyrimidin-4-*yl*-hydrazine (**321**) (0.15 g, 0.9 mmol);

White solid;

Yield: 0.23 g (96%)

Melting Point: 239-243°C

MS (ESI^+): 271.0 $[M+H]^+$

Two species observed.Major/minor species ratio: 5/1.

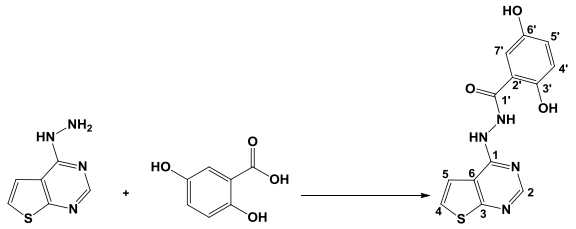
^{1}H-NMR (DMSO-d$_6$), δ: (major species) 2.52 (s, 3H, H-2'), 7.79 (d, J= 5.9 Hz, 1H, H-aromatic), 8.08 (d, J= 5.9 Hz, 1H, H-aromatic), 8.60 (s, 1H, H-2), 8.64-8.67 (m, 1H, H-aromatic), 8.69-8.70 (m, 1H, H-aromatic), 9.26 (s, 1H, H-aromatic), 11.21 (bs, 1H, NH).

^{1}H-NMR (DMSO-d$_6$), δ: (minor species) 2.52 (s, 3H, H-2'), 7.47 (d, J= 5.3 Hz, 1H, H-aromatic), 7.62 (d, J= 5.3 Hz, 1H, H-aromatic), 7.98-8.00 (m, 1H, H-aromatic), 8.60 (s, 1H, H-2), 8.64-8.67 (m, 1H, H-aromatic), 9.81 (s, 1H, H-aromatic), 12.06 (bs, 1H, NH).

^{13}C-NMR (DMSO-d$_6$), δ: (major and minor species) 11.96, 12.33 (CH_3, C-2'), 115.01 (C, C-aromatic), 121.12, 122.33, 124.25, 141.88, 142.16, 143.30, 143.51, 143.65, 143.84, 144.61 (CH, C-aromatic), 144.76, 148.35, 150.71 (C, C-aromatic), 152.69 (CH, C-aromatic), 156.08 (C, C-aromatic).

6.4.2.2 *N*'-(Thieno[2,3-*d*]pyrimidin-4-*yl*)arylcarbohydrazide (326-329)

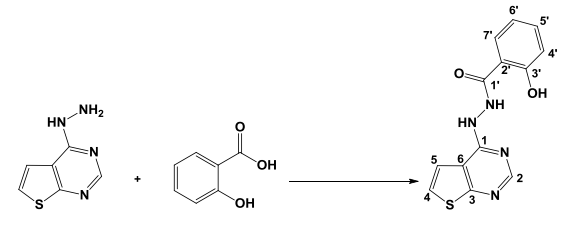
2,5-Dihydroxy-*N*'-(thieno[2,3-*d*]pyrimidin-4-*yl*)benzohydrazide (326)
($C_{13}H_{10}N_4O_3S$; M.W.= 302.3)



321 **274** **326**

General procedure 22;
Reagent: thieno[2,3-*d*]pyrimidin-4-*yl*-hydrazine (**321**) (0.15 g, 0.9 mmol);
Purification: re-crystallisation from EtOH.
Light brown solid;
Yield: 0.05 g (15%)
Melting Point: >260°C
MS (ESI^+): 303.1 $[M+H]^+$
^{1}H-NMR (DMSO-d_6), δ: 6.83 (d, J= 8.8 Hz, 1H, H-4'), 6.93 (dd, J_1= 8.8 Hz, J_2= 2.8 Hz, 1H, H-5'), 7.35 (d, J= 2.8 Hz, 1H, H-7'), 7.69-7.72 (m, 2H, H-aromatic), 8.43 (s, 1H, H-2), 9.11 (bs, 1H, NH), 10.18 (bs, 1H, OH), 10.73 (bs 1H, NH), 11.07 (bs, 1H, OH).
^{13}C-NMR (DMSO-d_6), δ: 113.93 (CH, C-aromatic), 114.78, 115.32 (C, C-aromatic), 117.90, 119.02, 121.65, 124.11 (CH, C-aromatic), 128.52, 149.45, 149.61, 151.28 (C, C-aromatic), 153.41 (CH, C-aromatic), 167.26 (C, C-1').

2-Hydroxy-*N*'-(thieno[2,3-*d*]pyrimidin-4-*yl*)benzohydrazide (327)
($C_{13}H_{10}N_4O_2S$; M.W.= 286.3)



321 **275** **327**

General procedure 22;

Reagent: thieno[2,3-*d*]pyrimidin-4-*yl*-hydrazine (**321**) (0.15 g, 0.9 mmol);

Purification: re-crystallisation from EtOH.

White solid;

Yield: 0.05 g (19%)

Melting Point: 180-183°C

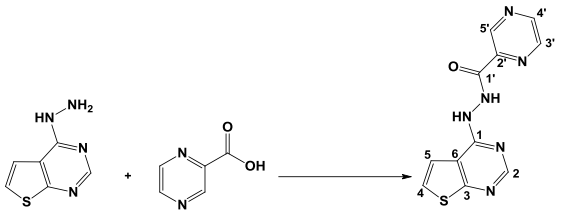
MS (ESI^+): 287.0 $[M+H]^+$

^{1}H-NMR (DMSO-d$_6$), δ: 6.96-7.01 (m, 2H, H-aromatic), 7.46-7.50 (m, 1H, H-aromatic), 7.68-7.74 (m, 2H, H-aromatic), 7.97 (dd, J_1= 7.9 Hz, J_2= 1.0 Hz, 1H, H-aromatic), 8.44 (s, 1H, H-2), 10.18 (bs, 1H, NH), 10.85 (bs 1H, NH), 11.85 (bs, 1H, OH).

^{13}C-NMR (DMSO-d$_6$), δ: 114.77 (C, C-aromatic), 117.37, 118.97, 119.09 (CH, C-aromatic), 120.64 (C, C-aromatic), 128.39, 134.15 (CH, C-aromatic), 134.49 (C, C-aromatic), 153.43 (CH, C-aromatic), 159.12 (C, C-aromatic), 167.99 (C, C-1').

***N*'-(Thieno[2,3-*d*]pyrimidin-4-*yl*)pyrazine-2-carbohydrazide (328)**

($C_{11}H_8N_6OS$; M.W.= 272.3)



321 **276** **328**

General procedure 22;

Reagent: thieno[2,3-*d*]pyrimidin-4-*yl*-hydrazine (**321**) (0.15 g, 0.9 mmol);

Purification: re-crystallisation from EtOH.

White solid;

Yield: 0.08 g (31%)

Melting Point: 193-197°C

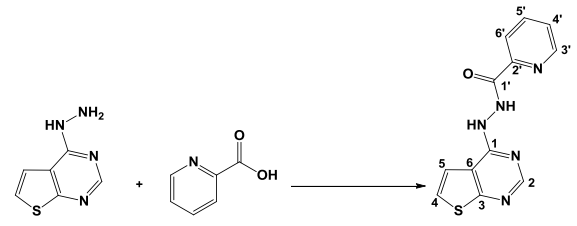
MS (ESI^+): 272.9 $[M+H]^+$

^{1}H-NMR (DMSO-d$_6$), δ: 7.68-7.72 (m, 2H, H-aromatic), 8.41 (s, 1H, H-2), 8.83 (dd, J_1= 2.5 Hz, J_2= 1.5 Hz, 1H, H-4'), 8.96 (d, J= 2.5 Hz, 1H, H-3'), 9.23 (d, J= 1.5 Hz, 1H, H-5'), 10.14 (bs, 1H, NH), 11.06 (bs 1H, NH).

^{13}C-NMR (DMSO-d$_6$), δ: 114.83 (C, C-aromatic), 119.03, 124.09 (CH, C-aromatic), 123.14 138.47 (C, C-aromatic), 143.67, 143.77 (CH, C-aromatic), 144.18 (C, C-aromatic), 148.10, 153.44 (CH, C-aromatic), 162.66 (C, C-1').

***N*'-(thieno[2,3-*d*]pyrimidin-4-*yl*)picolinohydrazide (329)**

(C$_{12}$H$_9$N$_5$OS; M.W.= 271.3)



321 **277** **329**

General procedure 22;

Reagent: thieno[2,3-*d*]pyrimidin-4-*yl*-hydrazine (**321**) (0.15 g, 0.9 mmol);

Purification: re-crystallisation from EtOH.

White solid;

Yield: 0.08 g (30%)

Melting Point: 191-194°C

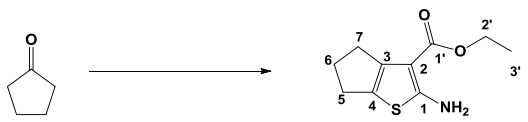
MS (ESI$^+$): 271.9 $[M+H]^+$

^{1}H-NMR (DMSO-d$_6$), δ: 7.67-7.71 (m, 3H, H-aromatic), 8.03-8.09 (m, 2H, H-aromatic), 8.40 (s, 1H, H-2), 8.74 (d, J= 4.6 Hz, 1H, H-aromatic), 10.09 (bs, 1H, N<u>H</u>), 10.87 (bs 1H, N<u>H</u>).

^{13}C-NMR (DMSO-d$_6$), δ: 114.79 (C, C-aromatic), 119.12 (CH, C-aromatic), 121.12 (C, C-aromatic), 122.46, 123.90, 127.08, 137.89 (CH, C-aromatic), 138.05 (C, C-aromatic), 148.74 (CH, C-aromatic), 149.20 (C, C-aromatic), 153.44 (CH, C-aromatic), 163.50 (C, C-1').

6.4.4 Cyclopentane[b]thienopyrimidines

Ethyl 2-amino-5,6-dihydro-4*H*-cyclopentane[*b*]thiophene-3-carboxylate (331)
($C_{10}H_{13}NO_2S$; M.W.= 211.2)[42]



330 **331**

General procedure 16;

Reagent: cyclopentanone (**330**) (1 g, 11.9 mmol);

T.L.C. System: *n*hexane-EtOAc 8:2 v/v, Rf: 0.55.

Purification: flash column chromatography (*n*hexane:EtOAc 100:0 v/v increasing to *n*hexane-EtOAc 90:10 v/v);

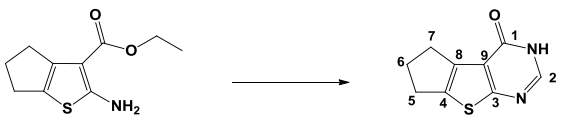
White solid;

Yield: 1.39 g (55%)

^{1}H-NMR ($CDCl_3$), δ: 1.34 (t, J= 7.1 Hz, 3H, H-3'), 2.29-2.36 (m, 2H, H-6), 2.71-2.75 (m, 2H, C$\underline{H}_2$), 2.82-2.86 (m, 2H, C$\underline{H}_2$), 4.26 (q, J= 7.1 Hz, 2H, H-2'), 5.88 (bs, 2H, N$\underline{H}_2$).

^{13}C-NMR ($CDCl_3$), δ: 14.44 (CH_3, C-3'), 27.23, 28.87, 30.78 (CH_2, C-5, 6, 7), 59.38 (CH_2, C-2'), 102.89, 121.28, 142.67, 165.78 (C, C-1, 2, 3, 4), 166.39 (C, C-1').

1,2,3,5-Tetrahydro-8-thia-5,7-diaza-cyclopenta[*a*]inden-4-one (332)[43]
($C_9H_8N_2OS$; M.W.= 192.2)



331 **332**

General procedure 17;

Reagent: ethyl 2-amino-5,6-dihydro-4*H*-cyclopentane[*b*]thiophene-3-carboxylate (**331**) (1.39 g, 6.6 mmol);

Purification: re-crystallisation from 40% EtOH/H_2O;

Brown solid;

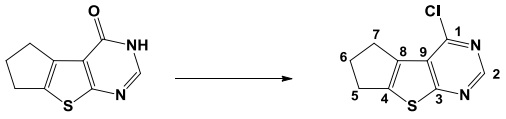
Yield: 0.98 g (77%)

^{1}H-NMR (DMSO-d$_6$), δ: 2.34-2.41 (m, 2H, H-6), 2.88-2.92 (m, 4H, H-5, 7), 7.99 (s, 1H, H-2), 12.37 (bs, 1H, N<u>H</u>).

^{13}C-NMR (DMSO-d$_6$), δ: 27.39, 28.63, 28.90 (CH_2, C-5, 6, 7), 120.31, 137.46, 139.56 (C, C-aromatic), 144.56 (CH, C-2), 157.32 (C, C-aromatic), 167.67 (C, C-1).

4-Chloro-2,3-dihydro-1*H*-8-thia-5,7-diaza-cyclopenta[*a*]indene (333)[44]

($C_9H_7ClN_2S$; M.W.= 210.6)



332 **333**

General procedure 18;

Reagent: 1,2,3,5-tetrahydro-8-thia-5,7-diaza-cyclopenta[a]inden-4-one (**332**) (0.98 g, 5.1 mmol);

T.L.C. System: *n*hexane-EtOAc 1:1 v/v, Rf: 0.66.

Purification: flash column chromatography (*n*hexane:EtOAc 100:0 v/v increasing to *n*hexane-EtOAc 90:10 v/v);

White solid;

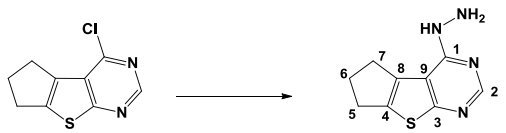
Yield: 0.73 g (68%)

^{1}H-NMR (CDCl$_3$), δ: 2.50-2.58 (m, 2H, H-6), 3.07 (t, J= 7.4 Hz, 2H, C<u>H</u>$_2$), 3.16 (t, J= 7.4 Hz, 2H, C<u>H</u>$_2$), 8.71 (s, 1H, H-2).

^{13}C-NMR (CDCl$_3$), δ: 27.41, 29.42, 30.19 (CH_2, C-5, 6, 7), 126.24, 136.32, 144.84 (C, C-aromatic), 151.44 (CH, C-2), 152.98, 173.66 (C, C-aromatic).

(2,3-Dihydro-1*H*-8-thia-5,7-diaza-cyclopenta[*a*]inden-4-*yl*)-hydrazine (334)[45]

($C_9H_{10}N_4S$; M.W.= 206.3)



333 **334**

General procedure 19;

Reagent: 4-chloro-2,3-dihydro-1*H*-8-thia-5,7-diaza-cyclopenta[*a*]indene (**333**) (0.73 g, 3.5 mmol);

Purification: re-crystallisation from 40% EtOH/H_2O;

White solid;

Yield: 0.55 g (78%)

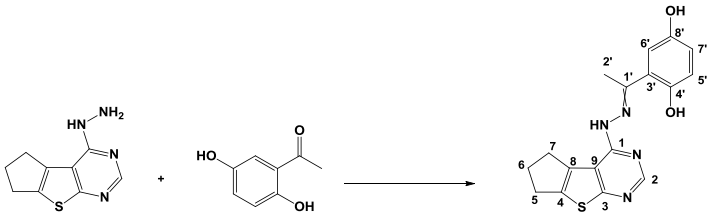
^{1}H-NMR (DMSO-d$_6$), δ: 2.36-2.43 (m, 2H, H-6), 2.93 (t, J= 7.2 Hz, 2H, C$\underline{H}_2$), 3.06 (t, J= 7.2 Hz, 2H, C$\underline{H}_2$), 4.65 (bs, 2H, N$\underline{H}_2$), 8.12 (bs, 1H, N$\underline{H}$), 8.32 (s, 1H, H-2).

^{13}C-NMR (DMSO-d$_6$), δ: 27.39, 28.97, 29.09 (CH_2, C-5, 6, 7), 135.79, 136.94 (C, C-aromatic), 152.47 (CH, C-2), 157.76 (C, C-aromatic).

6.4.3.1 *N*-(2,3-Dihydro-1*H*-8-thia-5,7-diaza-cyclopenta[*a*]inden-4-*yl*)-*N*'-(1-phenyl-ethylidene)-hydrazines (335-340)

2-{1-[(2,3-Dihydro-1*H*-8-thia-5,7-diaza-cyclopenta[*a*]inden-4-*yl*)-hydrazono]-ethyl}-benzene-1,4-diol (335)

($C_{17}H_{16}N_4O_2S$; M.W.= 340.4)



334 **194** **335**

General procedure 20;

Reagent: (2,3-dihydro-1*H*-8-thia-5,7-diaza-cyclopenta[*a*]inden-4-*yl*)-hydrazine (**334**) (0.15 g, 0.7 mmol);

Yellow solid;

Yield: 0.18 g (74%)

Melting Point: 308-310°C

MS (ESI^+): 341.1 $[M+H]^+$

Two species observed. Major/minor species ratio: 3/2.

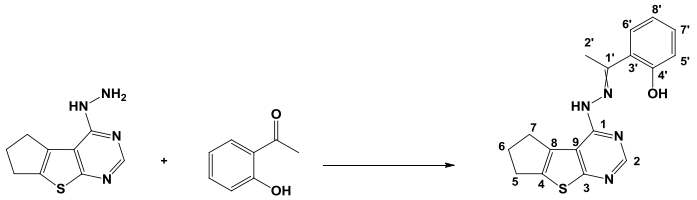
^{1}H-NMR (DMSO-d$_6$), δ: (major species) 2.41 (s, 3H, H-2'), 2.44-2.49 (m, 2H, C<u>H</u>$_2$), 2.98-3.02 (m, 2H, C<u>H</u>$_2$), 3.17-3.21 (m, 2H, C<u>H</u>$_2$), 6.74-6.76 (m, 2H, H-aromatic), 6.98-7.00 (m, 1H, H-aromatic), 8.52 (s, 1H, H-aromatic), 8.88 (bs, 1H, O<u>H</u>), 9.51 (bs, 1H, N<u>H</u>), 12.38 (bs, 1H, O<u>H</u>).

^{1}H-NMR (DMSO-d$_6$), δ: 2.41 (s, 3H, H-2'), 2.44-2.49 (m, 2H, C<u>H</u>$_2$), 2.90-2.94 (m, 2H, C<u>H</u>$_2$), 3.17-3.21 (m, 2H, C<u>H</u>$_2$), 6.74-6.76 (m, 2H, H-aromatic), 6.98-7.00 (m, 1H, H-aromatic), 7.73 (s, 1H, H-aromatic), 8.88 (bs, 1H, O<u>H</u>), 11.54 (bs, 1H, N<u>H</u>), 11.79 (bs, 1H, O<u>H</u>).

^{13}C-NMR (DMSO-d$_6$), δ: (major and minor species) 13.03, 14.45 (CH_3, C-2'), 27.32, 28.67, 29.07, 29.33, 29.59 (CH_2, C-5, 6, 7), 112.96 (C, C-aromatic), 113.56, 114.17, 116.92, 117.61, 118.09, 118.22 (CH, C-aromatic), 119.70, 135.42, 139.20 (C, C-aromatic), 143.83 (CH, C-aromatic), 144.79, 149.18, 151.02 (C, C-aromatic), 152.53 (CH, C-aromatic), 153.56, 154.96, 171.33 (C, C-aromatic).

2-{1-[(2,3-Dihydro-1*H*-8-thia-5,7-diaza-cyclopenta[*a*]inden-4-*yl*)-hydrazono]-ethyl}-phenol (336)

($C_{17}H_{16}N_4OS$; M.W.= 324.4)



334 **202** **336**

General procedure 20;

Reagent: (2,3-dihydro-1*H*-8-thia-5,7-diaza-cyclopenta[*a*]inden-4-*yl*)-hydrazine (**334**) (0.15 g, 0.7 mmol);

Pale yellow solid;

Yield: 0.17 g (71%)

Melting Point: 267-269°C

MS (ESI^+): 325.1 $[M+H]^+$

Microanalysis: Calculated for $C_{17}H_{16}N_4OS$ (324.4); Theoretical: %C = 62.94, %H = 4.97, %N = 17.26; Found: %C = 62.92, %H = 4.83, %N = 17.36.

Two species observed. Major/minor species ratio: 2/1.

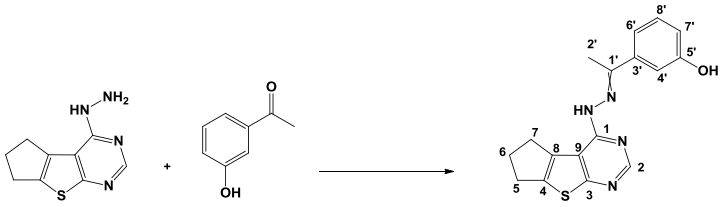
^{1}H-NMR (DMSO-d$_6$), δ: (major species) 2.37 (s, 3H, H-2'), 2.43-2.48 (m, 2H, C<u>H</u>$_2$), 2.98-3.07 (m, 2H, C<u>H</u>$_2$), 3.18-3.25 (m, 2H, C<u>H</u>$_2$), 6.88-6.92 (m, 2H, H-aromatic), 7.27-7.32 (m, 1H, H-aromatic), 7.59-7.67 (m, 1H, H-aromatic), 8.54 (s, 1H, H-aromatic), 9.59 (bs, 1H, N<u>H</u>), 13.16 (bs, 1H, O<u>H</u>).

^{1}H-NMR (DMSO-d$_6$), δ: (minor species) 2.37 (s, 3H, H-2'), 2.43-2.48 (m, 2H, C<u>H</u>$_2$), 2.90-2.94 (m, 2H, C<u>H</u>$_2$), 2.98-3.07 (m, 2H, C<u>H</u>$_2$), 6.88-6.92 (m, 2H, H-aromatic), 7.27-7.32 (m, 1H, H-aromatic), 7.59-7.67 (m, 1H, H-aromatic), 7.75 (s, 1H, H-aromatic), 11.49 (bs, 1H, N<u>H</u>), 12.55 (bs, 1H, O<u>H</u>).

^{13}C-NMR (DMSO-d$_6$), δ: (major and minor species) 13.06, 14.48 (CH_3, C-2'), 27.36, 28.69, 29.12, 29.35, 29.57 (CH_2, C-5, 6, 7), 112.92 (C, C-aromatic), 116.50, 117.23, 118.58 (CH, C-aromatic), 119.69, 135.40 (C, C-aromatic), 128.13, 128.72, 130.82 (CH, C-aromatic), 135.47, 139.33 (C, C-aromatic), 143.85, 152.57 (CH, C-aromatic), 153.71, 155.47, 158.31 (C, C-aromatic).

3-{1-[(2,3-Dihydro-1*H*-8-thia-5,7-diaza-cyclopenta[*a*]inden-4-*yl*)-hydrazono]-ethyl}-phenol (337)

($C_{17}H_{16}N_4OS$; M.W.= 324.4)



334 **203** **337**

General procedure 20;

Reagent: (2,3-dihydro-1*H*-8-thia-5,7-diaza-cyclopenta[*a*]inden-4-*yl*)-hydrazine (**334**) (0.15 g, 0.7 mmol);

Pale pink solid;

Yield: 0.15 g (64%)

Melting Point: 320-322°C

MS (ESI^+): 325.1 $[M+H]^+$

Two species observed. Major/minor species ratio: 5/2.

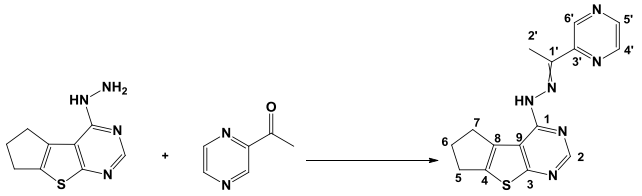
^{1}H-NMR (DMSO-d_6), δ: (major species) 2.41 (s, 3H, H-2'), 2.89-3.02 (m, 6H, C$\underline{H}_2$), 6.80-6.83 (m, 1H, H-aromatic), 7.17-7.25 (m, 1H, H-aromatic), 7.38-7.40 (m, 1H, H-aromatic), 7.47-7.49 (m, 1H, H-aromatic), 7.75 (s, 1H, H-aromatic), 9.39 (bs, 1H, N$\underline{H}$), 11.56 (bs, 1H, O$\underline{H}$).

^{1}H-NMR (DMSO-d_6), δ: (minor species) 2.24-2.30 (m, 2H, C$\underline{H}_2$), 2.33-2.36 (m, 4H, C$\underline{H}_2$), 2.37 (s, 3H, H-2'), 6.80-6.83 (m, 1H, H-aromatic), 7.17-7.25 (m, 3H, H-aromatic), 8.45 (s, 1H, H-aromatic), 9.50 (bs, 1H, N$\underline{H}$), 9.80 (bs, 1H, O$\underline{H}$).

^{13}C-NMR (DMSO-d_6), δ: (major and minor species) 13.99, 14.08 (CH_3, C-2'), 27.05, 27.41, 29.07, 29.38, 29.52, 30.98 (CH_2, C-5, 6, 7), 112.92 (CH, C-aromatic), 113.03 (C, C-aromatic), 113.50, 115.91, 116.17 (CH, C-aromatic), 117.19 (C, C-aromatic), 117.42, 117.49, 128.86, 129.27 (CH, C-aromatic), 136.89, 137.51, 138.17, 139.40, 139.78, 140.21 (C, C-aromatic), 143.82 (CH, C-aromatic), 146.70, 151.06 (C, C-aromatic), 151.65 (CH, C-aromatic), 157.15, 157.26, 157.65, 161.78 (C, C-aromatic).

4-(2-(1-(Pyrazin-2-*yl*)ethylidene)hydrazinyl)-6,7-dihydro-5*H*-cyclopenta[4,5] thieno[2,3-*d*]pyrimidine (338)

($C_{15}H_{14}N_6S$; M.W.= 310.3)



General procedure 20;

Reagent: (2,3-dihydro-1*H*-8-thia-5,7-diaza-cyclopenta[*a*]inden-4-*yl*)-hydrazine (**334**) (0.15 g, 0.7 mmol);

Yellow solid;

Yield: 0.09 g (42%)

Melting Point: 182-185°C

MS (ESI^+): 311.1 $[M+H]^+$

Two species observed. Major/minor species ratio: 4/1.

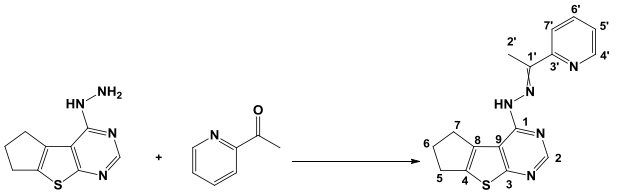
^{1}H-NMR ($CDCl_3$), δ: (major species) 2.48-2.56 (m, 5H, H-2', C<u>H</u>$_2$), 3.05-3.09 (m, 2H, C<u>H</u>$_2$), 3.11-3.17 (m, 2H, C<u>H</u>$_2$), 8.53-8.56 (m, 3H, H-aromatic, N<u>H</u>), 8.61 (s, 1H, H-2), 9.40 (d, J= 1.4 Hz, 1H, H-aromatic).

^{1}H-NMR ($CDCl_3$), δ: (minor species) 2.48-2.56 (m, 2H, C<u>H</u>$_2$), 2.61 (s, 3H, C<u>H</u>$_3$), 2.97-3.01 (m, 2H, C<u>H</u>$_2$), 3.11-3.17 (m, 2H, C<u>H</u>$_2$), 7.75 (s, 1H, H-aromatic), 8.49 (d, J= 2.5 Hz, 1H, H-aromatic), 8.53-8.56 (m, 1H, H-aromatic), 9.34 (d, J= 1.4 Hz, 1H, H-aromatic), 10.39 (bs, 1H, N<u>H</u>).

^{13}C-NMR ($CDCl_3$), δ: (major and minor species) 10.63, 13.07 (CH_3, C-2'), 27.90, 28.10, 29.70, 29.77, 29.88, 30.95 (CH_2, C-5, 6, 7), 113.57, 134.80 (C, C-aromatic), 140.85 (CH, C-aromatic), 141.18 (C, C-aromatic), 142.95, 143.00, 143.24, 143.34, 143.45, 143.79 (CH, C-aromatic), 147.34, 150.50 (C, C-aromatic), 152.30 (CH, C-aromatic), 153.86, 173.68 (C, C-aromatic).

4-(2-(1-(Pyridin-2-*yl*)ethylidene)hydrazinyl)-6,7-dihydro-5*H*-cyclopenta[4,5] thieno[2,3-*d*]pyrimidine (339)

($C_{16}H_{15}N_5S$; M.W.= 309.3)



334 **251** **339**

General procedure 20;

Reagent: (2,3-dihydro-1*H*-8-thia-5,7-diaza-cyclopenta[*a*]inden-4-*yl*)-hydrazine (**334**) (0.15 g, 0.7 mmol);

Orange solid;

Yield: 0.08 g (36%)

Melting Point: 153-157°C

MS (ESI^+): 310.1 $[M+H]^+$

Two species observed. Major/minor species ratio: 5/2.

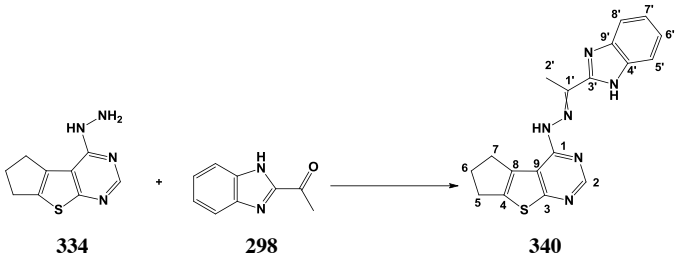
^{1}H-NMR ($CDCl_3$), δ: (major species) 2.59 (s, 3H, H-2'), 2.60-2.65 (m, 2H, C$\underline{H}_2$), 3.04-3.08 (m, 2H, C$\underline{H}_2$), 3.31-3.35 (m, 2H, C$\underline{H}_2$), 7.40-7.43 (m, 1H, H-aromatic), 7.62-7.64 (m, 1H, H-aromatic), 7.91-7.95 (m, 1H, H-aromatic), 8.65 (s, 1H, H-2), 8.68-8.70 (m, 1H, H-aromatic), 15.07 (bs, 1H, N$\underline{H}$).

^{1}H-NMR ($CDCl_3$), δ: (minor species) 2.54 (s, 3H, H-2'), 2.49-2.57 (m, 2H, C$\underline{H}_2$), 3.04-3.08 (m, 2H, C$\underline{H}_2$), 3.12-3.16 (m, 2H, C$\underline{H}_2$), 7.27-7.29 (m, 1H, H-aromatic), 7.71-7.75 (m, 1H, H-aromatic), 8.20-8.22 (m, 1H, H-aromatic), 8.48 (bs, 1H, N$\underline{H}$), 8.61 (s, 1H, H-2), 8.62-8.63 (m, 1H, H-aromatic).

^{13}C-NMR ($CDCl_3$), δ: (major and minor species) 10.91, 22.68 (CH_3, C-2'), 27.91, 27.98, 29.82, 29.87, 30.57 (CH_2, C-5, 6, 7), 113.57 (C, C-aromatic), 121.25, 123.63, 123.73, 123.78 (CH, C-aromatic), 134.91 (C, C-aromatic), 136.24, 137.78 (CH, C-aromatic), 139.42, 139.87, 140.69 (C, C-aromatic), 146.97, 148.58 (CH, C-aromatic), 149.70 (C, C-aromatic), 152.66, 153.45 (CH, C-aromatic), 153.63, 153.94, 155.09, 172.48 (C, C-aromatic).

4-(2-(1-(1*H*-Benzo[*d*]imidazol-2-*yl*)ethylidene)hydrazinyl)-6,7-dihydro-5*H*-cyclopenta[4,5]thieno[2,3-*d*]pyrimidine (340)

($C_{19}H_{14}N_6S$; M.W.= 348.4)



334 **298** **340**

General procedure 20;

Reagent: (2,3-dihydro-1*H*-8-thia-5,7-diaza-cyclopenta[*a*]inden-4-*yl*)-hydrazine (**334**) (0.15 g, 0.7 mmol);

Light brown solid;

Yield: 0.14 g (58%)

Melting Point: charring > 230°C

MS (ESI^+): 349.1 $[M+H]^+$

Two species observed. Major/minor species ratio: 5/1.

^{1}H-NMR (DMSO-d_6), δ: (major species) 2.36-2.42 (m, 2H, C<u>H</u>$_2$), 2.52 (s, 3H, H-2'), 2.89-2.94 (m, 2H, C<u>H</u>$_2$), 2.99-3.07 (m, 2H, C<u>H</u>$_2$), 7.16-7.24 (m, 2H, H-aromatic), 7.52-7.60 (m, 1H, H-aromatic), 7.62-7.71 (m, 1H, H-aromatic), 8.04 (s, 1H, H-2), 12.09 (bs, 1H, N<u>H</u>), 12.54 (bs, 1H, N<u>H</u>).

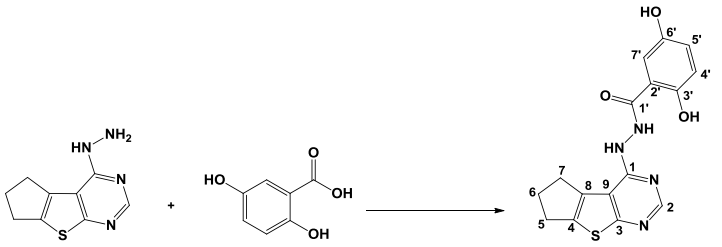
^{1}H-NMR (DMSO-d_6), δ: (minor species) 2.29-2.33 (m, 2H, C<u>H</u>$_2$), 2.52 (s, 3H, H-2'), 2.89-2.94 (m, 2H, C<u>H</u>$_2$), 2.99-3.07 (m, 2H, C<u>H</u>$_2$), 7.35-7.41 (m, 2H, H-aromatic), 7.62-7.71 (m, 1H, H-aromatic), 7.73-7.76 (m, 1H, H-aromatic), 8.47 (s, 1H, H-2), 13.00 (bs, 1H, N<u>H</u>), 14.40 (bs, 1H, N<u>H</u>).

^{13}C-NMR (DMSO-d_6), δ: (major and minor species) 12.65, 18.52 (CH_3, C-2'), 27.11, 27.14, 29.13, 29.39, 29.45, 29.78 (CH_2, C-5, 6, 7), 111.06, 111.91, 113.74 (CH, C-aromatic), 117.24, 118.66 (C, C-aromatic), 119.17, 119.38, 121.45, 123.47, 124.56 (CH, C-aromatic), 132.36, 132.92, 134.25, 135.08, 135.47, 136.79, 138.23, 138.95 (C, C-aromatic), 139.74 (CH, C-aromatic), 141.62, 143.32, 144.35, 146.36, 149.58, 151.82, 155.20, 172.74 (C, C-aromatic).

6.4.3.2 *N*'-(6,7-Dihydro-5*H*-cyclopenta[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)arylcarbo hydrazides (341-346)

N'-(6,7-dihydro-5*H*-cyclopenta[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-2,5-dihydroxy benzohydrazide (341)

($C_{16}H_{14}N_4O_3S$; M.W.= 342.3)



General procedure 22;

Reagent: (2,3-dihydro-1*H*-8-thia-5,7-diaza-cyclopenta[*a*]inden-4-*yl*)-hydrazine (**334**) (0.2 g, 1.0 mmol);

Purification: re-crystallisation from EtOH.

Light grey solid;

Yield: 0.09 g (26%)

Melting Point: charring > 248°C

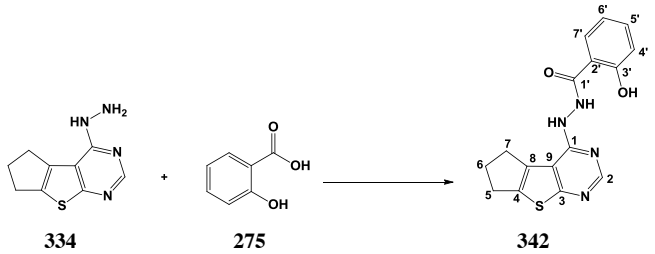
MS (ESI^+): 343.1 $[M+H]^+$

^{1}H-NMR (DMSO-d$_6$), δ: 2.42-2.48 (m, 2H, H-6), 2.99 (t, J=7.1 Hz, 2H, C$\underline{H}_2$), 3.12 (, J=6.9 Hz, 2H, C$\underline{H}_2$), 6.82 (d, J= 8.8 Hz, 1H, H-4'), 6.92 (dd, J_1= 8.8 Hz, J_2= 2.8 Hz, 1H, H-5'), 7.37 (d, J= 2.8 Hz, 1H, H-7'), 8.35 (s, 1H, H-2), 8.98 (bs, 1H, N$\underline{H}$), 9.09 (bs, 1H, O$\underline{H}$), 10.66 (bs 1H, N$\underline{H}$), 11.18 (bs, 1H, O$\underline{H}$).

^{13}C-NMR (DMSO-d$_6$), δ: 27.45, 29.23 (CH_2), 111.91 (C, C-aromatic), 113.85 (CH, C-aromatic), 115.22 (C, C-aromatic), 117.89, 121.65 (CH, C-aromatic), 135.33, 138.53, 149.56, 151.47 (C, C-aromatic), 152.26 (CH, C-aromatic), 156.43, 167.37 (C, C-aromatic), 170.94 (C, C-1').

***N*'-(6,7-Dihydro-5*H*-cyclopenta[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-2-hydroxybenzo hydrazide (342)**

($C_{16}H_{14}N_4O_2S$; M.W.= 326.3)



General procedure 22;

Reagent: (2,3-dihydro-1*H*-8-thia-5,7-diaza-cyclopenta[*a*]inden-4-*yl*)-hydrazine (**334**) (0.2 g, 1.0 mmol);

T.L.C. System: 100% EtOAc, Rf: 0.70.

Purification: flash column chromatography (*n*hexane:EtOAc 100:0 v/v increasing to *n*hexane:EtOAc 0:100 v/v).

White solid;

Yield: 0.10 g (30%)

Melting Point: charring > 230°C

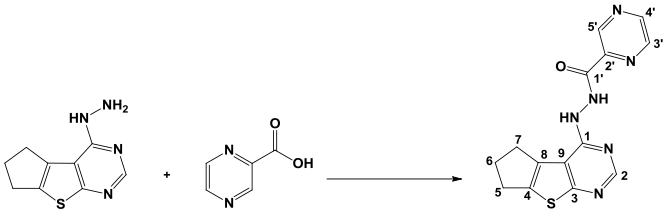
MS (ESI^+): 327.1 $[M+H]^+$

^{1}H-NMR (DMSO-d$_6$), δ: 2.43-2.49 (m, 2H, H-6), 3.00 (t, J=7.1 Hz, 2H, C$\underline{H}_2$), 3.13 (, J=7.1 Hz, 2H, C$\underline{H}_2$), 6.96-7.00 (m, 2H, H-aromatic), 7.46-7.50 (m, 1H, H-aromatic), 7.99 (dd, J_1= 7.9 Hz, J_2= 1.2 Hz, 1H, H-aromatic), 8.36 (s, 1H, H-2), 9.01 (bs, 1H, N$\underline{H}$), 10.79 (bs 1H, N$\underline{H}$), 11.98 (bs, 1H, N$\underline{H}$).

^{13}C-NMR (DMSO-d$_6$), δ: 27.44, 29.20, 29.24 (CH_2), 111.92, 114.59 (C, C-aromatic), 117.41, 119.02, 128.21, 134.14 (CH, C-aromatic), 135.31, 138.62 (C, C-aromatic), 152.28 (CH, C-aromatic), 156.52, 159.39, 168.15 (C, C-aromatic), 171.00 (C, C-1').

***N*'-(6,7-Dihydro-5*H*-cyclopenta[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)pyrazine-2-carbo hydrazide (343)**

($C_{14}H_{12}N_6OS$; M.W.= 312.3)



334 **276** **343**

General procedure 22;

Reagent: (2,3-dihydro-1*H*-8-thia-5,7-diaza-cyclopenta[*a*]inden-4-*yl*)-hydrazine (**334**) (0.2 g, 1.0 mmol);

Purification: re-crystallisation from EtOH.

Light brown solid;

Yield: 0.05 g (15%)

Melting Point: 174-178°C

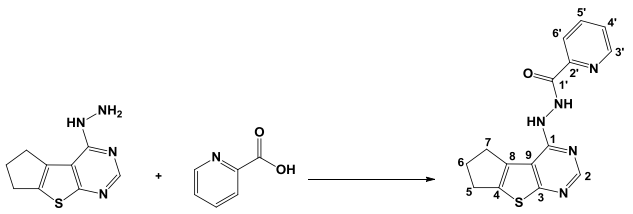
MS (ESI^+): 313.1 $[M+H]^+$

^{1}H-NMR (DMSO-d$_6$), δ: 2.41-2.47 (m, 2H, H-6), 2.98 (t, J= 7.1 Hz, 2H, C<u>H</u>$_2$), 3.12 (, J= 6.7 Hz, 2H, C<u>H</u>$_2$), 8.33 (s, 1H, H-2), 8.82-8.83 (m, 1H, H-4'), 8.95 (d, J= 2.4 Hz, 1H, H-3'), 9.00 (bs, 1H, N<u>H</u>), 9.22 (d, J= 1.2 Hz, 1H, H-5'), 10.93 (bs 1H, N<u>H</u>).

^{13}C-NMR (DMSO-d$_6$), δ: 27.46, 29.23, 29.31 (CH_2), 111.92, 135.34, 138.46 (C, C-aromatic), 143.67, 143.69 (CH, C-aromatic), 144.34 (C, C-aromatic), 148.01, 152.26 (CH, C-aromatic), 156.60, 162.59 (C, C-aromatic), 169.97 (C, C-1').

***N*'-(6,7-Dihydro-5*H*-cyclopenta[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)picolinohydrazide (344)**

($C_{15}H_{13}N_5OS$; M.W.= 311.3)



334 **277** **344**

General procedure 22;

Reagent: (2,3-dihydro-1*H*-8-thia-5,7-diaza-cyclopenta[*a*]inden-4-*yl*)-hydrazine (**334**) (0.2 g, 1.0 mmol);

Purification: re-crystallisation from EtOH.

Grey solid;

Yield: 0.09 g (30%)

Melting Point: 219-222°C

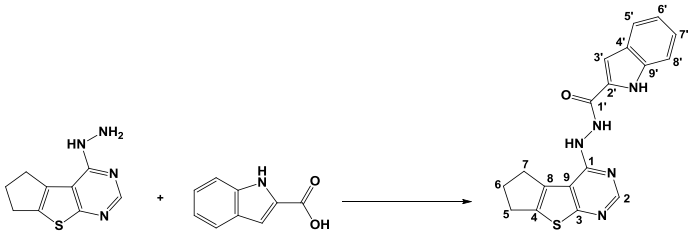
MS (ESI^+): 312.1 $[M+H]^+$

^{1}H-NMR (DMSO-d$_6$), δ: 2.40-2.46 (m, 2H, H-6), 2.98 (t, J= 7.1 Hz, 2H, C$\underline{H}_2$), 3.12 (, J= 6.9 Hz, 2H, C$\underline{H}_2$), 7.66-7.69 (m, 1H, H-aromatic), 8.02-8.05 (m, 2H, H-aromatic), 8.31 (s, 1H, H-2), 8.72-8.74 (m, 1H, H-aromatic), 8.96 (bs, 1H, N$\underline{H}$), 10.72 (bs 1H, N$\underline{H}$).

^{13}C-NMR (DMSO-d$_6$), δ: 27.46, 29.23, 29.36 (CH_2), 111.93, 114.89 (C, C-aromatic), 122.36, 126.97 (CH, C-aromatic), 135.47 (C, C-aromatic), 137.84 (CH, C-aromatic), 138.27 (C, C-aromatic), 148.69 (CH, C-aromatic), 149.37 (C, C-aromatic), 152.26 (CH, C-aromatic), 156.21 (C, C-aromatic), 169.74 (C, C-1').

***N*'-(6,7-Dihydro-5*H*-cyclopenta[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-1*H*-indole-2-carbohydrazide (345)**

($C_{18}H_{15}N_5OS$; M.W.= 349.4)



334 **297** **345**

General procedure 22;

Reagent: (2,3-dihydro-1*H*-8-thia-5,7-diaza-cyclopenta[*a*]inden-4-*yl*)-hydrazine (**334**) (0.2 g, 1.0 mmol);

Purification: re-crystallisation from EtOH.

White solid;

Yield: 0.13 g (38%)

Melting Point: 258-260°C

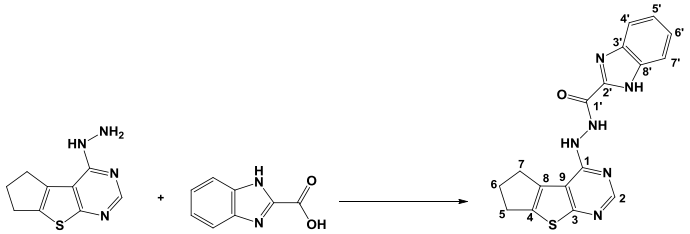
MS (ESI^+): 350.1 $[M+H]^+$

^{1}H-NMR (DMSO-d$_6$), δ: 2.41-2.48 (m, 2H, C<u>H</u>$_2$), 2.97-3.01 (m, 2H, C<u>H</u>$_2$), 3.12-3.16 (, m, 2H, C<u>H</u>$_2$), 7.06-7.10 (m, 1H, H-aromatic), 7.20-7.24 (m, 1H, H-aromatic), 7.33 (s, 1H, H-3'), 7.47 (d, J= 8.2 Hz, 1H, H-aromatic), 7.68 (d, J= 7.9 Hz, 1H, H-aromatic), 8.34 (s, 1H, H-2), 8.96 (bs, 1H, N<u>H</u>), 10.59 (bs 1H, N<u>H</u>), 11.70 (bs, 1H, N<u>H</u>).

^{13}C-NMR (DMSO-d$_6$), δ: 27.46, 29.23, 29.31 (CH_2), 111.92, 135.34, 138.46 (C, C-aromatic), 143.67, 143.69 (CH, C-aromatic), 144.34 (C, C-aromatic), 148.01, 152.26 (CH, C-aromatic), 156.60, 162.59 (C, C-aromatic), 169.97 (C, C-1').

_N_'-(6,7-Dihydro-5_H_-cyclopenta[4,5]thieno[2,3-_d_]pyrimidin-4-_yl_)-1_H_-benzo[_d_] imidazole-2-carbohydrazide (346)

($C_{17}H_{14}N_6OS$; M.W.= 350.4)



334 **298** **346**

General procedure 22;

Reagent: (2,3-dihydro-1_H_-8-thia-5,7-diaza-cyclopenta[_a_]inden-4-_yl_)-hydrazine (**334**) (0.2 g, 1.0 mmol);

Purification: re-crystallisation from EtOH.

White solid;

Yield: 0.16 g (47%)

Melting Point: 230-234°C

MS (ESI^+): 373.1 $[M+Na]^+$

^{1}H-NMR (DMSO-d$_6$), δ: 2.40-2.47 (m, 2H, C<u>H</u>$_2$), 2.96-3.00 (m, 2H, C<u>H</u>$_2$), 3.11-3.14 (, m, 2H, C<u>H</u>$_2$), 7.30-7.38 (m, 2H, H-aromatic), 7.56-7.60 (m, 1H, H-aromatic), 7.78-7.82 (m, 1H, H-aromatic), 8.33 (s, 1H, H-2), 9.04 (bs, 1H, N<u>H</u>), 10.96 (bs 1H, N<u>H</u>), 13.40 (bs, 1H, N<u>H</u>).

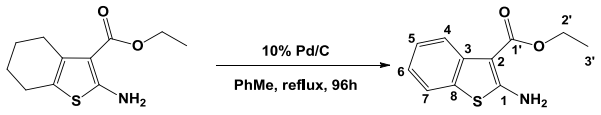
^{13}C-NMR (DMSO-d$_6$), δ: 27.47, 29.23, 29.36 (CH_2), 111.88 (C, C-aromatic), 112.57,

120.09, 122.73, 124.39 (CH, C-aromatic), 135.23, 138.40, 142.25, 144.31 (C, C-aromatic), 152.23 (CH, C-aromatic), 156.63, 158.72 (C, C-aromatic), 171.01 (C, C-1').

6.4.5 Benzo[*b*]thienopyrimidines

Ethyl 2-aminobenzo[*b*]thiophene-3-carboxylate (347)[46]

($C_{11}H_{11}NO_2S$; M.W.= 221.2)



190 **347**

To a mixture of compound **190** (1.8 g, 7.9 mmol) and 10% Pd/C (1.8 g) in toluene (130 mL) was stirred under reflux for 96 h. The reaction mixture was then cooled to r.t. and filtered over celite. The filtrate was concentrated at reduced pressure to afford a yellow solid residue, which was purified was by flash column chromatography (*n*hexane:EtOAc 100:0 v/v increasing to *n*hexane-EtOAc 90:10 v/v) to give pure ethyl 2-aminobenzo[b]thiophene-3-carboxylate (**347**) as a white solid.

T.L.C. System: *n*hexane-EtOAc 9:1 v/v, Rf: 0.28.

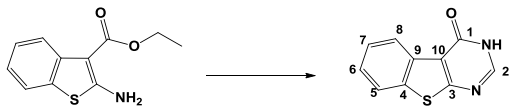
Yield: 0.72 g (41%)

^{1}H-NMR ($CDCl_3$), δ: 1.49 (t, J= 7.1 Hz, 3H, H-3'), 4.44 (q, J= 7.1 Hz, 2H, H-2'), 6.54 (bs, 2H, $N\underline{H}_2$), 7.14-7.17 (m, 1H, H-aromatic), 7.32-7.35 (m, 1H, H-aromatic), 7.51-7.53 (m, 1H, H-aromatic), 8.12-8.14 (m, 1H, H-aromatic).

^{13}C-NMR ($CDCl_3$), δ: 14.56 (CH_3, C-3'), 59.89 (CH_2, C-2'), 99.80 (C, C-aromatic), 121.29, 122.37, 122.50, 125.46 (CH, C-aromatic), 128.84, 137.41, 164.35 (C, C-aromatic), 166.31 (C, C-1').

Benzo[4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (348)[46]

($C_{10}H_6N_2OS$; M.W.= 202.2)



347 **348**

General procedure 17;

Reagent: ethyl 2-aminobenzo[*b*]thiophene-3-carboxylate (**347**) (0.7 g, 3.5 mmol);

Purification: re-crystallisation from 40% EtOH/H_2O.

Brown solid;

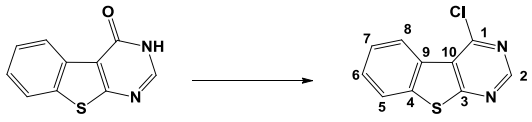
Yield: 0.57 g (81%)

^{1}H-NMR (DMSO-d$_6$), δ: 7.49-7.57 (m, 2H, H-aromatic), 8.07 (d, J= 7.7 Hz, 1H, H-aromatic), 8.31 (s, 1H, H-2), 12.86 (bs, 1H, NH).

^{13}C-NMR (DMSO-d$_6$), δ: 117.75 (C, C-aromatic), 122.82, 123.95, 125.73, 126.16 (CH, C-aromatic), 133.48, 134.69 (C, C-aromatic), 147.76 (CH, C-2), 157.35 (C, C-aromatic), 165.87 (C, C-1).

4-Chlorobenzo[4,5]thieno[2,3-*d*]pyrimidine (349)[46]

($C_{10}H_5ClN_2S$; M.W.= 220.6)



348 **349**

General procedure 18;

Reagent: benzo[4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (**348**) (0.7 g, 3.5 mmol);

T.L.C. System: *n*hexane-EtOAc 8:2 v/v, Rf: 0.69.

Purification: flash column chromatography (*n*hexane:EtOAc 100:0 v/v increasing to *n*hexane-EtOAc 95:5 v/v).

White solid;

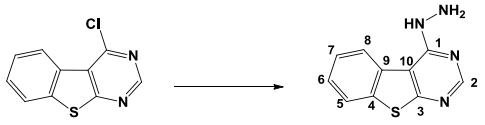
Yield: 0.37 g (74%)

^{1}H-NMR (CDCl$_3$), δ: 7.61-7.67 (m, 2H, H-aromatic), 7.94- 7.97 (m, 1H, H-aromatic), 8.76-8.78 (m, 1H, H-aromatic), 8.93 (s, 1H, H-2).

^{13}C-NMR (CDCl$_3$), δ: 123.00 (CH, C-aromatic), 125.10 (C, C-aromatic), 125.96, 126.00, 128.92 (CH, C-aromatic), 130.05, 137.06 (C, C-aromatic), 154.01 (CH, C-aromatic), 154.50, 171.01 (C, C-aromatic).

4-Hydrazinylbenzo[4,5]thieno[2,3-*d*]pyrimidine (350)[47]

($C_{10}H_8N_4S$; M.W.= 216.2)



349 **350**

General procedure 19;

Reagent: 4-chlorobenzo[4,5]thieno[2,3-*d*]pyrimidine (**349**) (0.37 g, 1.7 mmol);

Purification: re-crystallisation from 40% EtOH/H_2O.

Pale yellow solid;

Yield: 0.27 g (74%)

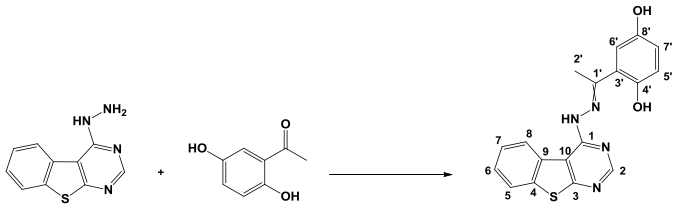
^{1}H-NMR (DMSO-d_6), δ: 4.86 (bs, 2H, N<u>H</u>$_2$), 7.49-7.56 (m, 2H, H-aromatic), 8.06-8.08 (m, 1H, H-aromatic), 8.45-8.47 (m, 1H, H-aromatic), 8.56 (s, 1H, H-2), 8.83 (bs, 1H, N<u>H</u>).

^{13}C-NMR (DMSO-d_6), δ: 109.24 (C, C-aromatic), 122.95, 123.97, 125.41, 125.96 (CH, C-aromatic), 131.66, 134.13 (C, C-aromatic), 154.85 (CH, C-2), 157.99, 166.45 (C, C-aromatic).

6.4.4.1 4-(2-(1-Arylethylidene)hydrazinyl)benzo[4,5]thieno[2,3-*d*]pyrimidines (351-354)

2-(1-(2-(Benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)hydrazono)ethyl)benzene-1,4-diol (351)

($C_{18}H_{14}N_4O_2S$; M.W.= 350.4)



350 **194** **351**

General procedure 20;

Reagent: 4-hydrazinylbenzo[4,5]thieno[2,3-*d*]pyrimidine (**350**) (0.15 g, 0.7 mmol);

Yellow solid;

Yield: 0.18 g (73%)

Melting Point: 246-249°C

MS (ESI^+): 351.1 $[M+H]^+$

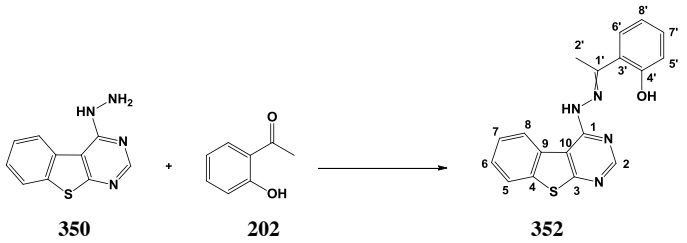
Two species observed. Major/minor species ratio: 6/1.

^{1}H-NMR (DMSO-d_6), δ: (major species) 2.62 (s, 3H, H-2'), 6.77-6.79 (m, 2H, H-aromatic), 7.04-7.07 (m, 1H, H-aromatic), 7.50-7.52 (m, 1H, H-aromatic), 7.57-7.63 (m, 1H, H-aromatic), 8.03-8.08 (m, 2H, H-aromatic), 8.74 (d, J= 7.9 Hz, 1H, H-aromatic), 8.91 (bs, 1H, OH), 11.66 (bs, 1H, NH), 12.06 (bs, 1H, OH).

^{1}H-NMR (DMSO-d_6), δ: (minor species) 2.62 (s, 3H, H-2'), 6.77-6.79 (m, 2H, H-aromatic), 7.04-7.07 (m, 1H, H-aromatic), 7.57-7.63 (m, 1H, H-aromatic), 7.66-7.71 (m, 1H, H-aromatic), 8.15-8.19 (m, 1H, H-aromatic), 8.33-8.37 (m, 1H, H-aromatic), 8.74 (d, J= 7.9 Hz, 1H, H-aromatic), 8.91 (bs, 1H, OH), 10.42 (bs, 1H, NH), 12.70 (bs, 1H, OH).

^{13}C-NMR (DMSO-d_6), δ: (major and minor species) 15.27 (CH_3, C-2'), 114.14 (C, C-aromatic), 114.39, 117.05, 118.30 (CH, C-aromatic), 121.16 (C, C-aromatic), 122.71, 125.21, 125.60, 125.70 (CH, C-aromatic), 133.21, 135.19, 145.01 (C, C-aromatic), 147.01 (CH, C-aromatic), 149.36, 150.84, 160.85, 163.32 (C, C-aromatic).

2-(1-(2-(Benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)hydrazono)ethyl)phenol (352)
($C_{18}H_{14}N_4OS$; M.W.= 334.4)



350 **202** **352**

General procedure 20;

Reagent: 4-hydrazinylbenzo[4,5]thieno[2,3-*d*]pyrimidine (**350**) (0.15 g, 0.7 mmol);

Yellow solid;

Yield: 0.19 g (81%)

Melting Point: 195-199°C

MS (ESI^+): 335.1 $[M+H]^+$

Two species observed. Major/minor species ratio: 6/1.

Microanalysis: Calculated for $C_{18}H_{14}N_4OS$ (334.4); Theoretical: %C = 64.65, %H = 4.22, %N = 16.75; Found: %C = 64.74, %H = 4.10, %N = 16.89.

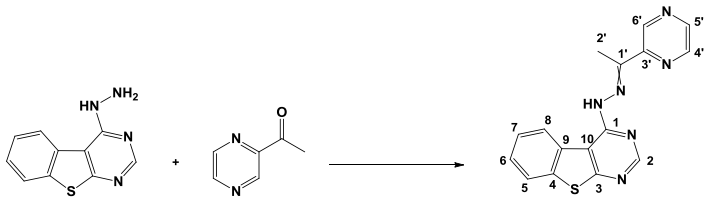
^{1}H-NMR (DMSO-d_6), δ: (major species) 2.66 (s, 3H, H-2'), 6.92-6.97 (m, 2H, H-aromatic), 7.30-7.34 (m, 1H, H-aromatic), 7.50-7.52 (m, 1H, H-aromatic), 7.58-7.60 (m, 1H, H-aromatic), 7.65-7.70 (m, 1H, H-aromatic), 8.04-8.09 (m, 2H, H-aromatic), 8.73-8.79 (m, 1H, H-aromatic), 12.04 (bs, 1H, NH), 12.39 (bs, 1H, OH).

^{1}H-NMR (DMSO-d_6), δ: (minor species) 2.66 (s, 3H, H-2'), 6.92-6.97 (m, 2H, H-aromatic), 7.30-7.34 (m, 1H, H-aromatic), 7.58-7.60 (m, 1H, H-aromatic), 7.65-7.70 (m, 2H, H-aromatic), 8.15-8.19 (m, 1H, H-aromatic), 8.34-8.38 (m, 1H, H-aromatic), 8.73-8.79 (m, 1H, H-aromatic), 10.46 (bs, 1H, NH), 13.46 (bs, 1H, OH).

^{13}C-NMR (DMSO-d_6), δ: (major and minor species) 15.17 (CH_3, C-2'), 114.16 (C, C-aromatic), 116.60, 118.75 (CH, C-aromatic), 121.10 (C, C-aromatic), 122.71, 125.23, 125.61, 125.71, 128.91, 130.88 (CH, C-aromatic), 133.21, 135.20, 145.11 (C, C-aromatic), 146.94 (CH, C-aromatic), 158.16, 160.93, 163.47 (C, C-aromatic).

4-(2-(1-(Pyrazin-2-*yl*)ethylidene)hydrazinyl)benzo[4,5]thieno[2,3-*d*]pyrimidine (353)

($C_{16}H_{12}N_6OS$; M.W.= 320.4)



350 **250** **353**

General procedure 20;

Reagent: 4-hydrazinylbenzo[4,5]thieno[2,3-*d*]pyrimidine (**350**) (0.15 g, 0.7 mmol);

Yellow solid;

Yield: 0.16 g (73%)

Melting Point: 218-221°C

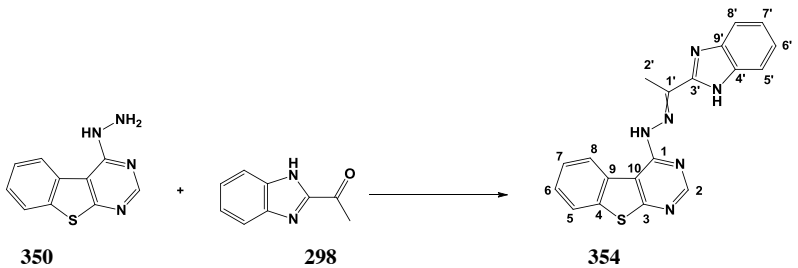
MS (ESI^+): 321.1 $[M+H]^+$

^{1}H-NMR (DMSO-d$_6$), δ: 2.62 (s, 3H, H-2'), 7.50-7.54 (m, 1H, H-aromatic), 7.58-7.62 (m, 1H, H-aromatic), 8.08 (d, J= 7.9 Hz, 1H, H-aromatic), 8.15 (d, J= 3.7 Hz, 1H, H-aromatic), 8.61 (s, 1H, H-2), 8.65-8.66 (m, 1H, H-aromatic), 8.78 (d, J= 7.9 Hz, 1H, H-aromatic), 9.87 (d, J= 1.4 Hz, 1H, H-aromatic), 12.44 (bs, 1H, NH).

^{13}C-NMR (DMSO-d$_6$), δ: 12.82 (CH_3, C-2'), 114.42 (C, C-aromatic), 122.71, 125.51, 125.65, 125.82 (CH, C-aromatic), 133.16, 135.26 (C, C-aromatic), 143.24, 143.43, 143.69, 146.65 (CH, C-aromatic), 148.23, 151.12, 157.56, 161.84 (C, C-aromatic).

4-(2-(1-(1*H*-Benzo[*d*]imidazol-2-*yl*)ethylidene)hydrazinyl)benzo[4,5]thieno[2,3-*d*] pyrimidine (354)

($C_{19}H_{14}N_6S$; M.W.= 358.4)



350 **298** **354**

General procedure 20;

Reagent: 4-hydrazinylbenzo[4,5]thieno[2,3-*d*]pyrimidine (**350**) (0.15 g, 0.7 mmol);

Yellow solid;

Yield: 0.16 g (62%)

Melting Point: charring > 250°C

MS (ESI^+): 359.1 $[M+H]^+$

Two species observed. Major/minor species ratio: 5/1.

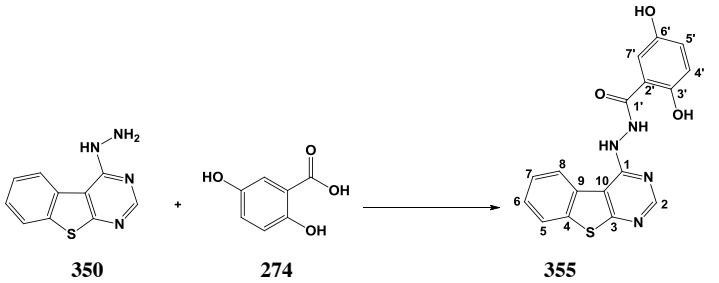
^{1}H-NMR (DMSO-d$_6$), δ: (major species) 2.67 (s, 3H, H-2'), 7.22-7.32 (m, 2H, H-aromatic), 7.52-7.55 (m, 1H, H-aromatic), 7.61-7.72 (m, 3H, H-aromatic), 8.10 (d, J= 7.9 Hz, 1H, H-aromatic), 8.36 (s, 1H, H-2), 8.80 (d, J= 7.9 Hz, 1H, H-aromatic), 12.60 (bs, 1H, NH), 12.68 (bs, 1H, NH).

^{1}H-NMR (DMSO-d$_6$), δ: (minor species) 2.63 (s, 3H, H-2'), 7.45-7.48 (m, 2H, H-aromatic), 7.61-7.72 (m, 2H, H-aromatic), 7.91-8.00 (m, 2H, H-aromatic), 8.22 (d, J= 7.9 Hz, 1H, H-aromatic), 8.75 (s, 1H, H-2), 9.31 (d, J= 7.9 Hz, 1H, H-aromatic), 13.21 (bs, 1H, NH), 13.49 (bs, 1H, NH).

^{13}C-NMR (DMSO-d$_6$), δ: (major and minor species) 13.30 (CH_3, C-2'), 114.38 (C, C-aromatic), 121.57, 123.25, 124.85, 125.70, 125.79, 125.87, 126.91 (CH, C-aromatic), 130.40, 133.11, 134.80, 135.26 (C, C-aromatic), 146.36 (CH, C-aromatic), 148.60, 150.42, 151.66 (C, C-aromatic), 155.23 (CH, C-aromatic), 161.81 (C, C-aromatic).

6.4.4.2 *N*'-(Benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)arylcarbohydrazides (355-358)

N'-(Benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-2,5-dihydroxybenzohydrazide (355) ($C_{17}H_{12}N_4O_3S$; M.W.= 352.4)



350 **274** **355**

General procedure 22;

Reagent: 4-hydrazinylbenzo[4,5]thieno[2,3-*d*]pyrimidine (**350**) (0.2 g, 0.9 mmol);

T.L.C. System: 100% EtOAc, Rf: 0.65.

Purification: flash column chromatography (*n*hexane:EtOAc 100:0 v/v increasing to *n*hexane:EtOAc 30:700 v/v).

Light grey solid;

Yield: 0.05 g (17%)

Melting Point: 241-244°C

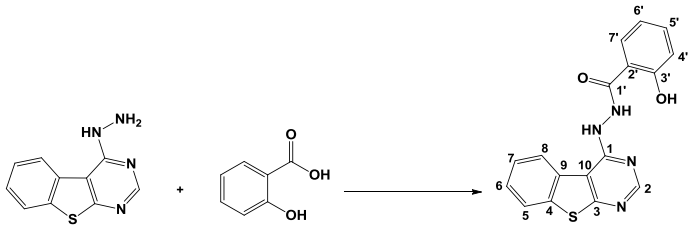
MS (ESI^+): 353.0 $[M+H]^+$

^{1}H-NMR (DMSO-d$_6$), δ: 6.84 (d, J= 8.8 Hz, 1H, H-4'), 6.93 (dd, J_1= 8.8 Hz, J_2= 2.8 Hz, 1H, H-5'), 7.41 (d, J= 2.8 Hz, 1H, H-7'), 7.58-7.66 (m, 2H, H-aromatic), 8.16 (d, J= 7.6 Hz, 1H, H-aromatic), 8.56-8.61 (m, 2H, H-aromatic), 9.11 (bs, 1H, NH), 9.64 (bs, 1H, OH), 10.92 (bs, 2H, NH, OH).

^{13}C-NMR (DMSO-d$_6$), δ: 110.08, 114.69 (C, C-aromatic), 117.42, 119.06, 123.30, 124.52, 125.67, 126.64, 128.34 (CH, C-aromatic), 130.55 (C, C-aromatic), 134.16 (CH, C-aromatic), 134.56 (C, C-aromatic), 154.90 (CH, C-aromatic), 157.41, 159.36 (C, C-aromatic), 167.98 (C, C-1').

***N*'-(Benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)-2-hydroxybenzohydrazide (356)**

($C_{17}H_{12}N_4O_2S$; M.W.= 336.4)



General procedure 22;

Reagent: 4-hydrazinylbenzo[4,5]thieno[2,3-*d*]pyrimidine (**350**) (0.2 g, 0.9 mmol);

Purification: recrystallization from EtOH.

Pale yellow solid;

Yield: 0.04 g (14%)

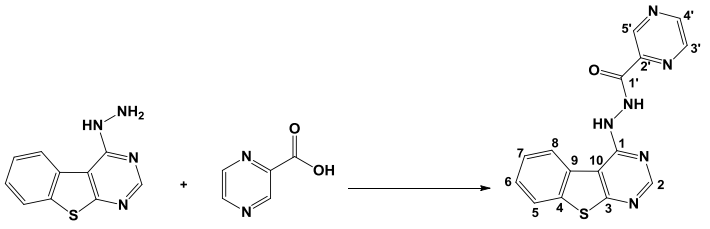
Melting Point: >260°C

MS (ESI^+): 337.0 $[M+H]^+$

^{1}H-NMR (DMSO-d_6), δ: 6.98-7.03 (m, 2H, H-aromatic), 7.48-7.52 (m, 1H, H-aromatic), 7.59-7.67 (m, 2H, H-aromatic), 8.04 (d, J= 7.5 Hz, 1H, H-aromatic), 8.18 (d, J= 7.3 Hz, 1H, H-aromatic), 8.59-8.63 (m, 2H, H-aromatic), 9.65 (bs, 1H, NH), 11.00 (bs, 1H, OH), 12.00 (bs, 1H, NH).

^{13}C-NMR (DMSO-d_6), δ: 110.07, 114.69 (C, C-aromatic), 117.88, 121.58, 123.25, 124.51, 125.63, 126.65 (CH, C-aromatic), 130.55 (C, C-aromatic), 134.16 (CH, C-aromatic), 134.56 (C, C-aromatic), 154.90 (CH, C-aromatic), 157.41, 159.36 (C, C-aromatic), 167.07 (C, C-1').

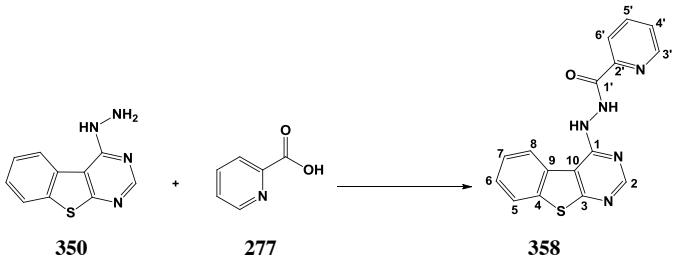
***N*'-(Benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)pyrazine-2-carbohydrazide (357)**
($C_{15}H_{10}N_6OS$; M.W.= 322.3)



350 **276** **357**

General procedure 22;
Reagent: 4-hydrazinylbenzo[4,5]thieno[2,3-*d*]pyrimidine (**350**) (0.2 g, 0.9 mmol);
Purification: recrystallization from $EtOH/H_2O$.
Pale yellow solid;
Yield: 0.06 g (21%)
Melting Point: 115-118°C
MS (ESI^+): 323.0 $[M+H]^+$
^{1}H-NMR (DMSO-d$_6$), δ: 7.57-7.65 (m, 2H, H-aromatic), 8.15 (d, J= 7.6 Hz, 1H, H-aromatic), 8.56 (s, 1H, H-2), 8.63 (d, J= 7.3 Hz, 1H, H-aromatic), 8.84-8.85 (m, 1H, H-aromatic), 8.96 (d, J= 2.3 Hz, 1H, H-aromatic), 9.26 (d, J= 1.3 Hz, 1H, H-aromatic), 9.64 (bs, 1H, NH), 11.14 (bs, 1H, NH).
^{13}C-NMR (DMSO-d$_6$), δ: 110.12 (C, C-aromatic), 123.23, 124.58, 125.61, 125.95 (CH, C-aromatic), 126.62, 130.70, 134.48 (C, C-aromatic), 143.67, 143.72 (CH, C-aromatic), 144.04, 144.38 (C, C-aromatic), 148.02 (CH, C-aromatic), 167.83 (C, C-1').

***N*'-(Benzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)picolinohydrazide (358)**
($C_{16}H_{11}N_5OS$; M.W.= 321.4)



350 **277** **358**

General procedure 22;

Reagent: 4-hydrazinylbenzo[4,5]thieno[2,3-*d*]pyrimidine (**350**) (0.2 g, 0.9 mmol);
Purification: recrystallization from EtOH.
White solid;
Yield: 0.08 g (28%)
Melting Point: 220-223°C
MS (ESI^+): 322.0 $[M+H]^+$

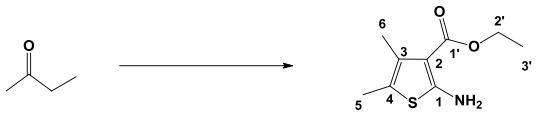
^{1}H-NMR (DMSO-d$_6$), δ: 7.57-7.65 (m, 2H, H-aromatic), 7.68-7.71 (m, 1H, H-aromatic), 8.04-8.08 (m, 1H, H-aromatic), 8.09-8.12 (m, 1H, H-aromatic), 8.15 (d, J= 7.6 Hz, 1H, H-aromatic), 8.56 (s, 1H, H-2), 8.63 (d, J= 6.6 Hz, 1H, H-aromatic), 8.76 (d, J= 4.3 Hz, 1H, H-aromatic), 9.57 (bs, 1H, NH), 10.92 (bs, 1H, NH).

^{13}C-NMR (DMSO-d$_6$), δ: 110.05 (C, C-aromatic), 122.39, 123.21, 125.58, 126.60, 126.67, 126.99 (CH, C-aromatic), 130.70, 134.44 (C, C-aromatic), 137.87, 148.70 (CH, C-aromatic), 149.38 (C, C-aromatic), 154.88 (CH, C-aromatic), 157.52, 163.27 (C, C-aromatic), 167.77 (C, C-1').

6.4.6 Dimethyl[*b*]thienopyrimidines

Ethyl 2-amino-4,5-dimethylthiophene-3-carboxylate (363)[48]

($C_9H_{13}NO_2S$; M.W.= 180.2)



361 **363**

General procedure 16;

Reagent: 2-butanone (**361**) (1 g, 13.9 mmol);

T.L.C. System: *n*hexane -EtOAc 8:2 v/v, Rf: 0.50.

Purification: flash column chromatography (*n*hexane:EtOAc 100:0 v/v increasing to *n*hexane -EtOAc 85:15 v/v);

Pale yellow solid;

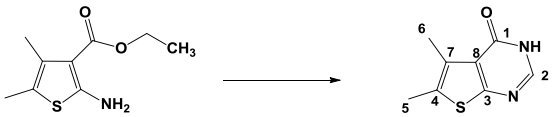
Yield: 1.30 g (47%)

^{1}H-NMR ($CDCl_3$), δ: 1.37 (t, J= 7.1 Hz, 3H, H-3'), 2.17 (s, 3H, C<u>H</u>$_3$), 2.19 (s, 3H, C<u>H</u>$_3$), 4.30 (q, J= 7.1 Hz, 2H, H-2'), 5.92 (bs, 2H, N<u>H</u>$_2$).

^{13}C-NMR ($CDCl_3$), δ: 12.29, 14.37, 14.75 (CH_3, C-3', 5, 6), 59.43 (CH_2, C-2'), 106.98, 113.84, 130.35, 160.94 (C, C-1, 2, 3, 4), 166.08 (C, C-1').

5,6-Dimethylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (365)[44]

($C_8H_8N_2OS$; M.W.= 180.2)



363 **365**

General procedure 17;

Reagent: ethyl 2-amino-4,5-dimethylthiophene-3-carboxylate (**363**) (1.03 g, 5.2 mmol);

Purification: recrystallization from EtOH/H_2O;

Pale yellow solid;

Yield: 0.45 g (49%)

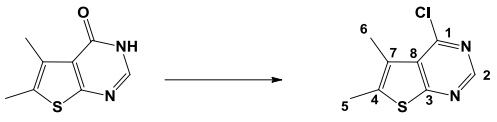
^{1}H-NMR (DMSO-d_6), δ: 2.35 (s, 3H, C<u>H</u>$_3$), 2.38 (s, 3H, C<u>H</u>$_3$), 7.98 (s, 1H, H-2), 12.28

(bs, 1H, N<u>H</u>).

^{13}C-NMR (DMSO-d$_6$), δ: 12.51, 12.81 (CH_3, C-5, 6), 123.45, 128.68, 129.23 (C, C-aromatic), 144.65 (CH, C-2), 157.90 (C, C-aromatic), 161.62 (C, C-1).

4-Chloro-5,6-dimethylthieno[2,3-*d*]pyrimidine (367)[44]

($C_8H_7ClN_2S$; M.W.= 198.7)



365 **367**

General procedure 18;

Reagent: 5,6-dimethylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (**365**) (1.03 g, 5.2 mmol);

T.L.C. System: *n*hexane -EtOAc 1:1 v/v, Rf: 0.67.

Purification: flash column chromatography (*n*hexane:EtOAc 100:0 v/v increasing to *n*hexane -EtOAc 90:10 v/v);

White solid;

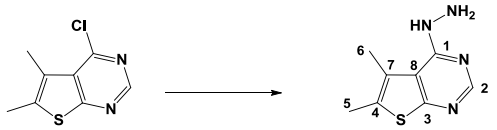
Yield: 0.34 g (91%)

^{1}H-NMR (CDCl$_3$), δ: 2.54 (s, 3H, C<u>H</u>$_3$), 2.57 (s, 3H, C<u>H</u>$_3$), 8.73 (s, 1H, H-2).

^{13}C-NMR (CDCl$_3$), δ: 14.08, 14.18 (CH_3, C-5, 6), 125.15, 129.43, 136.15 (C, C-aromatic), 151.48 (CH, C-2), 153.44, 168.30 (C, C-aromatic).

4-Hydrazinyl-5,6-dimethylthieno[2,3-*d*]pyrimidine (369)[49]

($C_8H_{10}N_4S$; M.W.= 194.3)



367 **369**

General procedure 19;

Reagent: 4-chloro-5,6-dimethylthieno[2,3-*d*]pyrimidine (**367**) (0.34 g, 1.7 mmol);

Purification: recrystallization from EtOH/H_2O;

Yellow solid;

Yield: 0.24 g (73%)

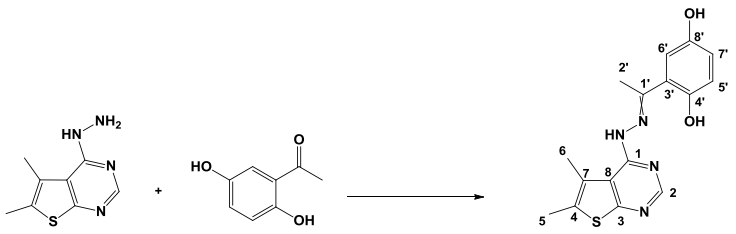
^{1}H-NMR (DMSO-d_6), δ: 2.38 (s, 3H, C<u>H</u>$_3$), 2.42 (s, 3H, C<u>H</u>$_3$), 4.59 (bs, 2H, N<u>H</u>$_2$), 8.03 (bs, 1H, N<u>H</u>), 8.33 (s, 1H, H-2).

^{13}C-NMR (DMSO-d_6), δ: 13.00, 13.83 (CH_3, C-5, 6), 115.92, 124.65, 128.34 (C, C-aromatic), 152.32 (CH, C-2), 158.30, 166.38 (C, C-aromatic).

6.4.5.1 4-(2-(1-Arylethylidene)hydrazinyl)-5,6-dimethylthieno[2,3-*d*]pyrimidines (371-372)

2-(1-(2-(5,6-Dimethylthieno[2,3-*d*]pyrimidin-4-*yl*)hydrazono)ethyl)benzene-1,4-diol (371)

($C_{16}H_{16}N_4O_2S$; M.W.= 328.4)



369 **194** **371**

General procedure 20;

Reagent: 4-hydrazinyl-5,6-dimethylthieno[2,3-*d*]pyrimidine (**369**) (0.15 g, 0.8 mmol);

Yellow solid;

Yield: 0.18 g (71%)

Melting Point: >260°C

MS (ESI^+): 329.1 $[M+H]^+$

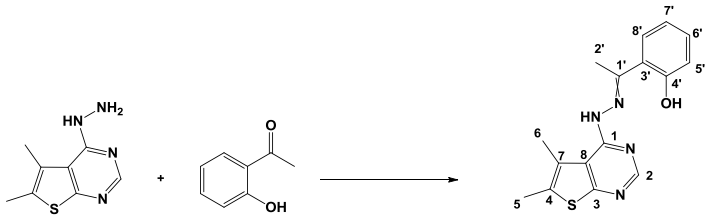
Two species observed. Major/minor species ratio: 3/1.

^{1}H-NMR (DMSO-d_6), δ: (major species) 2.36 (s, 3H, C<u>H</u>$_3$), 2.47 (s, 3H, C<u>H</u>$_3$), 2.49 (s, 3H, C<u>H</u>$_3$), 6.73-6.77 (m, 2H, H-aromatic), 6.97-7.02 (m, 1H, H-aromatic), 7.74 (s, 1H, H-2), 8.87 (bs, 1H, O<u>H</u>), 11.36 (bs, 1H, N<u>H</u>), 11.71 (bs, 1H, O<u>H</u>).

^{1}H-NMR (DMSO-d_6), δ: (minor species) 2.47 (s, 3H, C<u>H</u>$_3$), 2.49 (s, 3H, C<u>H</u>$_3$), 2.61 (s, 3H, C<u>H</u>$_3$), 6.73-6.77 (m, 2H, H-aromatic), 6.97-7.02 (m, 1H, H-aromatic), 8.52 (s, 1H, H-2), 8.87 (bs, 1H, O<u>H</u>), 9.51 (bs, 1H, N<u>H</u>), 12.60 (bs, 1H, O<u>H</u>).

^{13}C-NMR (DMSO-d_6), δ: (major and minor species) 12.86, 14.14, 15.02 (CH_3, C-5, 6), 112.55 (C, C-aromatic), 114.23, 116.95, 118.10 (CH, C-aromatic), 121.13, 128.80, 129.52 (C, C-aromatic), 143.87 (CH, C-aromatic), 144.63, 149.26, 150.87, 155.13, 163.04 (C, C-aromatic).

2-(1-(2-(5,6-Dimethylthieno[2,3-*d*]pyrimidin-4-*yl*)hydrazono)ethyl)phenol (372)
($C_{16}H_{16}N_4OS$; M.W.= 312.4)



369 **202** **372**

General procedure 20;

Reagent: 4-hydrazinyl-5,6-dimethylthieno[2,3-*d*]pyrimidine (**369**) (0.15 g, 0.8 mmol);

Pale yellow solid;

Yield: 0.13 g (54%)

Melting Point: 222-226°C

MS (ESI^+): 313.1 $[M+H]^+$

Two species observed. Major/minor species ratio: 3/1.

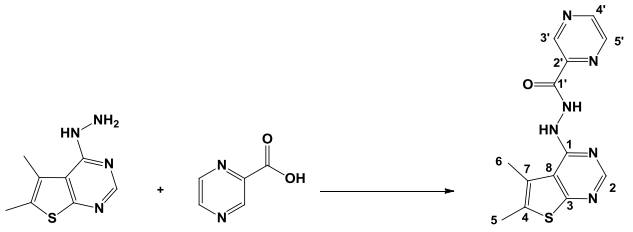
^{1}H-NMR (DMSO-d_6), δ: (major species) 2.36 (s, 3H, C<u>H</u>$_3$), 2.49 (s, 3H, C<u>H</u>$_3$), 2.53 (s, 3H, CH_3), 6.89-6.93 (m, 2H, H-aromatic), 7.27-7.30 (m, 1H, H-aromatic), 7.61 (d, J= 7.5 Hz, 1H, H-aromatic), 7.75 (s, 1H, H-2), 11.38 (bs, 1H, N<u>H</u>), 12.49 (bs, 1H, O<u>H</u>).

^{1}H-NMR (DMSO-d_6), δ: (minor species) 2.47 (s, 3H, C<u>H</u>$_3$), 2.53 (s, 3H, CH_3), 2.61 (s, 3H, C<u>H</u>$_3$), 6.89-6.93 (m, 2H, H-aromatic), 7.27-7.30 (m, 1H, H-aromatic), 7.64-7.67 (m, 1H, H-aromatic), 8.54 (s, 1H, H-2), 9.54 (bs, 1H, N<u>H</u>), 13.37 (bs, 1H, O<u>H</u>).

^{13}C-NMR (DMSO-d_6), δ: (major and minor species) 12.87, 13.05, 13.23, 13.55, 14.16, 14.95 (CH_3, C-5, 6, 2'), 116.52, 117.28, 118.50, 118.67 (CH, C-aromatic), 119.48, 120.10, 121.04, 124.27 (C, C-aromatic), 128.02, 128.74 (CH, C-aromatic), 128.81, 129.57 (C, C-aromatic), 130.69, 143.85 (CH, C-aromatic), 145.45 (C, C-aromatic), 152.51 (CH, C-aromatic), 153.97, 155.36, 155.89, 158.18, 158.48, 163.34, 165.11 (C, C-aromatic).

6.4.5.2 N'-(5,6-Dimethylthieno[2,3-d]pyrimidin-4-yl)pyrazine-2-carbohydrazide (374)

($C_{13}H_{12}N_6OS$; M.W.= 300.3)



369 **276** **374**

General procedure 22;

Reagent: 4-hydrazinyl-5,6-dimethylthieno[2,3-*d*]pyrimidine (**369**) (0.11 g, 0.6 mmol);

Purification: re-crystallisation from EtOH;

White solid;

Yield: 0.06 g (35%)

Melting Point: 194-197°C

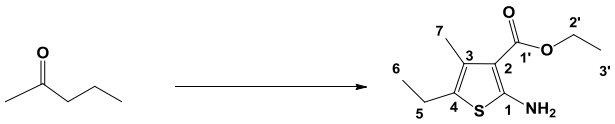
MS (ESI^+): 301.1 $[M+H]^+$

^{1}H-NMR (DMSO-d_6), δ: 2.44 (s, 3H, C<u>H</u>$_3$), 2.53 (s, 3H, C<u>H</u>$_3$), 8.31 (s, 1H, H-2), 8.81-8.81-8.82 (m, 1H, H-aromatic), 8.94 (d, J= 2.3 Hz, 1H, H-aromatic), 8.96(bs, 1H, N<u>H</u>), 9.22 (d, J= 1.2 Hz, 1H, H-aromatic), 10.90 (bs 1H, N<u>H</u>).

^{13}C-NMR (DMSO-d_6), δ: 13.14, 14.08 (CH_3, C-5, 6), 116.53, 124.60, 127.15, 129.76 (C, C-aromatic), 143.62 (CH, C-aromatic), 144.42, 146.77 (C, C-aromatic), 147.60, 147.91, 151.86 (CH, C-aromatic), 161.95 (C, C-1').

6.4.7 6-Ethyl-5-methylthieno[2,3-*d*]pyrimidines

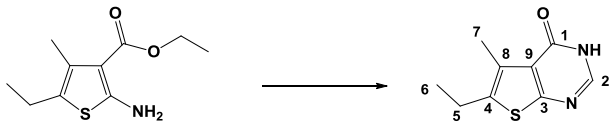
Ethyl 2-amino-5-ethyl-4-methylthiophene-3-carboxylate (364)[50]
($C_{10}H_{15}NO_2S$; M.W.= 213.3)



362 364

General procedure 16;
Reagent: 2-pentanone (**362**) (1 g, 11.6 mmol);
T.L.C. System: *n*hexane-EtOAc 8:2 v/v, Rf: 0.52;
Purification: flash column chromatography (*n*hexane:EtOAc 100:0 v/v increasing to *n*hexane -EtOAc 95:5 v/v);
Yellow oil;
Yield: 0.83 g (33%)
^{1}H-NMR ($CDCl_3$), δ: 1.17 (t, J= 7.5 Hz, 3H, H-6), 1.37 (t, J= 7.1 Hz, 3H, H-3'), 2.20 (s, 3H, H-7), 2.60 (q, J= 7.5 Hz, 2H, H-5), 4.30 (q, J= 7.1 Hz, 2H, H-2'), 5.92 (bs, 2H, $N\underline{H}_2$).
^{13}C-NMR ($CDCl_3$), δ: 14.44, 14.64, 15.76 (CH_3, C-3', 6, 7), 20.50 (CH_2, C-5), 59.43 (CH_2, C-2'), 107.03, 121.79, 129.43, 161.05 (C, C-1, 2, 3, 4), 166.16 (C, C-1').

6-Ethyl-5-methylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (366)[51]
($C_9H_{10}N_2OS$; M.W.= 194.3)



364 366

General procedure 17;
Reagent: ethyl 2-amino-5-ethyl-4-methylthiophene-3-carboxylate (**364**) (0.83 g, 3.8 mmol);
Purification: recrystallisation from 40% EtOH/H_2O;

Brown solid;

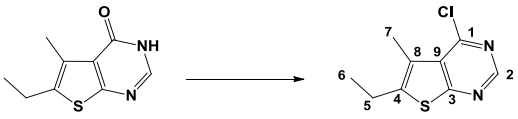
Yield: 0.61 g (83%)

^{1}H-NMR (DMSO-d$_6$), δ: 1.20 (t, J= 7.5 Hz, 3H, H-5), 2.40 (s, 3H, H-7), 2.77 (q, J= 7.5 Hz, 2H, H-5), 7.99 (s, 1H, H-2), 12.28 (bs, 1H, NH).

^{13}C-NMR (DMSO-d$_6$), δ: 12.77, 15.36 (CH_3, C-6, 7), 20.33 (CH_2, C-5), 123.49, 127.84, 136.67 (C, C-aromatic), 144.67 (CH, C-2), 158.03 (C, C-aromatic), 161.68 (C, C-1).

4-Chloro-6-ethyl-5-methylthieno[2,3-*d*]pyrimidine (368)[51]

($C_9H_9ClN_2S$; M.W.= 212.7)



366 **368**

General procedure 18;

Reagent: 6-ethyl-5-methylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (**366**) (0.61 g, 3.1 mmol);

T.L.C. System: *n*hexane-EtOAc 1:1 v/v, Rf: 0.67.

Purification: flash column chromatography (*n*hexane:EtOAc 100:0 v/v increasing to *n*hexane-EtOAc 90:10 v/v);

White solid;

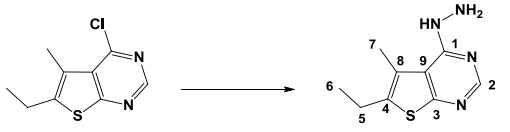
Yield: 0.37 g (50%)

^{1}H-NMR ($CDCl_3$), δ: 1.36 (t, J= 7.5 Hz, 3H, H-6), 2.59 (s, 3H, H-7), 2.94 (q, J= 7.5 Hz, 2H, H-5), 8.73 (s, 1H, H-2).

^{13}C-NMR ($CDCl_3$), δ: 14.16, 15.20 (CH_3, C-6, 7), 21.96 (CH_2, C-5), 124.16, 129.55, 143.66 (C, C-aromatic), 151.43 (CH, C-2), 153.58, 168.31 (C, C-aromatic).

6-Ethyl-4-hydrazinyl-5-methylthieno[2,3-*d*]pyrimidine (370)

($C_9H_{12}N_4S$; M.W.= 208.8)



368 **370**

General procedure 19;

Reagent: 4-chloro-6-ethyl-5-methylthieno[2,3-*d*]pyrimidine (**368**) (0.34 g, 1.7 mmol);

Purification: recrystallization from 40% EtOH/H_2O;

Yellow solid;

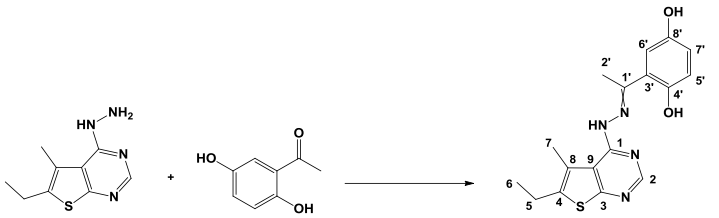
Yield: 0.11 g (46%)

^{1}H-NMR (DMSO-d$_6$), δ: 1.19 (t, J= 7.5 Hz, 3H, H-6), 2.43 (s, 3H, H-7), 2.79 (q, J= 7.5 Hz, 2H, H-5), 4.59 (bs, 2H, N$\underline{H}_2$), 8.07 (bs, 1H, N$\underline{H}$), 8.33 (s, 1H, H-2).

^{13}C-NMR (DMSO-d$_6$), δ: 13.77, 15.49 (CH_3, C-6, 7), 20.65 (CH_2, C-5), 115.96, 123.80, 135.72 (C, C-aromatic), 152.31 (CH, C-2), 158.48, 163.22 (C, C-aromatic).

6.4.6.1 2-(1-(2-(6-Ethyl-5-methylthieno[2,3-*d*]pyrimidin-4-*yl*)hydrazono)ethyl) benzene-1,4-diol (373)

($C_{17}H_{18}N_4O_2S$; M.W.= 342.4)



370 **194** **373**

General procedure 20;

Reagent: 6-ethyl-4-hydrazinyl-5-methylthieno[2,3-*d*]pyrimidine (**370**) (0.09 g, 0.4 mmol);

Yellow solid;

Yield: 0.07 g (53%)

Melting Point: 235-237°C

MS (ESI^+): 343.1 $[M+H]^+$

Two species observed. Major/minor species ratio: 3/1.

Microanalysis: Calculated for $C_{17}H_{18}N_4O_2S$ (342.4); Theoretical: %C = 59.63, %H = 5.30, %N = 16.35; Found: %C = 59.69, %H = 5.31, %N = 16.51.

^{1}H-NMR (DMSO-d$_6$), δ: (major species) 1.22 (t, J= 7.4 Hz, 3H, H-6), 2.46 (s, 3H, C<u>H</u>$_3$), 2.52 (s, 3H, C<u>H</u>$_3$), 2.78 (q, J= 7.4 Hz, 2H, H-5), 6.73-6.76 (m, 2H, H-aromatic), 6.97-6.99 (m, 1H, H-aromatic), 7.74 (s, 1H, H-2), 8.87 (bs, 1H, O<u>H</u>), 11.36 (bs, 1H, N<u>H</u>), 11.70 (bs, 1H, O<u>H</u>).

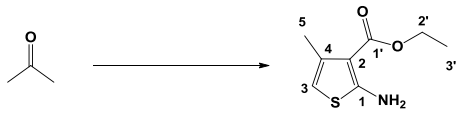
^{1}H-NMR (DMSO-d$_6$), δ: (minor species) 1.26 (t, J= 7.4 Hz, 3H, H-6), 2.45 (s, 3H, C<u>H</u>$_3$), 2.63 (s, 3H, C<u>H</u>$_3$), 2.89 (q, J= 7.4 Hz, 2H, H-5), 6.73-6.76 (m, 2H, H-aromatic), 7.00-7.02 (m, 1H, H-aromatic), 8.53 (s, 1H, H-2), 8.87 (bs, 1H, O<u>H</u>), 9.53 (bs, 1H, N<u>H</u>), 12.60 (bs, 1H, O<u>H</u>).

^{13}C-NMR (DMSO-d$_6$), δ: (major and minor species) 14.08, 15.04, 15.39 (CH_3, C-6, 7), 20.62 (CH_2, C-5), 114.24, 116.95, 118.09 (CH, C-aromatic), 120.75, 121.12, 127.96, 137.06 (C, C-aromatic), 143.89 (CH, C-aromatic), 144.75, 149.28, 150.86, 155.24, 163.07 (C, C-aromatic).

6.4.8 6-Chloro-5-methyl-thieno[2,3-*d*]pyrimidines

Ethyl 2-amino-4-methylthiophene-3-carboxylate (376)[55]

($C_8H_{11}NO_2S$; M.W.= 199.3)



375 **376**

General procedure 16;

Reagent: acetone (**375**) (1 g, 17.2 mmol);

Morpholine used instead of NEt_3;

T.L.C. System: *n*hexane-EtOAc 8:2 v/v, Rf: 0.46.

Purification: flash column chromatography (*n*hexane:EtOAc 100:0 v/v increasing to *n*hexane-EtOAc 85:15 v/v);

Pale yellow solid;

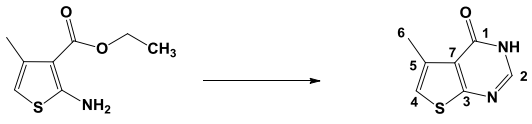
Yield: 1.3 g (41%)

^{1}H-NMR ($CDCl_3$), δ: 1.37 (t, J= 7.1 Hz, 3H, H-3'), 2.29 (d, J= 1.2 Hz, 3H, H-5), 4.30 (q, J= 7.1 Hz, 2H, H-2'), 5.83 (q, J= 1.2 Hz, 1H, H-3), 6.08 (bs, 2H, $N\underline{H}_2$).

^{13}C-NMR ($CDCl_3$), δ: 14.08, 18.44 (CH_3, C-3', 6), 59.54 (CH_2, C-2'), 102.81 (CH, C-3), 106.59, 136.66, 166.13 (C, C-aromatic), 166.63 (C, C-1').

5-Methylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (377)[55]

($C_7H_6N_2OS$; M.W.= 166.2)



376 **377**

General procedure 17;

Reagent: ethyl 2-amino-4-methylthiophene-3-carboxylate (**376**) (1.3 g, 7 mmol);

Purification: recrystallization from $EtOH/H_2O$;

Yellow solid;

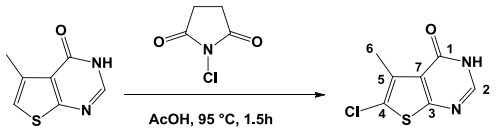
Yield: 0.73 g (62%)

^{1}H-NMR (DMSO-d$_6$), δ: 2.47 (s, 3H, H-6), 7.13 (s, 1H, H-4), 8.04 (s, 1H, H-2), 12.34 (bs, 1H, N<u>H</u>).

^{13}C-NMR (DMSO-d$_6$), δ: 16.10 (CH_3, C-6), 118.02 (CH, C-4), 122.83, 133.66 (C, C-aromatic), 145.52 (CH, C-2), 158.27 (C, C-aromatic), 164.71 (C, C-1).

6-Chloro-5-methylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (378)[56]

($C_7H_5ClN_2OS$; M.W.= 200.6)



377 **378**

5-Methylthieno[2,3-*d*]pyrimidin-4(3H)-one (**377**) (0.76 g, 4.4 mmol) and *N*-chlorosuccinimmide (0.7 g, 5.2 mmol) were dissolved in glacial AcOH (23 mL) and heated at 95°C for 1.5 h. The reaction mixture was then concentrated *in vacuo*. The residue was stirred with H_2O (25 mL), filtered, washed with H_2O and MeOH and finally dried to give 6-chloro-5-methylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (**378**) as a brown solid.

T.L.C. System: *n*hexane-EtOAc 2:3 v/v, Rf: 0.65.

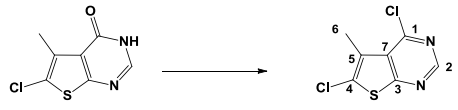
Yield: 0.56 g (63%)

^{1}H-NMR (DMSO-d$_6$), δ: 2.42 (s, 3H, H-6), 8.10 (s, 1H, H-2), 12.56 (bs, 1H, N<u>H</u>).

^{13}C-NMR (DMSO-d$_6$), δ: 12.92 (CH_3, C-6), 120.51, 122.55, 130.85 (C, C-aromatic), 146.38 (CH, C-2), 157.09 (C, C-aromatic), 161.55 (C, C-1).

4,6-Dichloro-5-methylthieno[2,3-*d*]pyrimidine (379)[56]

($C_7H_4Cl_2N_2S$; M.W.= 219.1)



378 **379**

General procedure 18;

Reagent: 6-chloro-5-methylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (**378**) (0.56 g, 2.7 mmol);

T.L.C. System: *n*hexane-EtOAc 8:2 v/v, Rf: 0.56.

Purification: flash column chromatography (*n*hexane:EtOAc 100:0 v/v increasing to *n*hexane-EtOAc 92.5:7.5 v/v);

White solid;

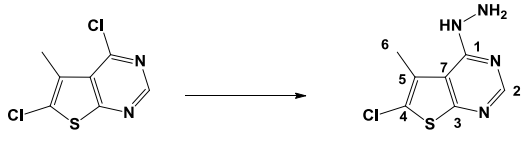
Yield: 0.29 g (49%)

^{1}H-NMR ($CDCl_3$), δ: 2.66 (s, 3H, H-6), 8.79 (s, 1H, H-2).

^{13}C-NMR ($CDCl_3$), δ: 14.43 (CH_3, C-6), 127.14, 128.29, 129.58 (C, C-aromatic), 152.48 (CH, C-2), 153.83, 167.30 (C, C-aromatic).

6-Chloro-4-hydrazinyl-5-methylthieno[2,3-*d*]pyrimidine (380)[56]

($C_7H_7ClN_4S$; M.W.= 214.7)



379 **380**

General procedure 19;

Reagent: 4,6-dichloro-5-methylthieno[2,3-*d*]pyrimidine (**379**) (0.29 g, 1.3 mmol);

Purification: recrystallization from 40 % EtOH/H_2O;

White crystals;

Yield: 0.25 g (87%)

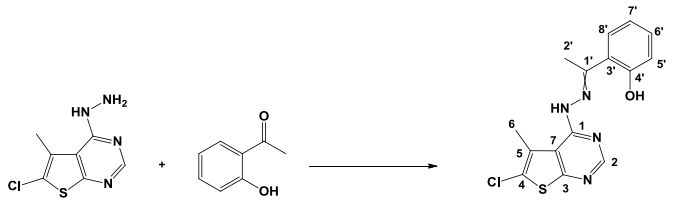
^{1}H-NMR (DMSO-d_6), δ: 2.49 (s, 3H, H-6), 4.66 (bs, 2H, N$\underline{H}_2$), 8.27 (bs, 1H, N$\underline{H}$), 8.39 (s, 1H, H-2).

^{13}C-NMR (DMSO-d_6), δ: 13.94 (CH_3, C-6), 129.96, 130.41, 131.75 (C, C-aromatic), 153.59 (CH, C-2), 159.46, 166.78 (C, C-aromatic).

6.4.8.1 6-Chloro-5-methyl-4-(2-(1-arylethylidene)hydrazinyl)thieno[2,3-*d*] pyrimidines (381-382)

2-(1-(2-(6-Chloro-5-methylthieno[2,3-*d*]pyrimidin-4-*yl*)hydrazono)ethyl)phenol (381)

($C_{15}H_{13}ClN_4OS$; M.W.= 332.8)



380 **202** **381**

General procedure 20;

Reagent: 6-chloro-4-hydrazinyl-5-methylthieno[2,3-*d*]pyrimidine (**380**) (0.05 g, 0.2 mmol);

Light green solid;

Yield: 0.06 g (77%)

Melting Point: 220-223°C

MS (ESI^+): 332.9 $[M+H]^+$

Two species observed. Major/minor species ratio: 5/1.

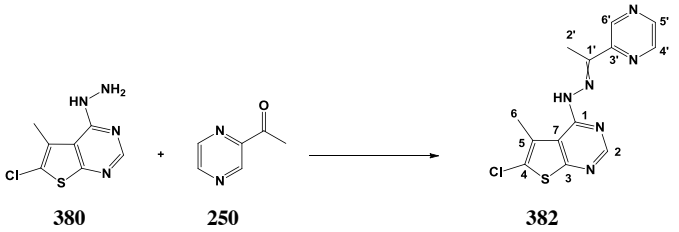
^{1}H-NMR (DMSO-d_6), δ: (major species) 2.51 (s, 3H, C<u>H</u>$_3$), 2.54 (s, 3H, C<u>H</u>$_3$), 6.89-6.94 (m, 2H, H-aromatic), 7.29-7.31 (m, 1H, H-aromatic), 7.60-7.67 (m, 1H, H-aromatic), 7.82 (s, 1H, H-2), 11.61 (bs, 1H, N<u>H</u>), 12.29 (bs, 1H, O<u>H</u>).

^{1}H-NMR (DMSO-d_6), δ: (minor species) 2.54 (s, 3H, C<u>H</u>$_3$), 2.69 (s, 3H, C<u>H</u>$_3$), 6.89-6.94 (m, 2H, H-aromatic), 7.29-7.31 (m, 1H, H-aromatic), 7.60-7.67 (m, 1H, H-aromatic), 8.63 (s, 1H, H-2), 9.68 (bs, 1H, N<u>H</u>), 13.26 (bs, 1H, O<u>H</u>).

^{13}C-NMR (DMSO-d_6), δ: (major and minor species) 14.35, 15.12 (CH_3, C-2', 6), 116.56, 118.71 (CH, C-aromatic), 119.24, 120.72, 121.02 (C, C-aromatic), 128.89, 130.91 (CH, C-aromatic), 144.52 (C, C-aromatic), 145.54 (CH, C-aromatic), 155.93, 158.14, 164.06 (C, C-aromatic).

6-Chloro-5-methyl-4-(2-(1-(pyrazin-2-*yl*)ethylidene)hydrazinyl)thieno[2,3-*d*]pyrimidine (382)

($C_{13}H_{11}ClN_6S$; M.W.= 318.8)



380 **250** **382**

General procedure 20;

Reagent: 6-chloro-4-hydrazinyl-5-methylthieno[2,3-*d*]pyrimidine (**380**) (0.05 g, 0.2 mmol);

Yellow solid;

Yield: 0.07 g (64%)

Melting Point: 234-238°C

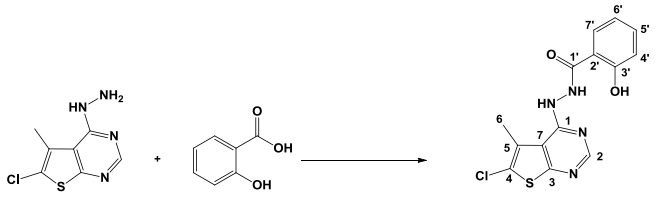
MS (ESI^+): 318.9 $[M+H]^+$

^{1}H-NMR (DMSO-d_6), δ: 2.48 (s, 3H, C<u>H</u>$_3$), 2.58 (s, 3H, C<u>H</u>$_3$), 7.95 (s, 1H, H-aromatic), 8.61 (d, J= 2.4 Hz, 1H, H-aromatic), 8.64-8.65 (m, 1H, H-aromatic), 9.79 (s, 1H, H-aromatic), 12.09 (bs, 1H, N<u>H</u>).

^{13}C-NMR (DMSO-d_6), δ: 12.77, 14.27 (CH_3, C-6, 2'), 115.78, 120.06 (C, C-aromatic), 143.27, 143.34, 143.72 (CH, C-aromatic), 144.90, 147.44, 148.74 (C, C-aromatic), 150.81 (CH, C-aromatic), 155.02, 164.93(C, C-aromatic).

6.4.8.2 N'-(6-Chloro-5-methylthieno[2,3-*d*]pyrimidin-4-*yl*)arylcarbohydrazides (383-384)

N'-(6-Ethylthieno[2,3-*d*]pyrimidin-4-*yl*)-2-hydroxybenzohydrazide (383) ($C_{14}H_{11}ClN_4O_2S$; M.W.= 334.8)



General procedure 22;

Reagent: 6-chloro-4-hydrazinyl-5-methylthieno[2,3-*d*]pyrimidine (**380**) (0.07 g, 0.3 mmol);

Purification: re-crystallisation from MeOH;

Light grey solid;

Yield: 0.02 g (19%)

Melting Point: 320-324°C

MS (ESI^+): 334.9 $[M+H]^+$

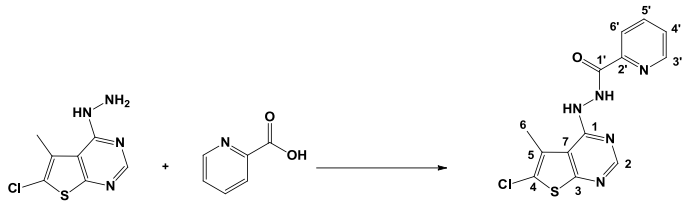
Microanalysis: Calculated for $C_{14}H_{11}ClN_4O_2S$ (334.8); Theoretical: %C = 50.23, %H = 3.31, %N = 16.73; Found: %C = 50.12, %H = 4.17, %N = 16.36.

^{1}H-NMR (DMSO-d$_6$), δ: 2.62 (s, 3H, H-6), 6.96-7.00 (m, 2H, H-aromatic), 7.45-7.50 (m, 1H, H-aromatic), 8.00 (d, J= 7.7 Hz, 1H, H-aromatic), 8.45 (s, 1H, H-2), 9.17 (bs, 1H, N<u>H</u>), 10.87 (bs, 1H, N<u>H</u>), 11.93 (bs 1H, O<u>H</u>).

^{13}C-NMR (DMSO-d$_6$), δ: 14.17 (CH_3, C-6), 114.78, 114.87 (C, C-aromatic), 117.34, 119.09 (CH, C-aromatic), 123.75, 126.82 (C, C-aromatic), 128.46, 134.09, 153.49 (CH, C-aromatic), 159.08 (C, C-aromatic), 168.13 (C, C-1').

N'-(6-Chloro-5-methylthieno[2,3-*d*]pyrimidin-4-*yl*)picolinohydrazide (384)

($C_{13}H_{10}ClN_5OS$; M.W.= 319.8)



380 **277** **384**

General procedure 17;

Reagent: 6-chloro-4-hydrazinyl-5-methylthieno[2,3-*d*]pyrimidine (**380**) (0.07 g, 0.3 mmol);

Purification: re-crystallisation from MeOH;

Pale yellow solid;

Yield: 0.07 g (61%)

Melting Point: 210-212 °C

MS (ESI^+): 319.9 $[M+H]^+$

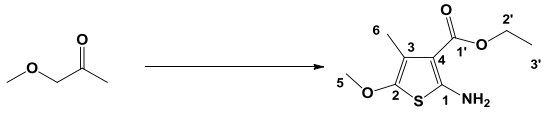
^{1}H-NMR (DMSO-d_6), δ: 2.60 (s, 3H, H-6), 7.66-7.69 (m, 1H, H-aromatic), 8.03-8.09 (m, 2H, H-aromatic), 8.37 (s, 1H, H-2), 8.72-8.74 (m, 1H, H-aromatic), 9.26 (bs, 1H, NH), 10.77 (bs, 1H, NH).

^{13}C-NMR (DMSO-d_6), δ: 14.22 (CH_3, C-6), 115.02, 121.46 (C, C-aromatic), 122.34, 126.98 (CH, C-aromatic), 131.97 (C, C-aromatic), 137.87 (CH, C-aromatic), 144.27 (C, C-aromatic), 148.69 (CH, C-aromatic), 149.30 (C, C-aromatic), 162.79 (C, C-1').

6.4.9 6-Methoxy-5-methyl-thieno[2,3-*d*]pyrimidines

Ethyl 2-amino-5-methoxy-4-methylthiophene-3-carboxylate (386)[57]

($C_9H_{13}NO_3S$; M.W.= 215.3)



385 **386**

General procedure 16;

Reagent: methoxyacetone (**385**) (0.5 g, 5.7 mmol);

Reaction stirred under reflux for 72 h;

T.L.C. System: *n*hexane -EtOAc 8:2 v/v, Rf: 0.40.

Purification: flash column chromatography (*n*hexane:EtOAc 100:0 v/v increasing to *n*hexane-EtOAc 95:5 v/v);

Brown oil;

Yield: 0.61 g (49%)

^{1}H-NMR ($CDCl_3$), δ: 1.35 (t, J= 7.1 Hz, 3H, H-3'), 2.14 (s, 3H, H-6), 3.74 (s, 3H, H-5), 4.26 (q, J= 7.1 Hz, 2H, H-2'), 5.93 (bs, 2H, $N\underline{H}_2$).

^{13}C-NMR ($CDCl_3$), δ: 12.52, 14.40 (CH_3, C-3', 6), 59.54 (CH_2, C-2'), 63.50 (CH_3, C-5), 103.10, 118.11, 142.62, 155.55 (C, C-aromatic), 166.31 (C, C-1').

6-Methoxy-5-methylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (387)

($C_8H_8N_2O_2S$; M.W.= 196.2)

386 **387**

General procedure 17a;

Reagent: ethyl 2-amino-5-methoxy-4-methylthiophene-3-carboxylate (**386**) (0.61 g, 2.8 mmol);

Brown solid;

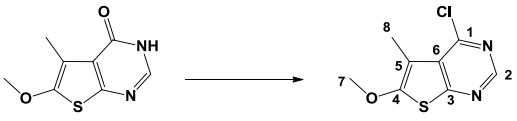
Yield: 0.29 g (52%)

^{1}H-NMR (DMSO-d$_6$), δ: 2.28 (s, 3H, H-8), 3.91 (s, 3H, H-7), 7.97 (s, 1H, H-2), 11.05 (bs, 1H, N<u>H</u>).

^{13}C-NMR (DMSO-d$_6$), δ: 10.72 (CH_3, C-8), 62.31 (CH_3, C-7), 112.12, 122.35 (C, C-aromatic), 144.27 (CH, C-2), 154.41, 154.69 (C, C-aromatic), 157.63 (C, C-1).

4-Chloro-6-methoxy-5-methylthieno[2,3-*d*]pyrimidine (388)

($C_8H_7ClN_2OS$; M.W.= 214.7)



387 **388**

General procedure 18;

Reagent: 6-methoxy-5-methylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (**387**) (0.29 g, 1.5 mmol);

T.L.C. System: *n*hexane-EtOAc 8:2 v/v, Rf: 0.59.

Purification: flash column chromatography (*n*hexane:EtOAc 100:0 v/v increasing to *n*hexane-EtOAc 85:15 v/v);

Light brown solid;

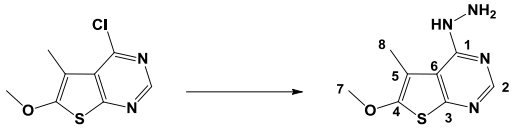
Yield: 0.25 g (79%)

^{1}H-NMR (CDCl$_3$), δ: 2.46 (s, 3H, H-8), 4.06 (s, 3H, H-7), 8.67 (s, 1H, H-2).

^{13}C-NMR (CDCl$_3$), δ: 11.56 (CH_3, C-8), 61.96 (CH_3, C-7), 108.17, 129.31 (C, C-aromatic), 150.55 (CH, C-2), 152.03, 159.30, 162.29 (C, C-aromatic).

4-Hydrazinyl-6-methoxy-5-methylthieno[2,3-*d*]pyrimidine (389)

($C_8H_{10}N_4OS$; M.W.= 210.3)



388 **389**

General procedure 19;

Reagent: 4-chloro-6-methoxy-5-methylthieno[2,3-*d*]pyrimidine (**388**) (0.25 g, 1.1

mmol);

Purification: recrystallization from 40 % EtOH/H_2O;

Light brown crystals;

Yield: 0.16 g (66%)

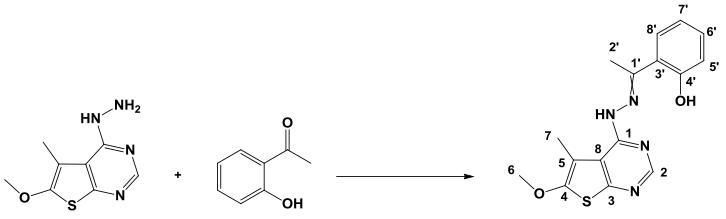
^{1}H-NMR (DMSO-d$_6$), δ: 2.33 (s, 3H, H-8), 3.91 (s, 3H, H-7), 4.55 (bs, 2H, N$\underline{H}_2$), 7.95 (bs, 1H, N$\underline{H}$), 8.31 (s, 1H, H-2).

^{13}C-NMR (DMSO-d$_6$), δ: 11.21 (CH_3, C-8), 62.24 (CH_3, C-7), 100.85, 108.27, 114.63 (C, C-aromatic), 151.79 (CH, C-2), 153.91, 158.03 (C, C-aromatic).

6.4.9.1 6-Methoxy-5-methyl-4-(2-(1-arylethylidene)hydrazinyl)thieno[2,3-*d*] pyrimidines (390-391)

2-(1-(2-(6-Methoxy-5-methylthieno[2,3-*d*]pyrimidin-4-*yl*)hydrazono)ethyl)phenol (390)

($C_{16}H_{16}N_4O_2S$; M.W.= 328.4)



389 **202** **390**

General procedure 20;

Reagent: 4-hydrazinyl-6-methoxy-5-methylthieno[2,3-*d*]pyrimidine (**389**) (0.04 g, 0.2 mmol);

Yellow crystals;

Yield: 0.03 g (50%)

Melting Point: 192-196°C

MS (ESI^+): 329.0 $[M+H]^+$

Two species observed. Major/minor species ratio: 3/2.

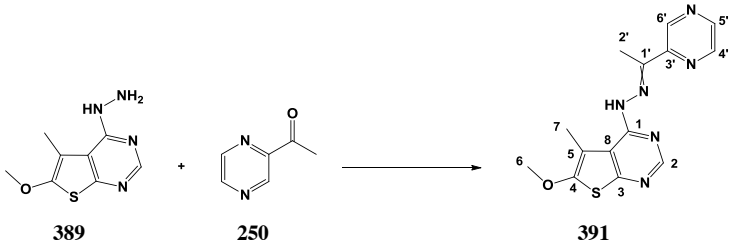
^{1}H-NMR (DMSO-d_6), δ: (major species) 2.41 (s, 3H, C<u>H</u>$_3$), 2.53 (s, 3H, C<u>H</u>$_3$), 3.94 (s, 3H, C<u>H</u>$_3$), 6.89-6.93 (m, 2H, H-aromatic), 7.27-7.31 (m, 1H, H-aromatic), 7.60-7.65 (m, 1H, H-aromatic), 7.74 (s, 1H, H-2), 11.45 (bs, 1H, N<u>H</u>), 12.52 (bs, 1H, O<u>H</u>).

^{1}H-NMR (DMSO-d_6), δ: (minor species) 2.41 (s, 3H, C<u>H</u>$_3$), 2.53 (s, 3H, C<u>H</u>$_3$), 3.94 (s, 3H, C<u>H</u>$_3$), 6.89-6.93 (m, 2H, H-aromatic), 7.27-7.31 (m, 1H, H-aromatic), 7.60-7.65 (m, 1H, H-aromatic), 8.52 (s, 1H, H-2), 9.50 (bs, 1H, N<u>H</u>), 13.38 (bs, 1H, O<u>H</u>).

^{13}C-NMR (DMSO-d_6), δ: (major and minor species) 11.85, 14.82 (CH_3, C-2', 7), 62.25 (CH_3, C-8), 116.58, 118.59 (CH, C-aromatic), 120.84, 121.93, 122.32 (C, C-aromatic), 128.05, 130.70 (CH, C-aromatic), 144.96 (C, C-aromatic), 144.87 (CH, C-aromatic), 155.45, 158.28, 163.91 (C, C-aromatic).

6-Methoxy-5-methyl-4-(2-(1-(pyrazin-2-*yl*)ethylidene)hydrazinyl)thieno[2,3-*d*]pyrimidine (391)

($C_{14}H_{14}N_6OS$; M.W.= 314.4)



389 **250** **391**

General procedure 20;

Reagent: 4-hydrazinyl-6-methoxy-5-methylthieno[2,3-*d*]pyrimidine (**389**) (0.04 g, 0.2 mmol);

Yellow solid;

Yield: 0.02 g (43%)

Melting Point: 237-240°C

MS (ESI^+): 315.0 $[M+H]^+$

Single species observed.

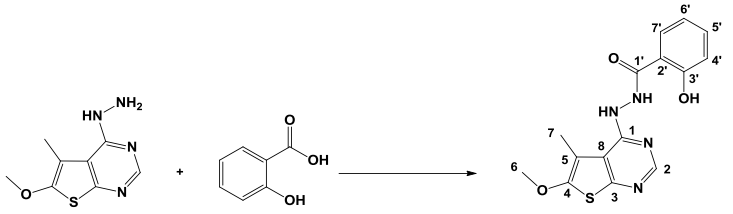
^{1}H-NMR (DMSO-d_6), δ: 2.43 (s, 3H, C<u>H</u>$_3$), 2.46 (s, 3H, C<u>H</u>$_3$), 3.93 (s, 3H, H-7), 7.85 (s, 1H, H-aromatic), 8.57-8.62 (m, 2H, H-aromatic), 9.78 (s, 1H, H-aromatic), 11.93 (bs, 1H, N<u>H</u>).

^{13}C-NMR (DMSO-d_6), δ: 11.79, 12.63 (CH_3, C-8, 2'), 62.22 (CH_3, C-7), 112.52, 119.19 (C, C-aromatic), 143.22, 143.28, 143.48 (CH, C-aromatic), 148.85, 149.81 (C, C-aromatic), 149.96 (CH, C-aromatic), 151.22, 155.58, 156.79 (C, C-aromatic).

6.4.9.2 *N*'-(6-Methoxy-5-methylthieno[2,3-*d*]pyrimidin-4-*yl*)arylhydrazides (392-393)

2-Hydroxy-*N*'-(6-methoxy-5-methylthieno[2,3-*d*]pyrimidin-4-*yl*)benzohydrazide (392)

($C_{15}H_{14}N_4O_3S$; M.W.= 330.4)



389 **275** **392**

General procedure 22;

Reagent: 4-hydrazinyl-6-methoxy-5-methylthieno[2,3-*d*]pyrimidine (**389**) (0.08 g, 0.4 mmol);

Purification: re-crystallisation from MeOH/H_2O;

Light grey solid;

Yield: 0.03 g (26%)

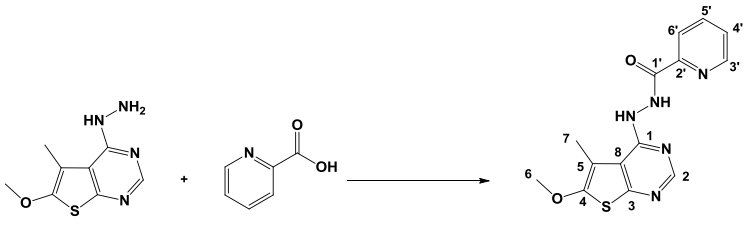
Melting Point: 199-201°C

MS (ESI^+): 330.9 $[M+H]^+$

^{1}H-NMR (DMSO-d_6), δ: 2.44 (s, 3H, H-7), 3.98 (s, 3H, H-6), 6.95-7.00 (m, 2H, H-aromatic), 7.44-7.49 (m, 1H, H-aromatic), 7.99 (d, J= 7.7 Hz, 1H, H-aromatic), 8.34 (s, 1H, H-2), 8.86 (bs, 1H, NH), 10.91 (bs, 1H, NH), 11.96 (bs 1H, OH).

^{13}C-NMR (DMSO-d_6), δ: 11.42 (CH_3, C-7), 63.36 (CH_3, C-6), 108.13, 114.82, 115.19 (C, C-aromatic), 117.34, 119.01 (CH, C-aromatic), 126.72 (C, C-aromatic), 128.33, 133.99, 151.58 (CH, C-aromatic), 154.93, 156.64, 159.19 (C, C-aromatic), 167.49 (C, C-1').

***N*'-(6-Methoxy-5-methylthieno[2,3-*d*]pyrimidin-4-*yl*)picolinohydrazide (393)**
($C_{14}H_{13}N_5O_2S$; M.W.= 315.4)



389 **277** **393**

General procedure 22;

Reagent: 4-hydrazinyl-6-methoxy-5-methylthieno[2,3-*d*]pyrimidine (**389**) (0.08 g, 0.4 mmol);

Purification: re-crystallisation from MeOH;

White solid;

Yield: 0.04 g (38%)

Melting Point: 169-172°C

MS (ESI^+): 315.9 $[M+H]^+$

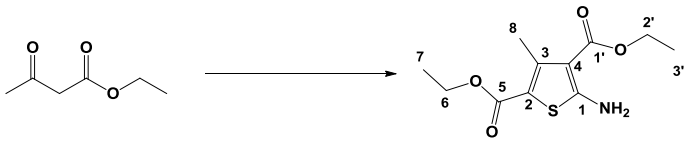
Microanalysis: Calculated for $C_{14}H_{13}N_5O_2S$ (315.4); Theoretical: %C = 53.32, %H = 4.15, %N = 22.20; Found: %C = 53.41, %H = 3.78, %N = 22.23.

^{1}H-NMR (DMSO-d_6), δ: 2.43 (s, 3H, H-7), 3.97 (s, 3H, H-6), 7.65-7.68 (m, 1H, H-aromatic), 8.02-8.08 (m, 2H, H-aromatic), 8.30 (s, 1H, H-2), 8.72-8.73 (m, 1H, H-aromatic), 8.82 (bs, 1H, NH), 10.66 (bs, 1H, NH).

^{13}C-NMR (DMSO-d_6), δ: 11.46 (CH_3, C-7), 62.34 (CH_3, C-6), 108.25, 115.28, (C, C-aromatic), 122.26, 126.91 (CH, C-aromatic), 132.06 (C, C-aromatic), 137.83, (CH, C-aromatic), 144.27 (C, C-aromatic), 148.67 (CH, C-aromatic), 149.38 (C, C-aromatic), 152.26 (CH, C-aromatic), 154.76 (C, C-aromatic), 163.41 (C, C-1').

6.4.10 Ethyl-5-methyl-thieno[2,3-*d*]pyrimidine-6-carboxylates

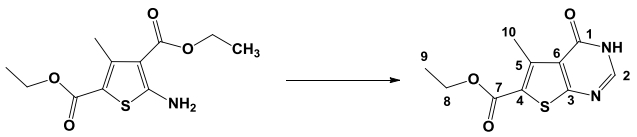
Diethyl 5-amino-3-methylthiophene-2,4-dicarboxylate (395)[58]
($C_{11}H_{15}NO_4S$; M.W.= 257.31)



394 395

General procedure 16;
Reagent: ethyl acetoacetate (**394**) (1.5 g, 11.5 mmol);
T.L.C. System: *n*hexane-EtOAc 7:3 v/v, Rf: 0.55.
Purification: flash column chromatography (*n*hexane:EtOAc 100:0 v/v increasing to *n*hexane-EtOAc 85:15 v/v);
Yellow solid;
Yield: 1.79 g (66%)
^{1}H-NMR ($CDCl_3$), δ: 1.34 (t, J= 7.1 Hz, 3H, C<u>H</u>$_3$), 1.39 (t, J= 7.1 Hz, 3H, C<u>H</u>$_3$), 2.71 (s, 3H, H-8), 4.28 (q, J= 7.1 Hz, 2H, C<u>H</u>$_2$), 4.33 (q, J= 7.1 Hz, 2H, C<u>H</u>$_2$), 6.55 (bs, 2H, N<u>H</u>$_2$).
^{13}C-NMR ($CDCl_3$), δ: 14.35, 14.41, 16.13 (CH_3, C-7, 8, 3'), 60.10, 60.42 (CH_2, C-6, 2'), 108.50, 108.58, 148.05, 162.89 (C, C-aromatic), 166.11, 166.13 (C, C-5, 1').

Ethyl 5-methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-6-carboxylate (396)[59]
($C_{10}H_{10}N_2O_3S$; M.W.= 238.3)



395 396

General procedure 17;
Reagent: diethyl 5-amino-3-methylthiophene-2,4-dicarboxylate (**395**) (1.86 g, 7.3 mmol);
0.5 mL of AcOH added, reaction stirred at 150°C for 5 days;

Purification: recrystallization from EtOH/H_2O;

Yellow solid;

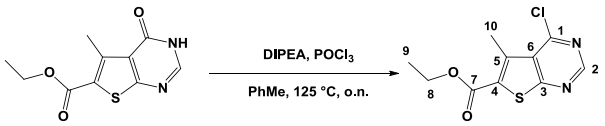
Yield: 1.73 g (71%)

^{1}H-NMR (DMSO-d$_6$), δ: 1.31 (t, J= 7.1 Hz, 3H, H-9), 2.28 (s, 3H, H-10), 4.30 (q, J= 7.1 Hz, 2H, H-8), 8.21 (s, 1H, H-2), 12.65 (bs, 1H, NH).

^{13}C-NMR (DMSO-d$_6$), δ: 14.10, 14.85 (CH_3, C-9, 10), 61.11 (CH_2, C-8), 121.41, 123.76, 143.33 (C, C-aromatic), 148.32 (CH, C-2), 158.28 (C, C-aromatic), 161.80, 165.73 (C, C-1, 7).

Ethyl 4-chloro-5-methylthieno[2,3-*d*]pyrimidine-6-carboxylate (397)[60]

($C_{10}H_9ClN_2O_2S$; M.W.= 256.7)



396 **397**

To a solution of intermediate ethyl 5-methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-6-carboxylate (**397**) (1.22 g, 5.1 mmol) in toluene (23 mL) were added DIPEA (0.7 mL, 4.1 mmol) and $POCl_3$ (0.6 mL, 6.1 mmol). The reaction mixture was stirred at 125°C o.n., then cooled to r.t., poured into ice-cold saturated $NaHCO_3$-H_2O-EtOAc (60 mL-60 mL-150 mL) and stirred to quence excess $POCl_3$. The organic layer was separated, washed with sat. aq. $NaHCO_3$, dried over $MgSO_4$ and concentrated under vacuum. The residue was purified by flash column chromatography (*n*hexane:EtOAc 100:0 v/v increasing to *n*hexane-EtOAc 90:10 v/v) to give pure ethyl 4-chloro-5-methylthieno[2,3-*d*]pyrimidine-6-carboxylate (**397**) as a yellow solid.

T.L.C. System: *n*hexane-EtOAc 7:3 v/v, Rf: 0.63.

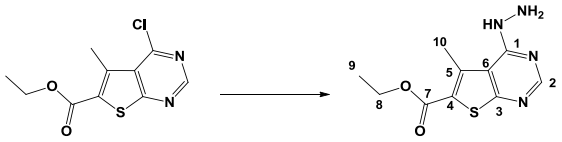
Yield: 1.05 g (79%)

^{1}H-NMR ($CDCl_3$), δ: 1.44 (t, J= 7.1 Hz, 3H, H-9), 3.07 (s, 3H, H-10), 4.44 (q, J= 7.1 Hz, 2H, H-8), 8.88 (s, 1H, H-2).

^{13}C-NMR ($CDCl_3$), δ: 14.25, 15.87 (CH_3, C-9, 10), 62.13 (CH_2, C-8), 128.51, 129.01, 138.94 (C, C-aromatic), 154.23 (CH, C-2), 157.32, 162.13 (C, C-aromatic), 169.24 (C, C-7).

Ethyl 4-hydrazinyl-5-methylthieno[2,3-*d*]pyrimidine-6-carboxylate (398)

($C_{10}H_{12}N_4O_2S$; M.W.= 252.3)



397 **398**

General procedure 19;

Reagent: ethyl 4-chloro-5-methylthieno[2,3-*d*]pyrimidine-6-carboxylate (**397**) (0.25 g, 1.1 mmol);

Purification: recrystallization from 40 % EtOH/H_2O;

Light orange crystals;

Yield: 0.72 g (69%)

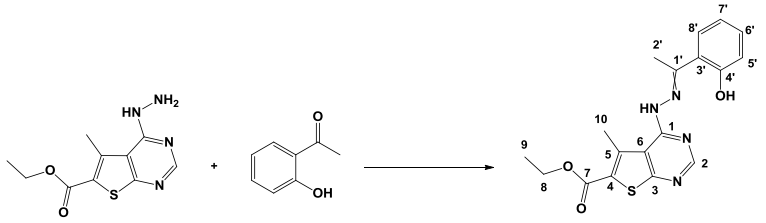
^{1}H-NMR (DMSO-d_6), δ: 1.31 (t, J= 7.1 Hz, 3H, H-9), 2.86 (s, 3H, H-10), 4.30 (q, J= 7.1 Hz, 2H, H-8), 4.89 (bs, 2H, N<u>H</u>$_2$), 8.40 (s, 1H, H-2), 8.53 (bs, 1H, N<u>H</u>).

^{13}C-NMR (DMSO-d_6), δ: 14.21, 15.57 (CH_3, C-9, 10), 61.05 (CH_2, C-8), 116.09, 120.11, 131.44, 140.00 (C, C-aromatic), 155.26 (CH, C-2), 160.05 (C, C-aromatic), 162.17 (C, C-7).

6.4.10.1 Ethyl-5-methyl-4-(2-(1-phenylethylidene)hydrazinyl)thieno[2,3-*d*] pyrimidine-6-carboxylates (399-400)

Ethyl-4-(2-(1-(2-hydroxyphenyl)ethylidene)hydrazinyl)-5-methylthieno[2,3-*d*]pyrimidine-6-carboxylate (399)

($C_{18}H_{18}N_4O_3S$; M.W.= 370.4)



398 **202** **399**

General procedure 20;

Reagent: ethyl 4-hydrazinyl-5-methylthieno[2,3-*d*]pyrimidine-6-carboxylate (**398**) (0.1 g, 0.25 mmol);

Yellow solid;

Yield: 0.05 g (29%)

Melting Point: 199-201°C

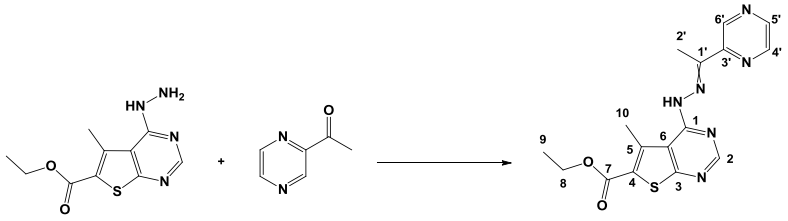
MS (ESI^+): 371.0 $[M+H]^+$

^{1}H-NMR (DMSO-d_6), δ: 1.30 (t, J= 7.1 Hz, 3H, H-9), 2.52 (s, 3H, C<u>H</u>$_3$), 2.92 (s, 3H, C<u>H</u>$_3$), 4.29 (s, 2H, C<u>H</u>$_2$), 6.89-6.93 (m, 2H, H-aromatic), 7.28-7.32 (m, 1H, H-aromatic), 7.63 (d, J= 7.1 Hz, 1H, H-aromatic), 7.88 (s, 1H, H-2), 11.69 (bs, 1H, N<u>H</u>), 12.22 (bs, 1H, O<u>H</u>).

^{13}C-NMR (DMSO-d_6), δ: 14.10, 15.27, 16.07 (CH_3, C-2', 9, 10), 60.95 (CH_3, C-8), 116.61, 118.72 (CH, C-aromatic), 120.55, 120.98, 121.33 (C, C-aromatic), 128.96, 131.03 (CH, C-aromatic), 143.06, 145.07 (C, C-aromatic), 147.36 (CH, C-aromatic), 158.17, 161.08, 162.23 (C, C-aromatic), 164.59 (C, C-7).

Ethyl-5-methyl-4-(2-(1-(pyrazin-2-*yl*)ethylidene)hydrazinyl)thieno[2,3-*d*] pyrimidine-6-carboxylate (400)

($C_{14}H_{14}N_6OS$; M.W.= 314.4)



398 **250** **400**

General procedure 20;

Reagent: ethyl 4-hydrazinyl-5-methylthieno[2,3-*d*]pyrimidine-6-carboxylate (**398**) (0.1 g, 0.25 mmol);

Yellow solid;

Yield: 0.07 g (45%)

Melting Point: 194-197°C

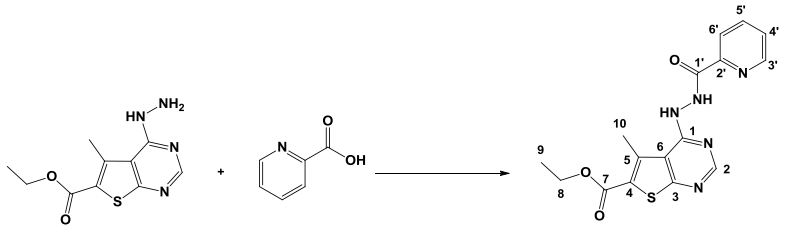
MS (ESI^+): 357.0 $[M+H]^+$

^{1}H-NMR (DMSO-d_6), δ: 1.28 (t, J= 7.1 Hz, 3H, H-9), 2.42 (s, 3H, C$\underline{H}_3$), 2.88 (s, 3H, C$\underline{H}_3$), 4.23 (q, J= 7.1 Hz, 2H, H-8), 7.95 (s, 1H, H-2), 8.58 (d, J= 2.5 Hz, 1H, H-4'), 8.60 (dd, J_1= 2.5 Hz, J_2= 1.5 Hz, 1H, H-5'), 9.75 (d, J= 1.5 Hz, 1H, H-6'), 12.04 (bs, 1H, N$\underline{H}$).

^{13}C-NMR (DMSO-d_6), δ: 12.84, 14.07, 15.97 (CH_3, C-9, 10, 2'), 60.93 (CH_2, C-8), 120.65, 121.53, 143.20 (C, C-aromatic), 143.22, 143.43, 143.85, 147.04 (CH, C-aromatic), 148.59, 150.81, 158.66, 162.07 (C, C-aromatic), 162.13 (C, C-7).

6.4.10.2 Ethyl 5-methyl-4-(2-picolinoylhydrazinyl)thieno[2,3-*d*]pyrimidine-6-carboxylate (401)

($C_{16}H_{15}N_5O_3S$; M.W.= 357.4)



398 **277** **401**

General procedure 22;

Reagent: ethyl 4-hydrazinyl-5-methylthieno[2,3-*d*]pyrimidine-6-carboxylate (**398**) (0.15 g, 0.6 mmol);

Purification: re-crystallisation from MeOH/H_2O;

Yellow solid;

Yield: 0.1 g (46%)

Melting Point: 118-122°C

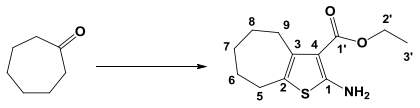
MS (ESI^+): 358.0 $[M+H]^+$

^{1}H-NMR (DMSO-d_6), δ: 1.33 (t, J= 7.1 Hz, 3H, H-9), 2.97 (s, 3H, H-10), 4.33 (q, 2H, H-8), 7.64-7.67 (m, 1H, H-aromatic), 8.02-8.09 (m, 2H, H-aromatic), 8.31 (s, 1H, H-2), 8.70-8.73 (m, 1H, H-aromatic), 10.11 (bs, 1H, NH), 11.05 (bs, 1H, NH).

^{13}C-NMR (DMSO-d_6), δ: 14.12, 15.86 (CH_3, C-79, 10), 61.12 (CH_2, C-8), 116.04, 121.56 (C, C-aromatic), 122.18, 126.75 (CH, C-aromatic), 135.24 (C, C-aromatic), 137.85, (CH, C-aromatic), 141.32 (C, C-aromatic), 148.61 (CH, C-aromatic), 149.60 (C, C-aromatic), 151.97 (CH, C-aromatic), 161.91, 162.06 (C, C-1').

6.4.11 6,7,8,9-Tetrahydro-3*H*-cyclohepta[4,5]thieno[2,3-*d*]pyrimidines

Ethyl 2-amino-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxylate (405)[44] ($C_{12}H_{17}NO_2S$; M.W.= 239.3)



402 **405**

General procedure 16;

Reagent: cycloheptanone (**402**) (1.5 g, 13.4 mmol);

Morpholine used instead of NEt_3;

T.L.C. System: *n*hexane-EtOAc 8:2 v/v, Rf: 0.49.

Purification: flash column chromatography (*n*hexane:EtOAc 100:0 v/v increasing to *n*hexane-EtOAc 95:5 v/v);

Pale yellow solid;

Yield: 1.98 g (62%)

^{1}H-NMR ($CDCl_3$), δ: 1.37 (t, J= 7.1 Hz, 3H, H-3'), 1.61-1.68 (m, 4H, C<u>H</u>$_2$), 1.80-1.85 (m, 2H, C<u>H</u>$_2$), 2.58-2.61 (m, 2H, C<u>H</u>$_2$), 2.98-3.01 (m, 2H, C<u>H</u>$_2$), 4.30 (q, J= 7.1 Hz, 2H, H-2'), 5.78 (bs, 2H, N<u>H</u>$_2$).

^{13}C-NMR ($CDCl_3$), δ: 14.44 (CH_3, C-3'), 26.91, 27.84, 28.61, 28.69, 32.10 (CH_2, C-5, 6, 7, 8, 9), 59.53 (CH_2, C-2'), 107.62, 121.26, 137.95, 159.85 (C, C-aromatic), 165.97 (C, C-1').

6,7,8,9-Tetrahydro-3*H*-cyclohepta[4,5]thieno[2,3-*d*]pyrimidin-4(5*H*)-one (408)[44] ($C_{11}H_{12}N_2OS$; M.W.= 220.3)

405 **408**

General procedure 17;

Reagent: ethyl 2-amino-5,6,7,8-tetrahydro-4H-cyclohepta[b]thiophene-3-carboxylate (**405**) (1.98 g, 8.3 mmol);

Brown solid;

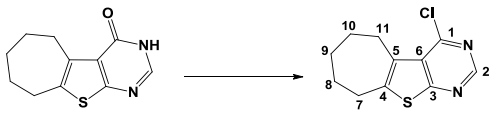
Yield: 1.5 g (82%)

^{1}H-NMR (DMSO-d$_6$), δ: 1.54-1.59 (m, 2H, C<u>H</u>$_2$), 1.60-1.65 (m, 2H, C<u>H</u>$_2$), 1.82-1.87 (m, 2H, C<u>H</u>$_2$), 2.81-2.84 (m, 2H, C<u>H</u>$_2$), 3.24-3.27 (m, 2H, C<u>H</u>$_2$), 7.98 (s, 1H, H-2), 12.27 (bs, 1H, N<u>H</u>).

^{13}C-NMR (DMSO-d$_6$), δ: 26.82, 27.22, 27.27, 28.98, 31.93 (CH_2, C-7, 8, 9 10, 11), 123.20, 136.41, 136.42 (C, C-aromatic), 144.46 (CH, C-2), 158.15 (C, C-aromatic), 160.82 (C, C-1).

4-Chloro-6,7,8,9-tetrahydro-5*H*-cyclohepta[4,5]thieno[2,3-*d*]pyrimidine (411)[44]

($C_{11}H_{11}ClN_2S$; M.W.= 238.7)



408 **411**

General procedure 18;

Reagent: 6,7,8,9-tetrahydro-3*H*-cyclohepta[4,5]thieno[2,3-*d*]pyrimidin-4(5*H*)-one (**408**) (1.5 g, 6.8 mmol);

T.L.C. System: *n*hexane-EtOAc 8:2 v/v, Rf: 0.64.

Purification: flash column chromatography (*n*hexane:EtOAc 100:0 v/v increasing to *n*hexane-EtOAc 95:5 v/v);

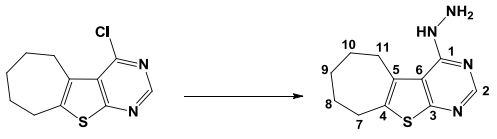
Yellow solid;

Yield: 1.1 g (67%)

^{1}H-NMR ($CDCl_3$), δ: 1.73-1.83 (m, 4H, C<u>H</u>$_2$), 1.94-2.00 (m, 2H, C<u>H</u>$_2$), 2.97-3.01 (m, 2H, C<u>H</u>$_2$), 3.36-3.39 (m, 2H, C<u>H</u>$_2$), 8.72 (s, 1H, H-2).

^{13}C-NMR ($CDCl_3$), δ: 26.43, 26.93, 28.12, 30.13, 31.83 (CH_2, C-7, 8, 9 10, 11), 129.02, 132.44, 143.90 (C, C-aromatic), 151.03 (CH, C-2), 153.11, 167.87 (C, C-aromatic).

4-Hydrazinyl-6,7,8,9-tetrahydro-5*H*-cyclohepta[4,5]thieno[2,3-*d*]pyrimidine (414)[64] ($C_{11}H_{14}N_4S$; M.W.= 234.3)



411 414

General procedure 15;

Reagent: 4-chloro-6,7,8,9-tetrahydro-5*H*-cyclohepta[4,5]thieno[2,3-*d*]pyrimidine (**411**) (1.1 g, 4.6 mmol);

Purification: recrystallization from 40 % EtOH/H_2O;

Yellow solid;

Yield: 0.84 g (78%)

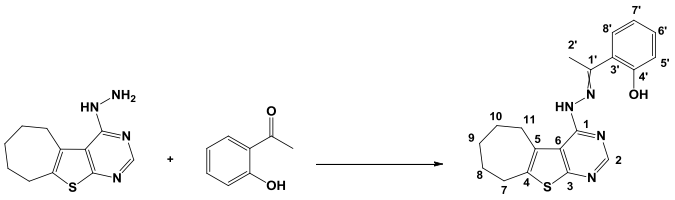
^{1}H-NMR (DMSO-d_6), δ: 1.60-1.69 (m, 4H, C$\underline{H}_2$), 1.80-1.86 (m, 2H, C$\underline{H}_2$), 2.83-2.87 (m, 2H, C$\underline{H}_2$), 3.01-3.05 (m, 2H, C$\underline{H}_2$), 4.59 (bs, 2H, N$\underline{H}_2$), 8.20 (bs, 1H, N$\underline{H}$), 8.32 (s, 1H, H-2).

^{13}C-NMR (DMSO-d_6), δ: 26.61, 26.85, 28.62, 28.73, 30.91 (CH_2, C-7, 8, 9 10, 11), 115.02, 126.72, 135.62 (C, C-aromatic), 151.90 (CH, C-2), 155.98, 163.92 (C, C-aromatic).

6.4.11.1 4-(2-(1-Arylethylidene)hydrazinyl)-6,7,8,9-tetrahydro-5*H*-cyclohepta[4,5] thieno[2,3-*d*]pyrimidines (417-418)

2-(1-(2-(6,7,8,9-Tetrahydro-5*H*-cyclohepta[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*) hydrazono)ethyl)phenol (417)

($C_{19}H_{20}N_4OS$; M.W.= 352.5)



414 **202** **417**

General procedure 20;

Reagent: 4-hydrazinyl-6,7,8,9-tetrahydro-5*H*-cyclohepta[4,5]thieno[2,3-*d*]pyrimidine (**414**) (0.15 g, 0.6 mmol);

Yellow solid;

Yield: 0.09 g (39%)

Melting Point: 170-173°C

MS (ESI^+): 353.0 $[M+H]^+$

Two species observed. Major/minor species ratio: 9:1.

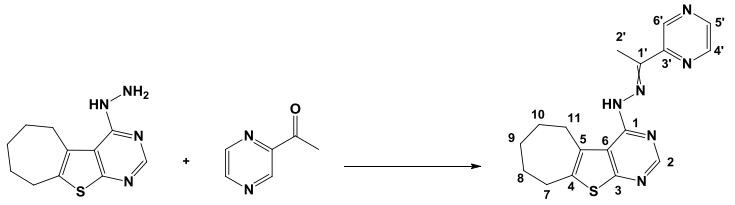
^{1}H-NMR (DMSO-d_6), δ: (major species) 1.59-1.68 (m, 4H, C$\underline{H}_2$), 1.83-1.89 (m, 2H, C$\underline{H}_2$), 2.49 (s, 3H, H-2'), 2.82-2.85 (m, 2H, C$\underline{H}_2$), 3.46-3.49 (m, 2H, C$\underline{H}_2$), 6.89-6.93 (m, 2H, H-aromatic), 7.26-7.30 (m, 1H, H-aromatic), 7.59-7.62 (m, 1H, H-aromatic), 7.74 (s, 1H, H-2), 11.37 (bs, 1H, N$\underline{H}$), 12.43 (bs, 1H, O$\underline{H}$).

^{1}H-NMR (DMSO-d_6), δ: (minor species) 1.73-1.79 (m, 4H, C$\underline{H}_2$), 1.83-1.89 (m, 2H, C$\underline{H}_2$), 2.49 (s, 3H, H-2'), 2.93-2.96 (m, 2H, C$\underline{H}_2$), 3.19-3.23 (m, 2H, C$\underline{H}_2$), 6.89-6.93 (m, 2H, H-aromatic), 7.26-7.30 (m, 1H, H-aromatic), 7.64-7.67 (m, 1H, H-aromatic), 8.54 (s, 1H, H-2), 9.82 (bs, 1H, N$\underline{H}$), 13.44 (bs, 1H, O$\underline{H}$).

^{13}C-NMR (DMSO-d_6), δ: (major and minor species) 15.00 (CH_3, C-2'), 26.70, 27.31, 27.93, 29.03, 31.93 (CH_2, C-7, 8, 9 10, 11), 116.53, 118.66 (CH, C-aromatic), 119.72, 121.09 (C, C-aromatic), 128.73, 130.69 (CH, C-aromatic), 136.58, 136.97 (C, C-aromatic), 143.60 (CH, C-aromatic), 145.66, 155.21, 158.17, 163.28 (C, C-aromatic).

4-(2-(1-(Pyrazin-2-*yl*)ethylidene)hydrazinyl)-6,7,8,9-tetrahydro-5*H*-cyclohepta [4,5]thieno[2,3-*d*]pyrimidine (418)

($C_{17}H_{18}N_6S$; M.W.= 338.4)



414 **250** **418**

General procedure 20;

Reagent: 4-hydrazinyl-6,7,8,9-tetrahydro-5*H*-cyclohepta[4,5]thieno[2,3-*d*]pyrimidine (**414**) (0.15 g, 0.6 mmol);

Yellow solid;

Yield: 0.09 g (43%)

Melting Point: 201-205°C

MS (ESI^+): 339.0 $[M+H]^+$

Microanalysis: Calculated for $C_{17}H_{18}N_6S$ (338.4); Theoretical: %C = 60.33, %H = 5.36, %N = 24.82; Found: %C = 60.13, %H = 5.29, %N = 24.31.

Single species observed.

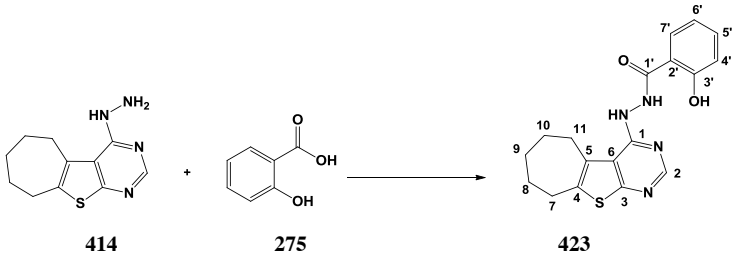
^{1}H-NMR (DMSO-d_6), δ: 1.59-1.68 (m, 4H, C$\underline{H}_2$), 1.83-1.89 (m, 4H, C$\underline{H}_2$), 2.46 (s, 3H, H-2'), 2.82-2.86 (m, 2H, C$\underline{H}_2$), 3.51-3.54 (m, 2H, C$\underline{H}_2$), 7.86 (d, J= 3.6 Hz, 1H, H-aromatic), 8.58-8.63 (m, 2H, H-aromatic), 9.80 (d, J= 1.3 Hz, 1H, H-aromatic), 11.94 (bs, 1H, N$\underline{H}$).

^{13}C-NMR (DMSO-d_6), δ: 12.74 (CH_3, C-2'), 26.74, 27.29, 27.86, 29.06, 31.95 (CH_2, C-7, 8, 9 10, 11), 119.93, 136.69, 137.21 (C, C-aromatic), 143.19, 143.33, 143.41, 143.51 (CH, C-aromatic), 149.22, 151.18, 156.62, 156.87 (C, C-aromatic).

6.4.11.2 *N*'-(6,7,8,9-Tetrahydro-5*H*-cyclohepta[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)aryl hydrazides (423-424)

2-Hydroxy-*N*'-(6,7,8,9-tetrahydro-5*H*-cyclohepta[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*) benzohydrazide (423)

($C_{18}H_{18}N_4O_2S$; M.W.= 354.4)



414 **275** **423**

General procedure 22;

Reagent: 4-hydrazinyl-6,7,8,9-tetrahydro-5*H*-cyclohepta[4,5]thieno[2,3-*d*]pyrimidine (**414**) (0.2 g, 0.9 mmol);

T.L.C. System: EtOAc 100%, Rf: 0.63.

Purification: flash column chromatography (*n*hexane:EtOAc 100:0 v/v increasing to *n*hexane-EtOAc 50:50 v/v);

White solid;

Yield: 0.1 g (38%)

Melting Point: charring >260°C

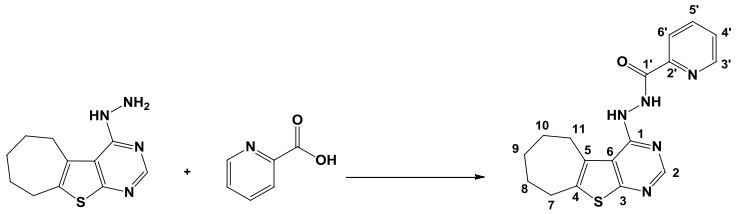
MS (ESI^+): 355.0 $[M+H]^+$

^{1}H-NMR (DMSO-d$_6$), δ: 1.67-1.76 (m, 4H, C$\underline{H}_2$), 1.85-1.92 (m, 2H, C$\underline{H}_2$), 2.91-2.96 (m, 2H, C$\underline{H}_2$), 3.14-3.19 (m, 2H, C$\underline{H}_2$), 6.95-7.01 (m, 2H, H-aromatic), 7.44-7.49 (m, 1H, H-aromatic), 7.97-7.01 (m, 1H, H-aromatic), 8.35 (s, 1H, H-2), 8.12 (bs, 1H, N$\underline{H}$), 10.83 (bs, 1H, N$\underline{H}$), 11.99 (bs 1H, O$\underline{H}$).

^{13}C-NMR (DMSO-d$_6$), δ: 26.64, 26.76, 28.73, 28.81, 29.11 (CH_2, C-7, 8, 9 10, 11), 114.83, 116.51 (C, C-aromatic), 117.34, 119.03, 129.25 (CH, C-aromatic), 131.81 (C, C-aromatic), 133.99 (CH, C-aromatic), 137.30 (C, C-aromatic), 151.77 (CH, C-aromatic), 156.66, 159.16, 164.09 (C, C-aromatic), 167.44 (C, C-1').

***N*'-(6,7,8,9-Tetrahydro-5*H*-cyclohepta[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)picolino hydrazide (424)**

($C_{17}H_{17}N_5OS$; M.W.= 339.4)



General procedure 22;

Reagent: 4-hydrazinyl-6,7,8,9-tetrahydro-5*H*-cyclohepta[4,5]thieno[2,3-*d*]pyrimidine (**414**) (0.2 g, 0.9 mmol);

Purification: re-crystallisation from EtOH;

White solid;

Yield: 0.09 g (30%)

Melting Point: 147-149°C

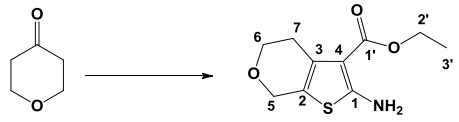
MS (ESI^+): 340.4 $[M+H]^+$

^{1}H-NMR (DMSO-d$_6$), δ: 1.69-1.74 (m, 4H, C<u>H</u>$_2$), 1.86-1.91 (m, 2H, C<u>H</u>$_2$), 2.90-2.95 (m, 2H, C<u>H</u>$_2$), 3.14-3.18 (m, 2H, C<u>H</u>$_2$), 7.66-7.69 (m, 1H, H-aromatic), 8.02-8.09 (m, 2H, H-aromatic), 8.33 (s, 1H, H-2), 8.71-8.75 (m, 1H, H-aromatic), 9.07 (bs, 1H, N<u>H</u>), 10.67 (bs, 1H, N<u>H</u>).

^{13}C-NMR (DMSO-d$_6$), δ: 26.69, 26.78, 28.75, 28.84, 30.92 (CH_2, C-7, 8, 9 10, 11), 116.59 (C, C-aromatic), 122.25, 126.92 (CH, C-aromatic), 131.84, 137.33 (C, C-aromatic), 137.85, 148.68 (CH, C-aromatic), 149.37 (C, C-aromatic), 151.78 (CH, C-aromatic), 156.79, 163.17 (C, C-aromatic), 169.21 (C, C-1').

6.4.12 5,6-Dihydro-3*H*-pyrano[4',3':4,5]thieno [2,3-*d*]pyrimidines

Ethyl 2-amino-5,7-dihydro-4*H*-thieno[2,3-*c*]pyran-3-carboxylate (406)[65]
($C_{10}H_{13}NO_3S$; M.W.= 227.3)



403 **406**

General procedure 16;

Reagent: tetrahydro-4H-pyran-4-one (**403**) (1.5 g, 15 mmol);

Morpholine used instead of NEt_3;

T.L.C. System: *n*hexane-EtOAc 6:4 v/v, Rf: 0.65.

Purification: flash column chromatography (*n*hexane:EtOAc 100:0 v/v increasing to *n*hexane-EtOAc 85:15 v/v);

White solid;

Yield: 3.02 g (89%)

^{1}H-NMR ($CDCl_3$), δ: 1.35 (t, J= 7.1 Hz, 3H, H-3'), 2.48 (tt, J_1= 5.6 Hz, J_2= 2.0 Hz, 2H, H-6), 3.92 (t, J= 5.6 Hz, 2H, H-7), 4.29 (q, J= 7.1 Hz, 2H, H-2'),4.57 (t, J= 2.0 Hz, 2H, H-5), 6.03 (bs, 2H, $N\underline{H}_2$).

^{13}C-NMR ($CDCl_3$), δ: 14.44 (CH_3, C-3'), 27.72, 59.56, 64.60, 65.12 (CH_2, C-5, 6, 7, 2'), 105.47, 114.81, 130.35, 162.21 (C, C-aromatic), 165.84 (C, C-1').

5,6-Dihydro-3*H*-pyrano[4',3':4,5]thieno[2,3-*d*]pyrimidin-4(8*H*)-one (409)[66]
($C_9H_8N_2O_2S$; M.W.= 208.2)

406 **409**

General procedure 17;

Reagent: ethyl 2-amino-5,7-dihydro-4*H*-thieno[2,3-*c*]pyran-3-carboxylate (**406**) (3.02 g, 13.3 mmol);

Brown solid;

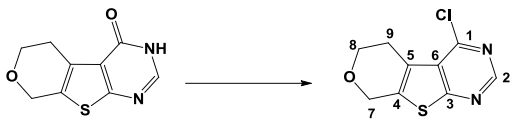
Yield: 2.2 g (80%)

^{1}H-NMR (DMSO-d$_6$), δ: 2.94 (t, J= 5.5 Hz, 2H, C<u>H</u>$_2$), 3.90 (t, J= 5.5 Hz, 2H, C<u>H</u>$_2$), 4.75 (s, 2H, H-7), 8.04 (s, 1H, H-2), 12.40 (bs, 1H, N<u>H</u>).

^{13}C-NMR (DMSO-d$_6$), δ: 26.01, 63.85, 64.23 (CH_2, C-7, 8, 9), 122.29, 128.41, 129.93 (C, C-aromatic), 145.22 (CH, C-2), 157.56 (C, C-aromatic), 163.11(C, C-1).

4-Chloro-6,8-dihydro-5*H*-pyrano[4',3':4,5]thieno[2,3-*d*]pyrimidine (412)[66]

($C_9H_7ClN_2OS$; M.W.= 226.7)



409 **412**

General procedure 18;

Reagent: 5,6-dihydro-3*H*-pyrano[4',3':4,5]thieno[2,3-*d*]pyrimidin-4(8*H*)-one (**409**) (2.2 g, 10.6 mmol);

T.L.C. System: *n*hexane-EtOAc 7:3 v/v, Rf: 0.46.

Purification: flash column chromatography (*n*hexane:EtOAc 100:0 v/v increasing to *n*hexane-EtOAc 80:20 v/v);

White solid;

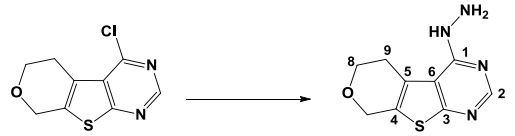
Yield: 2.28 g (95%)

^{1}H-NMR ($CDCl_3$), δ: 3.18 (tt, J_1= 5.5 Hz, J_2= 1.9 Hz, 2H, H-8), 4.05 (t, J= 5.5 Hz, 2H, H-9), 4.88 (t, J= 1.9 Hz, 2H, H-7), 8.73 (s, 1H, H-2).

^{13}C-NMR ($CDCl_3$), δ: 26.88, 64.66, 65.29 (CH_2, C-7, 8, 9), 125.04, 128.20, 137.02 (C, C-aromatic), 152.09 (CH, C-2), 153.55, 168.99 (C, C-aromatic).

4-Hydrazinyl-6,8-dihydro-5*H*-pyrano[4',3':4,5]thieno[2,3-*d*]pyrimidine (415)[66]

($C_9H_{10}N_4OS$; M.W.= 222.3)



412 **415**

General procedure 19;

Reagent: 4-chloro-6,8-dihydro-5*H*-pyrano[4',3':4,5]thieno[2,3-*d*]pyrimidine (**415**) (2.28 g, 10 mmol);

Purification: recrystallization from 40 % EtOH/H_2O;

White solid;

Yield: 1.24 g (55%)

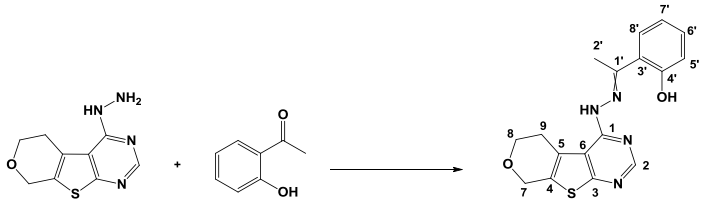
^{1}H-NMR (DMSO-d$_6$), δ: 3.03 (tt, J_1= 5.5 Hz, J_2= 1.8 Hz, 2H, H-8), 3.92 (t, J= 5.5 Hz, 2H, H-9), 4.60 (bs, 2H, N$\underline{H}_2$), 4.77 (t, J= 1.8 Hz, 2H, H-7), 8.01 (bs, 1H, N$\underline{H}$), 8.36 (s, 1H, H-2).

^{13}C-NMR (DMSO-d$_6$), δ: 25.97, 63.89, 64.57 (CH_2, C-7, 8, 9), 114.17, 124.63, 129.26 (C, C-aromatic), 152.78 (CH, C-2), 158.46, 164.63 (C, C-aromatic).

6.4.12.1 4-(2-(1-Phenylethylidene)hydrazinyl)-6,8-dihydro-5*H*-pyrano[4',3':4,5] thieno[2,3-*d*]pyrimidines (419-420)

2-(1-(2-(6,8-Dihydro-5*H*-pyrano[4',3':4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)hydrazono) ethyl)phenol (419)

($C_{17}H_{16}N_4O_2S$; M.W.= 340.4)



415 **202** **419**

General procedure 20;

Reagent: 4-hydrazinyl-6,8-dihydro-5*H*-pyrano[4',3':4,5]thieno[2,3-*d*]pyrimidine (**415**) (0.2 g, 0.9 mmol);

Yellow solid;

Yield: 0.29 g (94%)

Melting Point: 236-239°C

MS (ESI^+): 341.0 $[M+H]^+$

Microanalysis: Calculated for $C_{17}H_{16}N_4O_2S$ (340.4); Theoretical: %C = 59.98, %H = 4.74, %N = 16.45; Found: %C = 59.96, %H = 4.59, %N = 16.35.

Two species observed. Major/minor species ratio: 2:1.

^{1}H-NMR (DMSO-d_6), δ: (major species) 2.49 (s, 3H, H-2'), 3.05-3.09 (m, 2H, C<u>H</u>$_2$), 3.91-3.95 (m, 2H, C<u>H</u>$_2$), 4.75-4.77 (m, 2H, C<u>H</u>$_2$), 6.89-6.93 (m, 2H, H-aromatic), 7.26-7.31 (m, 1H, H-aromatic), 7.59-7.67 (m, 1H, H-aromatic), 7.78 (s, 1H, H-2), 11.51 (bs, 1H, N<u>H</u>), 12.46 (bs, 1H, O<u>H</u>).

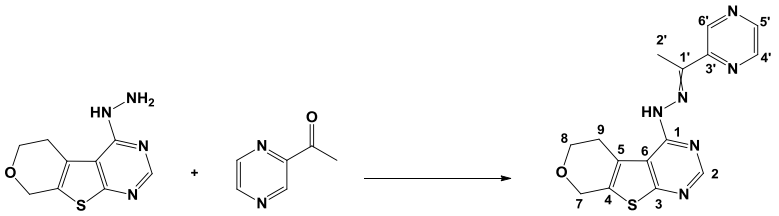
^{1}H-NMR (DMSO-d_6), δ: (minor species) 2.49 (s, 3H, H-2'), 3.25.3.29 (m, 2H, C<u>H</u>$_2$), 3.99-4.03 (m, 2H, C<u>H</u>$_2$), 4.84-4.87 (m, 2H, C<u>H</u>$_2$), 6.89-6.93 (m, 2H, H-aromatic), 7.26-7.31 (m, 1H, H-aromatic), 7.59-7.67 (m, 1H, H-aromatic), 8.59 (s, 1H, H-2), 9.48 (bs, 1H, N<u>H</u>), 13.30 (bs, 1H, O<u>H</u>).

^{13}C-NMR (DMSO-d_6), δ: (major and minor species) 14.84 (CH_3, C-2'), 25.54, 27.15, 64.11, 64.36 (CH_2, C-7, 8, 9), 112.85 (C, C-aromatic), 116.53, 117.29, 118.67 (CH, C-aromatic), 119.42, 120.93 (C, C-aromatic), 128.08 (CH, C-aromatic), 128.36 (C, C-

aromatic), 128.77 (CH, C-aromatic), 130.71 (C, C-aromatic), 130.43, 130.75 (CH, C-aromatic), 142.06 (C, C-aromatic), 144.44 (CH, C-aromatic), 145.40, 151.13, 158.22, 163.56, 168.71 (C, C-aromatic).

4-(2-(1-(Pyrazin-2-*yl*)ethylidene)hydrazinyl)-6,8-dihydro-5*H*-pyrano[4',3':4,5] thieno[2,3-*d*]pyrimidine (420)

($C_{15}H_{14}N_6S$; M.W.= 326.4)



415 **250** **420**

General procedure 20;

Reagent: 4-hydrazinyl-6,8-dihydro-5*H*-pyrano[4',3':4,5]thieno[2,3-*d*]pyrimidine (**415**) (0.2 g, 0.9 mmol);

Yellow solid;

Yield: 0.25 g (86%)

Melting Point: 219-223°C

MS (ESI^+): 327.0 $[M+H]^+$

Two species observed. Major/minor species ratio: 9:1.

^{1}H-NMR ($CDCl_3$), δ: (major species) 2.59 (s, 3H, H-2'), 3.21-3.25 (m, 2H, C<u>H</u>$_2$), 4.07 (t, J= 5.4 Hz, 2H, C<u>H</u>$_2$), 4.93-4.94 (m, 2H, C<u>H</u>$_2$), 7.77-7.78 (m, 1H, H-aromatic), 8.57-8.60 (m, 1H, H-aromatic), 9.01 (s, 1H, H-aromatic), 9.38 (s, 1H, H-aromatic), 10.45 (bs, 1H, N<u>H</u>).

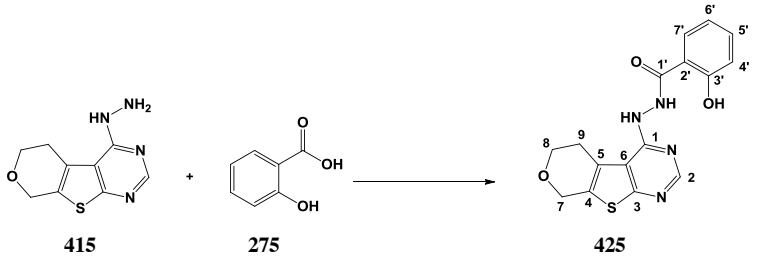
^{1}H-NMR ($CDCl_3$), δ: (minor species) 2.59 (s, 3H, H-2'), 3.30-3.34 (m, 2H, C<u>H</u>$_2$), 4.18 (t, J= 5.4 Hz, 2H, C<u>H</u>$_2$), 4.93-4.94 (m, 2H, C<u>H</u>$_2$), 8.57-8.60 (m, 1H, H-aromatic), 8.73-8.76 (m, 2H, H-aromatic), 9.01 (s, 1H, H-aromatic), 14.36 (bs, 1H, N<u>H</u>).

^{13}C-NMR ($CDCl_3$), δ: (major and minor species) 22.19 (CH_3, C-2'), 27.06, 27.23, 64.73, 64.95, 65.21, 65.52 (CH_2, C-7, 8, 9), 132.23, 135.11, 139.56 (C, C-aromatic), 140.89, 142.79, 143.25, 143.78, 144.44 (CH, C-aromatic), 144.96 (C, C-aromatic), 145.30 (CH, C-aromatic), 147.94, 151.82, 155.57, 157.44 (C, C-aromatic).

6.4.12.2 *N*'-(6,8-Dihydro-5*H*-pyrano[4',3':4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)aryl hydrazides (425-426)

_N_'-(6,8-Dihydro-5_H_-pyrano[4',3':4,5]thieno[2,3-_d_]pyrimidin-4-_yl_)-2-hydroxybenzo hydrazide (425)

($C_{16}H_{14}N_4O_3S$; M.W.= 342.4)



General procedure 22;

Reagent: 4-hydrazinyl-6,8-dihydro-5*H*-pyrano[4',3':4,5]thieno[2,3-*d*]pyrimidine (**415**) (0.3 g, 1.4 mmol);

Purification: recrystallization from EtOH/H_2O;

Light brown solid;

Yield: 0.11 g (23%)

Melting Point: 132-134°C

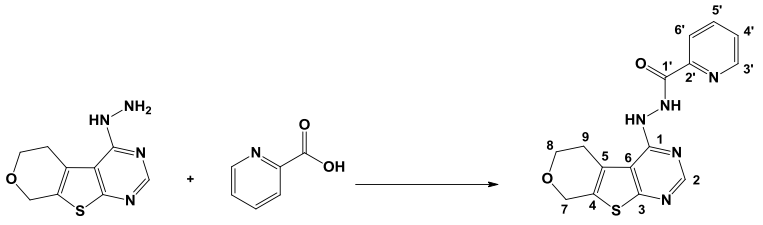
MS (ESI^+): 342.9 $[M+H]^+$

^{1}H-NMR (DMSO-d$_6$), δ: 3.12-3.16 (m, 2H, C<u>H</u>$_2$), 4.00 (t, J= 5.2 Hz, 2H, H-9), 4.83-4.85 (m, 2H, C<u>H</u>$_2$), 6.96-7.00 (m, 2H, H-aromatic), 7.45-7.49 (m, 1H, H-aromatic), 8.00 (d, J= 7.8 Hz, 1H, H-aromatic), 8.41 (s, 1H, H-2), 8.93 (bs, 1H, N<u>H</u>), 10.87 (bs, 1H, N<u>H</u>), 11.95 (bs 1H, O<u>H</u>).

^{13}C-NMR (DMSO-d$_6$), δ: 26.24, 63.84, 64.62 (CH_2, C-7, 8, 9), 114.59, 114.77 (C, C-aromatic), 117.35, 119.04 (CH, C-aromatic), 124.27 (C, C-aromatic), 128.37 (CH, C-aromatic), 130.87 (C, C-aromatic), 134.05, 152.68 (CH, C-aromatic), 156.84, 159.19, 165.89 (C, C-aromatic), 167.56 (C, C-1').

***N*'-(6,8-Dihydro-5*H*-pyrano[4',3':4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)picolino hydrazide (426)**

($C_{15}H_{13}N_5O_2S$; M.W.= 327.4)



415 **277** **426**

General procedure 22;

Reagent: 4-hydrazinyl-6,8-dihydro-5*H*-pyrano[4',3':4,5]thieno[2,3-*d*]pyrimidine (**415**) (0.3 g, 1.4 mmol);

Purification: re-crystallisation from EtOH;

White solid;

Yield: 0.37 g (83%)

Melting Point: 220-222°C

MS (ESI^+): 327.9 $[M+H]^+$

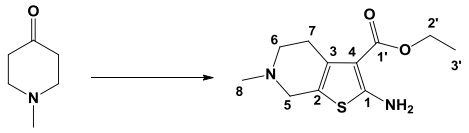
^{1}H-NMR (DMSO-d_6), δ: 3.13 (t, J= 5.4 Hz, 2H, C<u>H</u>$_2$), 3.99 (t, J= 5.4 Hz, 2H, C<u>H</u>$_2$), 4.84 (s, 2H, H-7), 7.66-7.69 (m, 1H, H-aromatic), 8.03-8.08 (m, 2H, H-aromatic), 8.36 (s, 1H, H-2), 8.72-8.74 (m, 1H, H-aromatic), 8.88 (bs, 1H, N<u>H</u>), 10.71 (bs, 1H, N<u>H</u>).

^{13}C-NMR (DMSO-d_6), δ: 26.32, 63.86, 64.33 (CH_2, C-7, 8, 9), 114.64 (C, C-aromatic), 122.31 (CH, C-aromatic), 124.35 (C, C-aromatic), 126.95 (CH, C-aromatic), 130.60 (C, C-aromatic), 137.85, 148.68 (CH, C-aromatic), 149.34 (C, C-aromatic), 152.65 (CH, C-aromatic), 157.05, 162.99 (C, C-aromatic), 166.31 (C, C-1').

6.4.13 7-Methyl-5,6,7,8-Tetrahydropyrido [4',3':4,5]thieno[2,3-*d*]pyrimidines

Ethyl 2-amino-6-methyl-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxylate (407)[67]

($C_{11}H_{16}N_2O_2S$; M.W.= 240.3)



404 **407**

General procedure 16;

Reagent: N-methyl-4-piperidone (**404**) (1.5 g, 13.3 mmol);

Morpholine used instead of NEt_3;

T.L.C. System: EtOAc-MeOH 9:1 v/v, Rf: 0.44.

Purification: recrystallization from 60% EtOH/H_2O;

Yellow solid;

Yield: 2.25 g (71%)

^{1}H-NMR ($CDCl_3$), δ: 1.34 (t, J= 7.1 Hz, 3H, H-3'), 2.45 (s, 3H, H-8), 2.67 (t, J= 5.8 Hz, 2H, H-7),2.85 (dd, J_1= 5.8 Hz, J_2= 1.9 Hz, 2H, H-6), 3.38 (t, J= 1.9 Hz, 2H, H-5), 4.27 (q, J= 7.1 Hz, 2H, H-2'), 6.01 (bs, 2H, $N\underline{H}_2$).

^{13}C-NMR ($CDCl_3$), δ: 14.44 (CH_3, C-3'), 27.40 (CH_2, C-aliphatic), 45.49 (CH_3, C-8), 52.44, 53.32, 59.44 (CH_2, C-aliphatic), 105.40, 114.60, 130.72, 162.07 (C, C-aromatic), 165.95 (C, C-1').

7-Methyl-5,6,7,8-tetrahydropyrido[4',3':4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (410)[68]

($C_{10}H_{11}N_3OS$; M.W.= 221.3)

407 **410**

General procedure 17a;

Reagent: ethyl 2-amino-6-methyl-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxylate (**407**) (0.51g, 2.1 mmol);

After drying under high vacuum, the crude reaction residue was used for the next step without further purification.

Brown oil;

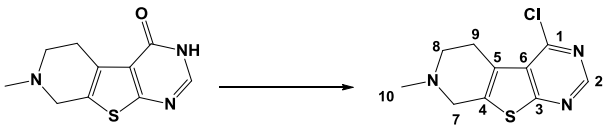
MS (ESI)$^+$: 221.9 $[M+H]^+$

^{1}H-NMR (DMSO-d$_6$), δ: 2.36 (s, 3H, H-10), 2.65 (t, J= 5.8 Hz, 2H, H-9), 2.93 (tt, J_1= 5.8 Hz, J_2= 1.7 Hz, 2H, H-8), 3.54 (t, J= 1.7Hz, 2H, H-7), 8.07 (s, 1H, H-2), 12.28 (bs, 1H, NH).

^{13}C-NMR (DMSO-d$_6$), δ: 25.71 (CH_2, C-aliphatic), 44.93 (CH_3, C-10), 51.17, 52.88 (CH_2, C-aliphatic), 122.28, 128.89, 129.70 (C, C-aromatic), 145.14 (CH, C-2), 157.63 (C, C-aromatic), 162.81 (C, C-1).

4-Chloro-7-methyl-5,6,7,8-tetrahydropyrido[4',3':4,5]thieno[2,3-*d*]pyrimidine (413)[68]

($C_{10}H_{10}ClN_3S$; M.W.= 239.7)



410 **413**

General procedure 14;

Reagent: 7-methyl-5,6,7,8-tetrahydropyrido[4',3':4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (**410**) (0.45 g, 2.1 mmol);

2 equivalents of NEt_3 added;

T.L.C. System: EtOAc-MeOH 9:1 v/v, Rf: 0.38.

Purification: flash column chromatography (EtOAc:MeOH 100:0 v/v increasing to EtOAc-MeOH 94:6 v/v);

White solid;

Yield: 0.25 g (49%)

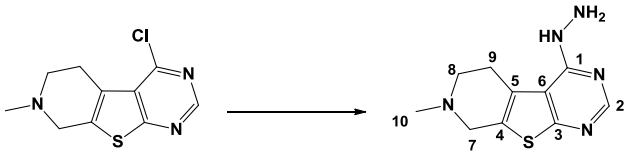
MS (ESI)$^+$: 239.9, 241.9 $[M+H]^+$

^{1}H-NMR ($CDCl_3$), δ: 2.55 (s, 3H, H-10), 2.85 (t, J= 5.8 Hz, 2H, H-9), 3.29 (tt, J_1= 5.8 Hz, J_2= 1.9 Hz, 2H, H-8), 3.75 (t, J= 1.9 Hz, 2H, H-7), 8.76 (s, 1H, H-2).

^{13}C-NMR ($CDCl_3$), δ: 26.74 (CH_2, C-aliphatic), 45.41 (CH_3, C-10), 51.75, 54.19 (CH_2, C-aliphatic), 125.44, 128.26, 136.79 (C, C-aromatic), 151.96 (CH, C-2), 153.46, 168.98 (C, C-aromatic).

4-Hydrazinyl-7-methyl-5,6,7,8-tetrahydropyrido[4',3':4,5]thieno[2,3-*d*]pyrimidine (416)

($C_{10}H_{13}N_5S$; M.W.= 235.3)



413 **416**

General procedure 19;

Reagent: 4-chloro-7-methyl-5,6,7,8-tetrahydropyrido[4',3':4,5]thieno[2,3-*d*]pyrimidine (**413**) (0.25 g, 1.1 mmol);

Purification: recrystallization from MeOH;

Yellow crystals;

Yield: 1.56 g (62%)

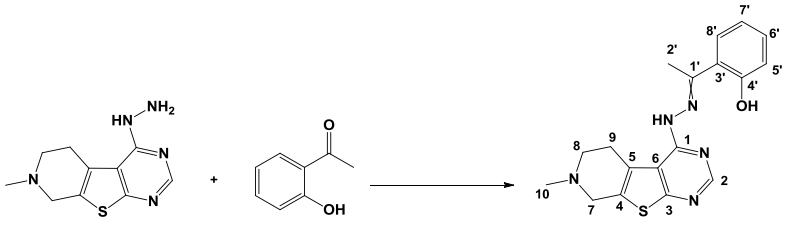
^{1}H-NMR (DMSO-d_6), δ: 2.37 (s, 3H, H-10), 2.67 (t, J= 5.7 Hz, 2H, C$\underline{H}_2$), 3.01 (t, J= 5.7 Hz, 2H, C$\underline{H}_2$), 3.47 (s, 2H, H-7), 4.57 (bs, 2H, N$\underline{H}_2$), 7.95 (bs, 1H, N$\underline{H}$), 8.35 (s, 1H, H-2).

^{13}C-NMR (DMSO-d_6), δ: 25.75 (CH_2, C-aliphatic), 44.84 (CH_3, C-10), 51.31, 53.31 (CH_2, C-aliphatic), 114.22, 125.07, 129.12 (C, C-aromatic), 152.70 (CH, C-2), 158.32, 164.24 (C, C-aromatic).

6.4.13.1 7-Methyl-4-(2-(1-phenylethylidene)hydrazinyl)-5,6,7,8-tetrahydropyrido [4',3':4,5]thieno[2,3-*d*]pyrimidines (421-422)

2-(1-(2-(7-Methyl-5,6,7,8-tetrahydropyrido[4',3':4,5]thieno[2,3-*d*]pyrimidin-4-*yl*) hydrazono)ethyl)phenol (421)

($C_{18}H_{19}N_5OS$; M.W.= 353.4)



416 **202** **421**

General procedure 20;

Reagent: 4-hydrazinyl-7-methyl-5,6,7,8-tetrahydropyrido[4',3':4,5]thieno[2,3-*d*] pyrimidine (**416**) (0.08 g, 0.3 mmol);

Light brown solid;

Yield: 0.03 g (23%)

Melting Point: 198-202°C

MS (ESI^+): 354.0 $[M+H]^+$

Two species observed. Major/minor species ratio: 3/1.

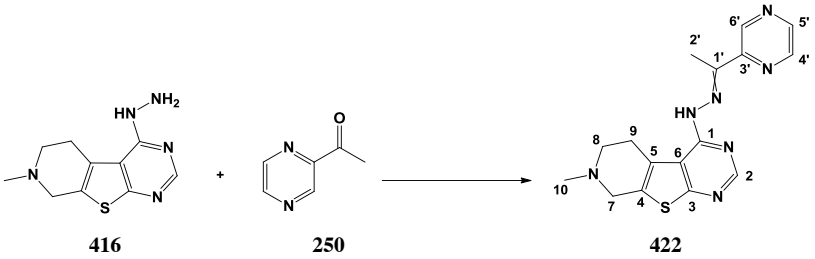
^{1}H-NMR (DMSO-d_6), δ: (major species) 2.42 (s, 3H, C<u>H</u>$_3$), 2.52 (s, 3H, C<u>H</u>$_3$), 2.77 (t, J= 5.2 Hz, 2H, C<u>H</u>$_2$), 3.10-3.19 (m, 2H, C<u>H</u>$_2$), 3.64-3.67 (m, 2H, C<u>H</u>$_2$), 6.89-6.93 (m, 1H, H-aromatic), 7.27-7.31 (m, 1H, H-aromatic), 7.63 (d, J= 7.6 Hz, 1H, H-aromatic), 7.81 (s, 1H, H-2), 8.50 (bs, 1H, N<u>H</u>), 11.97 (bs, 1H, O<u>H</u>).

^{1}H-NMR (DMSO-d_6), δ: (minor species) 2.42 (s, 3H, C<u>H</u>$_3$), 2.52 (s, 3H, C<u>H</u>$_3$), 2.77 (t, J= 5.2 Hz, 2H, C<u>H</u>$_2$), 3.10-3.19 (m, 2H, C<u>H</u>$_2$), 3.64-3.67 (m, 2H, C<u>H</u>$_2$), 6.89-6.93 (m, 1H, H-aromatic), 7.27-7.31 (m, 1H, H-aromatic), 7.63 (d, J= 7.6 Hz, 1H, H-aromatic), 7.81 (s, 1H, H-2), 9.48 (bs, 1H, N<u>H</u>), 13.26 (bs, 1H, O<u>H</u>).

^{13}C-NMR (DMSO-d_6), δ: (major and minor species) 39.86, 44.70 (CH_3, C-10, 2'), 51.32, 53.05, 55.99 (CH_2, C-7, 8, 9), 114.3 (C, C-aromatic), 116.78, 118.61 (CH, C-aromatic), 120.73, 122.94, 125.62 (C, C-aromatic), 127.21, 128.55, 130.77 (CH, C-aromatic), 138.34, 143.06, 153.52, 158.30 (C, C-aromatic).

7-Methyl-4-(2-(1-(pyrazin-2-*yl*)ethylidene)hydrazinyl)-5,6,7,8-tetrahydropyrido [4',3':4,5]thieno[2,3-*d*]pyrimidine (422)

($C_{16}H_{17}N_7S$; M.W.= 339.4)



416 **250** **422**

General procedure 20;

Reagent: 4-hydrazinyl-7-methyl-5,6,7,8-tetrahydropyrido[4',3':4,5]thieno[2,3-*d*] pyrimidine (**416**) (0.08 g, 0.3 mmol);

Yellow solid;

Yield: 0.205 g (53%)

Melting Point: 201-203 °C

MS (ESI^+): 340.0 $[M+H]^+$

Single species observed.

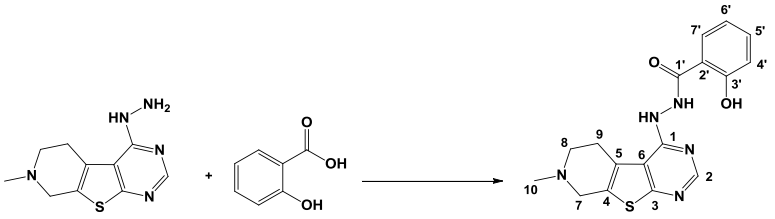
^{1}H-NMR ($CDCl_3$), δ: 2.38 (s, 3H, C<u>H</u>$_3$), 2.47 (s, 3H, C<u>H</u>$_3$), 2.69 (t, J= 5.7 Hz, 2H, C<u>H</u>$_2$), 3.10 (t, J= 5.7 Hz, 2H, C<u>H</u>$_2$), 3.57 (s, 2H, H-7), 7.88 (s, 1H, H-2), 8.58 (d, J= 2.6 Hz, 1H, H-4'), 8.62 (dd, J_1= 2.6 Hz, J_2= 1.5 Hz, 1H, H-5'), 9.79 (d, J= 1.5 Hz, 1H, H-6'), 11.98 (bs, 1H, N<u>H</u>).

^{13}C-NMR ($CDCl_3$), δ: 12.61 (CH_3, C-aliphatic), 26.76 (CH_2, C-aliphatic), 44.96 (CH_3, C-aliphatic), 51.47, 53.11 (CH_2, C-aliphatic), 119.22, 128.95, 130.53 (C, C-aromatic), 143.21, 143.30, 143.52, 144.11 (CH, C-aromatic), 148.72, 151.21, 157.06, 158.33 (C, C-aromatic).

6.4.13.2 *N*'-(7-Methyl-5,6,7,8-tetrahydropyrido[4',3':4,5]thieno[2,3-*d*]pyrimidin-4-*yl*) benzohydrazide (427-428)

2-Hydroxy-*N*'-(7-methyl-5,6,7,8-tetrahydropyrido[4',3':4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)benzohydrazides (427)

($C_{17}H_{17}N_5O_2S$; M.W.= 355.4)



416 **275** **427**

General procedure 22;

Reagent: 4-hydrazinyl-7-methyl-5,6,7,8-tetrahydropyrido[4',3':4,5]thieno[2,3-*d*] pyrimidine (**416**) (0.10 g, 0.4 mmol);

Purification: recrystallization from MeOH;

White solid;

Yield: 0.05 g (30%)

Melting Point: charring > 240°C

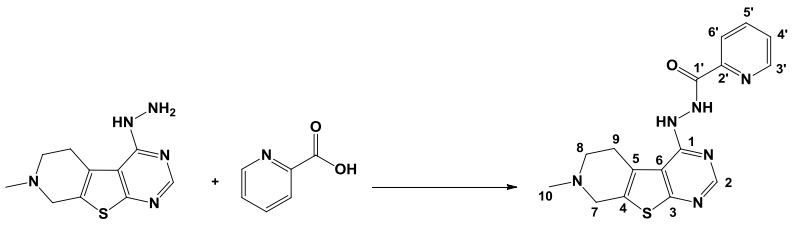
MS (ESI^+): 356.0 $[M+H]^+$

^{1}H-NMR (DMSO-d_6), δ: 2.42 (s, 3H, H-10), 2.75-2.79 (m, 2H, C<u>H</u>$_2$), 3.10-3.15 (m, 2H, C<u>H</u>$_2$), 3.67 (s, 2H, H-7), 6.99-6.99 (m, 2H, H-aromatic), 7.44-7.49 (m, 1H, H-aromatic), 7.97-8.01 (m, 1H, H-aromatic), 8.38 (s, 1H, H-2), 8.89 (bs, 1H, N<u>H</u>), 11.39 (bs, 2H, N<u>H</u>, O<u>H</u>).

^{13}C-NMR (DMSO-d_6), δ: 25.96 (CH_2, C-aliphatic), 44.76 (CH_3, C-10), 51.18, 53.28 (CH_2, C-aliphatic), 114.88, 114.97 (C, C-aromatic), 117.33, 118.98 (CH, C-aromatic), 124.48 (C, C-aromatic), 128.33 (CH, C-aromatic), 130.53 (C, C-aromatic), 133.95, 151.93 (CH, C-aromatic), 156.98, 159.22, 165.96 (C, C-aromatic), 168.02 (C, C-1').

***N*'-(7-Methyl-5,6,7,8-tetrahydropyrido[4',3':4,5]thieno[2,3-*d*]pyrimidin-4-*yl*) picolinohydrazide (428)**

($C_{16}H_{16}N_6OS$; M.W.= 340.4)



416 **277** **428**

General procedure 22;

Reagent: 4-hydrazinyl-7-methyl-5,6,7,8-tetrahydropyrido[4',3':4,5]thieno[2,3-*d*] pyrimidine (**416**) (0.10 g, 0.4 mmol);

Purification: re-crystallisation from EtOH;

Orange solid;

Yield: 0.06 g (41%)

Melting Point: charring > 200°C

MS (ESI^+): 341.0 $[M+H]^+$

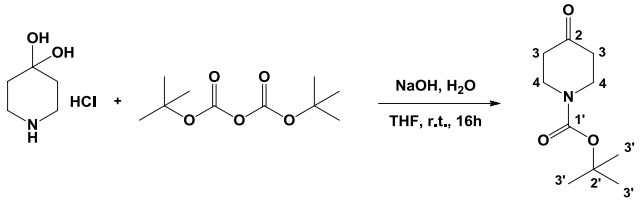
^{1}H-NMR (DMSO-d_6), δ: 2.41 (s, 3H, H-10), 2.75 (t, J= 5.5 Hz, 2H, C$\underline{H}_2$), 3.10-3.13 (m, 2H, C$\underline{H}_2$), 3.64 (s, 2H, H-7), 7.66-7.69 (m, 1H, H-aromatic), 8.02-8.08 (m, 2H, H-aromatic), 8.33 (s, 1H, H-2), 8.72-8.74 (m, 1H, H-aromatic), 8.85 (bs, 1H, N$\underline{H}$), 10.71 (bs, 1H, N$\underline{H}$).

^{13}C-NMR (DMSO-d_6), δ: 26.06 (CH_2, C-aliphatic), 44.81 (CH_3, C-10), 51.23, 53.32 (CH_2, C-aliphatic), 114.74 (C, C-aromatic), 122.30 (CH, C-aromatic), 124.83 (C, C-aromatic), 126.93 (CH, C-aromatic), 130.35 (C, C-aromatic), 137.84, 148.68 (CH, C-aromatic), 149.37 (C, C-aromatic), 152.46 (CH, C-aromatic), 157.12, 162.91 (C, C-aromatic), 166.27 (C, C-1').

6.4.14 5,6,7,8-Tetrahydropyrido[4',3':4,5]thieno [2,3-*d*]pyrimidines

tert-Butyl 4-oxopiperidine-1-carboxylate (430)[69]

($C_{10}H_{17}NO_3$; M.W.= 199.3)



429 **77** **430**

To a solution of 4-piperidone hydrate hydrochloride (**429**) (1 g, 6.5 mmol) in H_2O (10 mL) was added NaOH (0.29 g, 7.2 mmol), di-*tert*-butyl dicarbonate (**77**) (1.4 g, 6.5 mmol) and THF (10 mL). The reaction mixture was stirred at r.t. for 16 h, then extracted with diethyl ether (3 x 30 mL). The combined organic layers were washed with brine (40 mL), dried over $MgSO_4$ and concentrated under reduced pressure to give *tert*-butyl 4-oxopiperidine-1-carboxylate (**430**) as a waxy solid.

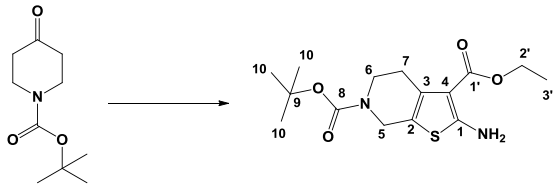
Yield: 1.2 g (98%)

^{1}H-NMR ($CDCl_3$), δ: 1.51 (s, 9H, H-3'), 2.45 (t, J= 6.2 Hz, 4H, C<u>H</u>$_2$), 3.73 (t, J= 6.2 Hz, 4H, C<u>H</u>$_2$).

^{13}C-NMR ($CDCl_3$), δ: 28.37 (CH_3, C-3'), 41.16, 42.99 (CH_2, C-aliphatic), 80.46 (C, C-2'), 154.50 (C, C-2), 207.79 (C, C-1').

6-_tert_-Butyl 3-ethyl 2-amino-4,5-dihydrothieno[2,3-*c*]pyridine-3,6(7*H*)-dicarboxylate (431)[30]

($C_{15}H_{22}N_2O_4S$; M.W.= 324.4)



430 **431**

General procedure 16;

Reagent: *tert*-butyl 4-oxopiperidine-1-carboxylate (**430**) (1.4 g, 7.1 mmol);

The reaction mixture was cooled to r.t. and the precipitate formed was collected by filtration and recrystallized from EtOH.

Pale yellow solid;

Yield: 1.4 g (61%)

^{1}H-NMR (CDCl$_3$), δ: 1.35 (t, J= 7.1 Hz, 3H, H-3'), 1.50 (s, 9H, H-10), 2.80-2.84 (m, 2H, C<u>H</u>$_2$), 3.63 (t, J= 5.7 Hz, 2H, H-7), 4.28 (q, J= 7.1 Hz, 2H, H-2'), 4.36 (s, 2H, H-5), 6.04 (bs, 2H, N<u>H</u>$_2$).

^{13}C-NMR (CDCl$_3$), δ: 14.14(CH$_3$, C-3'), 27.18 (CH$_2$, C-aliphatic), 28.46 (CH$_3$, C-10), 40.79, 59.58 (CH$_2$, C-aliphatic), 79.97 (C, C-9), 104.83, 113.71, 131.22, 154.66 (C, C-aromatic), 162.31, 165.81 (C, C-8, 1').

***tert*-Butyl 4-oxo-3,4,5,6-tetrahydropyrido[4',3':4,5]thieno[2,3-*d*]pyrimidine-7(8*H*)-carboxylate (432)[30]**

(C$_{14}$H$_{17}$N$_3$O$_3$S; M.W.= 307.4)

431 **432**

General procedure 17a;

Reagent: 6-*tert*-butyl 3-ethyl 2-amino-4,5-dihydrothieno[2,3-*c*]pyridine-3,6(7*H*)-dicarboxylate (**432**) (1.4, 4.3 mmol);

Yellow solid;

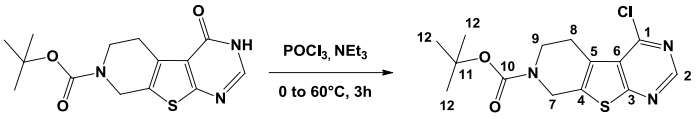
Yield: 1.33 g (83%).

^{1}H-NMR (DMSO-d$_6$), δ: 1.43 (s, 9H, H-12), 2.93 (t, J= 5.7 Hz, 2H, C<u>H</u>$_2$), 3.62 (t, J= 5.7 Hz, 2H, C<u>H</u>$_2$), 4.58 (s, 2H, H-7), 8.04 (s, 1H, H-2), 12.42 (bs, 1H, N<u>H</u>).

^{13}C-NMR (DMSO-d$_6$), δ: 25.42 (CH$_2$, C-aliphatic), 28.00 (CH$_3$, C-11), 40.55, 42.31 (CH$_2$, C-aliphatic), 79.38 (C, C-11), 108.46, 122.16, 129.49 (C, C-aromatic), 154.28 (CH, C-2), 155.86, 157.57 (C, C-aromatic), 165.31 (C, C-1).

***tert*-Butyl 4-chloro-5,6-dihydropyrido[4',3':4,5]thieno[2,3-*d*]pyrimidine-7(8*H*)-carboxylate (433)[30]**

($C_{14}H_{16}ClN_3O_2S$; M.W.= 325.8)



432 **433**

To a mixture of $POCl_3$ (3 mL) and NEt_3 (3 mL) at 0°C was added intermediate *tert*-butyl 4-oxo-3,4,5,6-tetrahydropyrido[4',3':4,5]thieno[2,3-d]pyrimidine-7(8H)-carboxylate (**432**) (1.1 g, 3.6 mmol). The reaction mixture was heated at 60 °C for 3 h, then cooled in an ice-bath and carefully neutralised with sat. Na_2CO_3 solution. The mixture was extracted with DCM (50 mL), the organic layer separated, dried over $MgSO_4$ and concentrated under vacuum. The residue was purified by flash column chromatography (*n*hexane -EtOAc: 100:0 v/v increasing to *n*hexane -EtOAc: 85:15 v/v) to give *tert*-butyl 4-chloro-5,6-dihydropyrido[4',3':4,5]thieno[2,3-*d*]pyrimidine-7(8*H*)-carboxylate (**433**) as a white solid.

T.L.C. System: *n*hexane-EtOAc 7:3 v/v, Rf: 0.48.

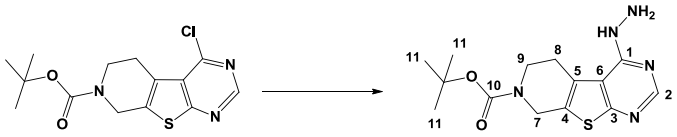
Yield: 1.26 g (96%)

^{1}H-NMR ($CDCl_3$), δ: 1.52 (s, 9H, H-11), 3.22 (t, J= 5.7 Hz, 2H, C<u>H</u>$_2$), 3.81 (t, J_1= 5.7 Hz, 2H,), 4.76 (s, 2H, H-7), 8.78 (s, 1H, H-2).

^{13}C-NMR ($CDCl_3$), δ: 26.51 (CH_2, C-aliphatic), 28.41 (CH_3, C-12), 41.12, 42.55 (CH_2, C-aliphatic), 78.99 (C, C-11), 125.11, 128.19, 133.24 (C, C-aromatic), 152.17 (CH, C-2), 155.47, 169.08 (C, C-aromatic).

***tert*-Butyl 4-hydrazinyl-5,6-dihydropyrido[4',3':4,5]thieno[2,3-*d*]pyrimidine-7(8*H*)-carboxylate (436)**

($C_{14}H_{19}N_5O_2S$; M.W.= 321.4)



433 **436**

General procedure 19;

Reagent: *tert*-butyl 4-chloro-5,6-dihydropyrido[4',3':4,5]thieno[2,3-*d*]pyrimidine-7(8*H*)-carboxylate (**433**) (0.23 g, 0.7 mmol);

Purification: recrystallization from 40% EtOH/H_2O;

Pale yellow crystals;

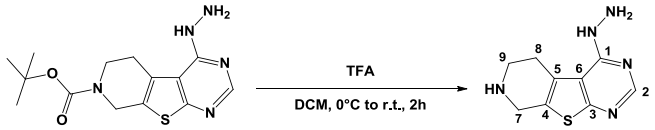
Yield: 0.11 g (46%)

^{1}H-NMR (DMSO-d$_6$), δ: 1.44 (s, 3H, H-11), 3.01 (t, J= 5.7 Hz, 2H, C$\underline{H}_2$), 3.63 (t, J= 5.7 Hz, 2H, C$\underline{H}_2$), 4.58-4.62 (bs, 4H, C$\underline{H}_2$, N$\underline{H}_2$), 8.03 (bs, 1H, N$\underline{H}$), 8.36 (s, 1H, H-2).

^{13}C-NMR (DMSO-d$_6$), δ: 25.75 (CH_2, C-aliphatic), 28.00 (CH_3, C-11), 79.46 (C, C-10), 114.17, 125.82 (C, C-aromatic), 152.90 (CH, C-2), 158.71, 164.12 (C, C-aromatic).

4-Hydrazinyl-5,6,7,8-tetrahydropyrido[4',3':4,5]thieno[2,3-*d*]pyrimidine (435)

($C_9H_{11}N_5S$; M.W.= 221.3)



436 **435**

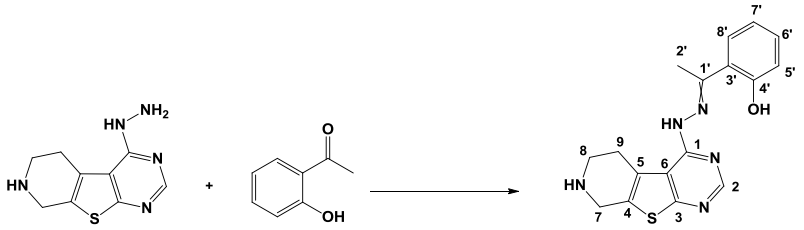
To a mixture of intermediate *tert*-butyl 4-hydrazinyl-5,6-dihydropyrido[4',3':4,5] thieno[2,3-*d*]pyrimidine-7(8*H*)-carboxylate (**436**) (0.1 g, 0.3 mmol) in DCM (2 mL) at 0°C was added TFA (1 mL) and then warmed to r.t. The reaction mixture was stirred for 2 h, then dried under vacuum. The mixture was neutralised by slow addition of sat. $NaHCO_3$ solution, then dried under vacuum. The resulting residue was added of MeOH and filtered. The filtrate was finally dried under vacuum to give crude 4-hydrazinyl-5,6,7,8-tetrahydropyrido[4',3':4,5]thieno[2,3-*d*]pyrimidine (**435**) as a brown oil which was used for the last reaction step without purification.

MS $(ESI)^+$: 221.9 $[M+H]^+$

6.4.14.1 4-(2-(1-Phenylethylidene)hydrazinyl)-5,6,7,8-tetrahydropyrido[4',3':4,5] thieno [2,3-*d*]pyrimidine (437-438)

2-(1-(2-(5,6,7,8-Tetrahydropyrido[4',3':4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)hydrazono) ethyl)phenol (437)

($C_{17}H_{17}N_5OS$; M.W.= 339.4)



General procedure 20;

Reagent: 4-hydrazinyl-5,6,7,8-tetrahydropyrido[4',3':4,5]thieno[2,3-*d*]pyrimidine (**435**) (0.07 g, 0.5 mmol);

Yellow solid;

Yield: 0.02 g (23%)

Melting Point: 241-244°C

MS (ESI^+): 340.0 $[M+H]^+$

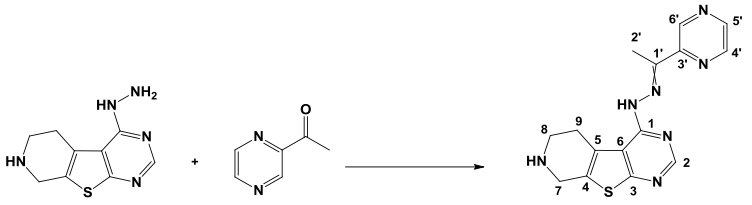
Single species observed.

^{1}H-NMR (DMSO-d_6), δ: 2.50 (s, 3H, H-2'), 3.04-3.09 (m, 4H, C<u>H</u>$_2$), 3.10-3.19 (m, 4H, C<u>H</u>$_2$), 3.94 (s,2H, H-7), 6.84-7.03 (m, 2H, H-aromatic), 7.26-7.31 (m, 1H, H-aromatic), 7.61-7.65 (m, 1H, H-aromatic), 8.04 (s, 2H, H-2).

^{13}C-NMR (DMSO-d_6), δ: 14.13 (CH_3, C-7), 26.73, 42.27, 44.07 (CH_2, C-7, 8, 9), 114.37 (C, C-aromatic), 116.80, 118.02 (CH, C-aromatic), 118.52, 123.07, 128.17 (C, C-aromatic), 128.42, 130.66 (CH, C-aromatic), 131.99, 143.43 (C, C-aromatic), 152.61 (CH, C-aromatic), 153.78, 158.42 (C, C-aromatic).

4-(2-(1-(Pyrazin-2-*yl*)ethylidene)hydrazinyl)-5,6,7,8-tetrahydropyrido[4',3':4,5] thieno[2,3-*d*]pyrimidine (438)

($C_{15}H_{15}N_7S$; M.W.= 325.4)



435 **250** **438**

General procedure 20;

Reagent: 4-hydrazinyl-5,6,7,8-tetrahydropyrido[4',3':4,5]thieno[2,3-*d*]pyrimidine (**435**) (0.07 g, 0.5 mmol);

Yellow solid;

Yield: 0.03 g (10%)

Melting Point: charring > 300°C

MS (ESI^+): 340.0 $[M+H]^+$

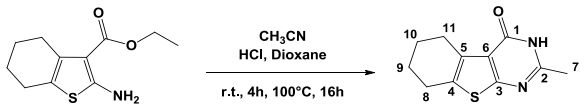
Single species observed.

^{1}H-NMR (DMSO-d_6), δ: 2.48 (s, 3H, C<u>H</u>$_3$), 3.32 (t, J= 6.1 Hz, 2H, C<u>H</u>$_2$), 3.44 (t, J= 6.1 Hz, 2H, C<u>H</u>$_2$), 34.39 (s, 2H, H-7), 7.96 (ds, 1H, H-2), 8.61 (d, J= 2.5 Hz, 1H, H-4'), 8.64 (dd, J_1= 2.5 Hz, J_2= 1.5 Hz, 1H, H-5'), 9.63 (bs, 1H, N<u>H</u>), 9.82 (d, J= 1.5 Hz, 1H, H-6'), 12.15 (bs, 1H, N<u>H</u>).

^{13}C-NMR (DMSO-d_6), δ: 12.71 (CH_3, C-aliphatic), 23.51, 40.34, 41.40 (CH_2, C-aliphatic), 118.60, 124.76, 128.28 (C, C-aromatic), 143.27, 143.36, 143.75, 145.11 (CH, C-aromatic), 148.46, 151.01, 157.69, 159.41 (C, C-aromatic).

6.4.15 2-Methyl-5,6,7,8-tetrahydro[1]benzothieno[2,3-*d*]pyrimidines

2-Methyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (439)[61] ($C_{11}H_{12}N_2OS$; M.W.= 220.3)



190 **439**

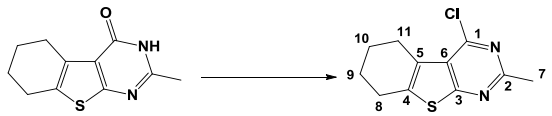
Intermediate ethyl 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (**190**) (0.86 g, 3.8 mmol) and acetonitrile (0.2 mL, 3.8 mmol) were placed in a pressure tube and saturated HCl solution in dioxane (8 mL) was added dropwise. The tube was carefully sealed and left at r.t. in ultrasonic bath for 4 h, and then heated at 100°C with stirring for 16 h. The reaction mixture was then cooled to r.t. and poured into water (70 mL). The precipitate formed was filtered and washed with water and *n*hexane to give 2-methyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (439) as a white solid.

Yield: 0.34 g (40%)

^{1}H-NMR (DMSO-d$_6$), δ: 1.72-1.77 (m, 2H, C$\underline{H}_2$), 1.77-1.82 (m, 2H, C$\underline{H}_2$), 2.31 (s, 3H, H-7), 2.70 (t, J= 5.8 Hz, 2H, C$\underline{H}_2$), 2.84 (t, J= 5.8 Hz, 2H, C$\underline{H}_2$), 12.18 (bs, 1H, N$\underline{H}$).

^{13}C-NMR (DMSO-d$_6$), δ: 20.78 (CH_3, C-7), 21.76, 22.49, 24.32, 25.23 (CH_2, C-8, 9, 10, 11), 120.13, 130.47, 130.69, 154.14, 158.44 (C, C-aromatic), 163.06 (C, C-1).

4-Chloro-2-methyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidine (440)[62] ($C_{11}H_{11}ClN_2O_2S$; M.W.= 256.7)



439 **440**

General procedure 18;

Reagent: 2-methyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (**439**) (0.4 g, 1.8 mmol);

T.L.C. System: *n*hexane-EtOAc 7:3 v/v, Rf: 0.69.

Purification: flash column chromatography (*n*hexane:EtOAc 100:0 v/v increasing to *n*hexane-EtOAc 85:15 v/v);

Light brown solid;

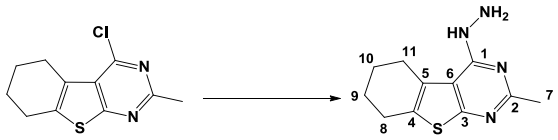
Yield: 0.31 g (72%)

^{1}H-NMR ($CDCl_3$), δ: 1.91-1.94 (m, 4H, C<u>H</u>$_2$), 2.77 (s, 3H, H-7), 2.85-2.88 (m, 2H, C<u>H</u>$_2$), 3.06-3.09 (m, 2H, C<u>H</u>$_2$).

^{13}C-NMR ($CDCl_3$), δ: 22.26, 22.53 (CH_2, C-aliphatic), 25.26 (CH_3, C-7), 25.93, 26.27 (CH_2, C-aliphatic), 126.14, 126.94, 137.90, 152.83, 161.76, 169.50.

4-Hydrazinyl-2-methyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidine (441)[63]

($C_{11}H_{14}N_4S$; M.W.= 234.3)



440 **441**

General procedure 19;

Reagent: 4-chloro-2-methyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidine (**440**) (0.23 g, 1 mmol);

Purification: recrystallization from 40 % EtOH/H_2O;

Yellow crystals;

Yield: 0.15 g (67%)

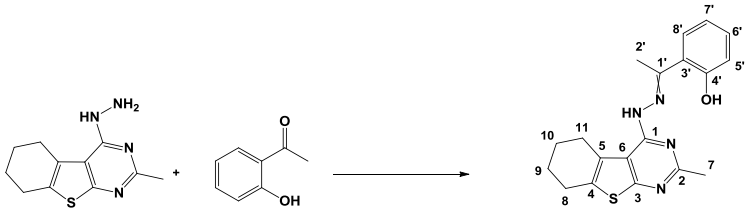
^{1}H-NMR (DMSO-d_6), δ: 1.77-1.80 (m, 4H, C<u>H</u>$_2$), 2.25 (s, 3H, H-7), 2.71-2.74 (m, 2H, C<u>H</u>$_2$), 2.87-2.90 (m, 2H, C<u>H</u>$_2$), 4.54 (bs, 2H, N<u>H</u>$_2$), 7.75 (bs, 1H, N<u>H</u>).

^{13}C-NMR (DMSO-d_6), δ: 22.03, 22.19, 24.77 (CH_2, C-aliphatic), 25.34 (CH_3, C-7), 25.46 (CH_2, C-aliphatic), 112.49, 126.54, 130.06, 158.19, 161.08, 164.61 (C, C-aromatic).

6.4.15.1 2-Methyl-4-(2-(1-arylethylidene)hydrazinyl)-5,6,7,8-tetrahydrobenzo[4,5] thieno[2,3-*d*]pyrimidines (442-443)

2-(1-(2-(2-Methyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*) hydrazono)ethyl)phenol (442)

($C_{19}H_{20}N_4OS$; M.W.= 352.5)



General procedure 20;

Reagent: 4-hydrazinyl-2-methyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidine (**441**) (0.05 g, 0.2 mmol);

Pale yellow solid;

Yield: 0.06 g (86%)

Melting Point: 257-260°C

MS (ESI^+): 353.0 $[M+H]^+$

Two species observed. Major/minor species ratio: 5/4.

^{1}H-NMR (DMSO-d_6), δ: (major species) 1.77-1.82 (m, 4H, C$\underline{H}_2$), 2.33 (s, 3H, C$\underline{H}_3$), 2.49 (s, 3H, C$\underline{H}_3$), 2.72-2.75 (m, 2H, C$\underline{H}_2$), 2.97-3.01 (m, 2H, C$\underline{H}_2$), 6.88-6.96 (m, 2H, H-aromatic), 7.26-7.31 (m, 1H, H-aromatic), 7.58-7.65 (m, 1H, H-aromatic), 10.96 (bs, 1H, N$\underline{H}$), 12.73 (bs, 1H, O$\underline{H}$).

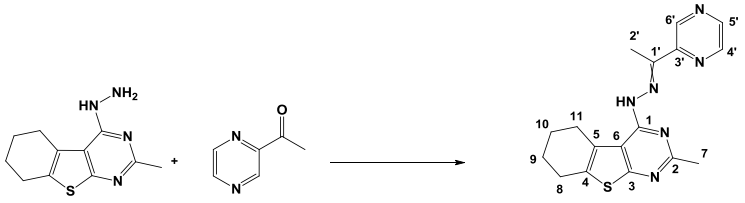
^{1}H-NMR (DMSO-d_6), δ: (minor species) 1.85-1.89 (m, 4H, C$\underline{H}_2$), 2.49 (s, 3H, C$\underline{H}_3$), 2.56 (s, 3H, C$\underline{H}_3$), 2.79-2.84 (m, 2H, C$\underline{H}_2$), 3.12-3.16 (m, 2H, C$\underline{H}_2$), 6.88-6.96 (m, 2H, H-aromatic), 7.26-7.31 (m, 1H, H-aromatic), 7.58-7.65 (m, 1H, H-aromatic), 9.32 (bs, 1H, N$\underline{H}$), 13.35 (bs, 1H, O$\underline{H}$).

^{13}C-NMR (DMSO-d_6), δ: (major and minor species) 12.88, 14.65, 21.21 (CH_3, C-aliphatic), 22.06, 22.13, 22.19, 22.41, 24.62, 24.91 (CH_2, C-aliphatic), 25.52 (CH_3, C-aliphatic), 26.46 (CH_2, C-aliphatic), 113.66 (C, C-aromatic), 116.58, 117.30 (CH, C-aromatic), 117.43 (C, C-aromatic), 118.42, 118.53 (CH, C-aromatic), 119.60, 121.08, 126.11 (C, C-aromatic), 127.93, 128.50, 130.53, 130.70 (CH, C-aromatic), 131.33,

132.46, 145.92, 152.88, 153.78, 154.50, 157.00, 158.47, 161.47, 162.68, 166.62 (C, C-aromatic).

2-Methyl-4-(2-(1-(pyrazin-2-*yl*)ethylidene)hydrazinyl)-5,6,7,8-tetrahydrobenzo [4,5]thieno[2,3-*d*]pyrimidine (443)

($C_{17}H_{18}N_6S$; M.W.= 338.4)



General procedure 20;

Reagent: 4-hydrazinyl-2-methyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidine (**441**) (0.05 g, 0.2 mmol);

Yellow solid;

Yield: 0.07 g (59%)

Melting Point: 189-191°C

MS (ESI^+): 339.0 $[M+H]^+$

Single species observed.

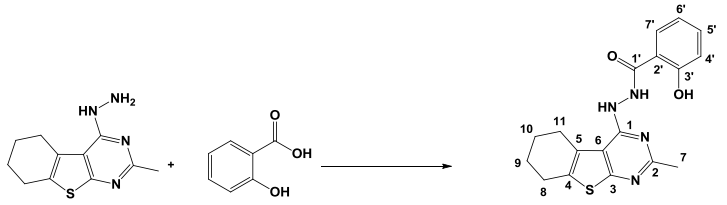
^{1}H-NMR (DMSO-d_6), δ: 1.76-1.82 (m, 4H, C<u>H</u>$_2$), 2.44 (s, 3H, C<u>H</u>$_3$), 2.46 (s, 3H, C<u>H</u>$_3$), 2.72-2.74 (m, 2H, C<u>H</u>$_2$), 3.00-3.03 (m, 2H, C<u>H</u>$_2$), 8.58 (d, J= 2.6 Hz, 1H, H-4'), 8.61 (dd, J_1= 2.6 Hz, J_2= 1.4 Hz, 1H, H-5'), 9.82 (d, J= 1.4 Hz, 1H, H-6'), 11.18 (bs, 1H, N<u>H</u>).

^{13}C-NMR (DMSO-d_6), δ: 12.83, 21.51 (CH_3, C-7, 2'), 22.14, 22.39, 24.64, 26.40 (CH_2, C-8, 9, 10, 11), 117.73, 130.61, 131.55 (C, C-aromatic), 143.13, 143.42, 143.66 (CH, C-aromatic), 148.97, 151.20, 152.79, 157.02, 158.28 (C, C-aromatic).

6.4.15.2 *N*'-(2-Methyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)aryl hydrazides (444-445)

2-Hydroxy-*N*'-(2-methyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*) benzohydrazide (444)

($C_{18}H_{18}N_4O_2S$; M.W.= 354.4)



441 **275** **444**

General procedure 22;

Reagent: 4-hydrazinyl-2-methyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidine (**441**) (0.09 g, 0.4 mmol);

Purification: re-crystallisation from 70% MeOH/H_2O;

Light orange solid;

Yield: 0.05 g (36%)

Melting Point: 209-212°C

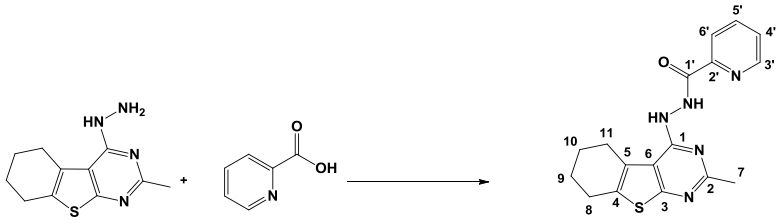
MS (ESI^+): 355.0 $[M+H]^+$

^{1}H-NMR (DMSO-d$_6$), δ: 1.82-1.87 (m, 4H, C<u>H</u>$_2$), 2.42 (s, 3H, H-7), 2.77-2.81 (m, 2H, C<u>H</u>$_2$), 2.99-3.03 (m, 2H, C<u>H</u>$_2$), 6.95-7.02 (m, 2H, H-aromatic), 7.44-7.48 (m, 1H, H-aromatic), 7.98 (d, J= 7.0 Hz, 1H, H-aromatic), 8.69 (bs, 1H, N<u>H</u>), 10.92 (bs, 1H, N<u>H</u>), 11.89 (bs 1H, O<u>H</u>).

^{13}C-NMR (DMSO-d$_6$), δ: 21.97, 22.17, 24.87 (CH_2, C-aliphatic), 25.41 (CH_3, C-7), 25.62 (CH_2, C-aliphatic), 112.87, 115.22 (C, C-aromatic), 117.27, 119.06 (CH, C-aromatic), 126.16 (C, C-aromatic), 128.46 (CH, C-aromatic), 131.63 (C, C-aromatic), 133.81 (CH, C-aromatic), 156.30, 158.83, 161.08, 166.11 (C, C-aromatic), 166.88 (C, C-1').

***N*'-(2-Methyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-*yl*)picolino hydrazide (445)**

($C_{17}H_{17}N_5OS$; M.W.= 339.4)



441 **277** **445**

General procedure 22;

Reagent: 4-hydrazinyl-2-methyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidine (**441**) (0.08 g, 0.3 mmol);

Purification: re-crystallisation from MeOH/H_2O;

Pale yellow solid;

Yield: 0.04 g (38%)

Melting Point: 99-101°C

MS (ESI^+): 340.0 $[M+H]^+$

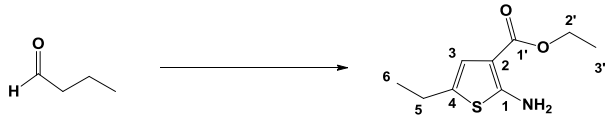
^{1}H-NMR (DMSO-d_6), δ: 1.83-1.86 (m, 4H, C$\underline{H}_2$), 2.38 (s, 3H, H-7), 2.78-2.81 (m, 2H, C$\underline{H}_2$), 2.98-3.02 (m, 2H, C$\underline{H}_2$), 7.65-7.69 (m, 1H, H-aromatic), 8.02-8.08 (m, 2H, H-aromatic), 8.58 (bs, 1H, N$\underline{H}$), 8.72-8.74 (m, 1H, H-aromatic), 10.63 (bs, 1H, N$\underline{H}$).

^{13}C-NMR (DMSO-d_6), δ: 22.00, 22.20, 24.88 (CH_2, C-aliphatic), 25.45 (CH_3, C-7), 25.69 (CH_2, C-aliphatic), 112.96 (C, C-aromatic), 122.25 (CH, C-aromatic), 126.22 (C, C-aromatic), 126.92 (CH, C-aromatic), 131.39 (C, C-aromatic), 137.86, 148.71 (CH, C-aromatic), 149.37, 156.69, 161.08, 162.74 (C, C-aromatic), 166.02 (C, C-1').

6.4.16 6-Ethylthieno[2,3-*d*]pyrimidines

Ethyl 2-amino-5-ethylthiophene-3-carboxylate (448)[52]

($C_9H_{13}NO_2S$; M.W.= 199.3)



447 **448**

General procedure 16;

Reagent: butyraldehyde (**447**) (1 g, 13.9 mmol);

T.L.C. System: *n*hexane-EtOAc 8:2 v/v, Rf: 0.52.

Purification: flash column chromatography (*n*hexane:EtOAc 100:0 v/v increasing to *n*hexane-EtOAc 95:5 v/v);

White solid;

Yield: 2.46 g (88%)

^{1}H-NMR ($CDCl_3$), δ: 1.24 (t, J= 7.5 Hz, 3H, H-6), 1.35 (t, J= 7.1 Hz, 3H, H-3'), 2.63 (qd, J_1= 7.5 Hz, J_2= 1.2 Hz, 2H, H-5), 4.27 (q, J= 7.1 Hz, 2H, H-2'), 5.80 (bs, 2H, $N\underline{H}_2$), 6.65 (t, J= 1.2 Hz, 1H, H-3).

^{13}C-NMR ($CDCl_3$), δ: 14.54, 15.37 (CH_3, C-6, 3'), 23.02, 59.43 (CH_2, C-5, 2'), 106.34 (C, C-aromatic), 120.66 (CH, C-3), 128.46, 161.14 (C, C-aromatic), 165.39 (C, C-1').

6-Ethylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (449)[53]

($C_8H_8N_2OS$; M.W.= 180.2)

448 **449**

General procedure 17;

Reagent: ethyl 2-amino-5-ethylthiophene-3-carboxylate (**448**) (2.46 g, 12.3 mmol);

Purification: recrystallization from EtOH/H_2O;

Light brown solid;

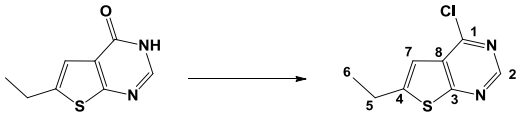
Yield: 1.64 g (73%)

^{1}H-NMR (DMSO-d$_6$), δ: 1.27 (t, J= 7.5 Hz, 3H, H-6), 2.85 (qd, J_1= 7.5 Hz, J_2= 1.1 Hz, 2H, H-5), 7.11 (t, J= 1.1 Hz, 1H, H-7), 8.05 (s, 1H, H-2), 12.39 (bs, 1H, NH).

^{13}C-NMR (DMSO-d$_6$), δ: 15.25 (CH_3, C-6), 23.12 (CH_2, C-5), 117.34 (CH, C-7), 124.77, 144.23 (C, C-aromatic), 145.00 (CH, C-2), 156.99 (C, C-aromatic), 162.76 (C, C-1).

4-Chloro-6-ethylthieno[2,3-*d*]pyrimidine (450)[54]

($C_8H_7ClN_2S$; M.W.= 198.7)



449 **450**

General procedure 18;

Reagent: 6-ethylthieno[2,3-d]pyrimidin-4(3H)-one (**449**) (1.64 g, 9.1 mmol);

T.L.C. System: *n*hexane-EtOAc 8:2 v/v, Rf: 0.63.

Purification: flash column chromatography (*n*hexane:EtOAc 100:0 v/v increasing to *n*hexane -EtOAc 95:5 v/v);

White solid;

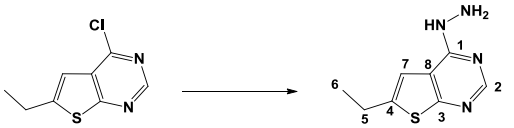
Yield: 1.42 g (78%)

^{1}H-NMR (CDCl$_3$), δ: 1.42 (t, J= 7.5 Hz, 3H, H-6), 3.01 (qd, J_1= 7.5 Hz, J_2= 1.2 Hz, 2H, H-5), 7.11 (t, J= 1.2 Hz, 1H, H-7), 8.78 (s, 1H, H-2).

^{13}C-NMR (CDCl$_3$), δ: 14.92 (CH_3, C-6), 24.59 (CH_2, C-5), 115.19 (CH, C-7), 130.49, 150.86 (C, C-aromatic), 152.02 (CH, C-2), 153.24, 168.45 (C, C-aromatic).

6-Ethyl-4-hydrazinylthieno[2,3-*d*]pyrimidine (451)[53]

($C_8H_{10}N_4S$; M.W.= 194.3)



450 **451**

General procedure 19;

Reagent: 4-chloro-6-ethylthieno[2,3-d]pyrimidine (**450**) (1.42 g, 7.1 mmol);

Purification: recrystallization from 40 % EtOH/H_2O;

White crystals;

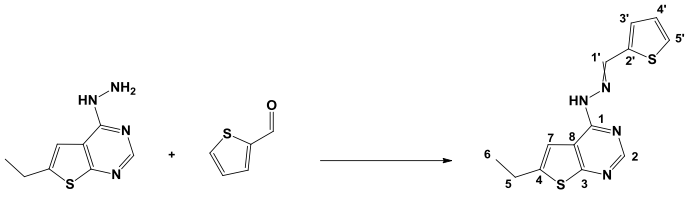
Yield: 0.98 g (70%)

^{1}H-NMR (DMSO-d$_6$), δ: 1.28 (t, J= 7.5 Hz, 3H, H-6), 2.86 (q, J= 7.5 Hz, 2H, H-5), 4.56 (bs, 2H, N$\underline{H}_2$), 7.33 (s, 1H, H-7), 8.30 (s, 1H, H-2), 8.99 (bs, 1H, N$\underline{H}$).

^{13}C-NMR (DMSO-d$_6$), δ: 15.11 (CH_3, C-6), 23.47 (CH_2, C-5), 114.75 (C, C-aromatic), 115.97 (CH, C-7), 128.47 (C, C-aromatic), 152.97 (CH, C-2), 158.67, 166.73 (C, C-aromatic).

6.4.16.1 6-Ethyl-4-(2-(1-arylethylidene)hydrazinyl)thieno[2,3-*d*]pyrimidines (446, 453-454)

6-Ethyl-4-(2-(thiophen-2-*yl*methylene)hydrazinyl)thieno[2,3-d]pyrimidine (446)[53] ($C_{13}H_{12}N_4S_2$; M.W.= 288.4)



451 **452** **446**

General procedure 20;

Reagent: 6-ethyl-4-hydrazinylthieno[2,3-*d*]pyrimidine (**451**) (0.10 g, 0.5 mmol);

Pale yellow solid;

Yield: 0.07 g (46%)

Melting Point: 164-168°C

MS (ESI^+): 289.0 $[M+H]^+$

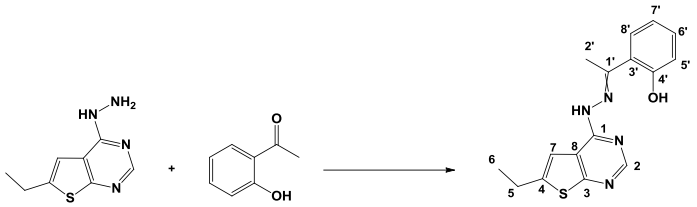
Single species observed.

^{1}H-NMR (DMSO-d_6), δ: 1.37 (t, J= 7.5 Hz, 3H, H-6), 2.94 (d, J= 7.5 Hz, 2H, H-5), 7.15 (dd, J_1= 5.1 Hz, J_2= 3.5 Hz, 1H, H-4'), 7.43 (d, J= 3.5 Hz, 1H, H-aromatic), 7.65 (d, J= 5.1 Hz, 1H, H-aromatic), 7.78 (s, 1H, H-aromatic), 8.40 (s, 2H, H-aromatic), 11.78 (bs, 1H, N<u>H</u>).

^{13}C-NMR (DMSO-d_6), δ: 14.60 (CH_3, C-6), 23.55 (CH_2, C-5), 115.02 (C, C-aromatic), 117.93, 127.94, 128.05, 129.57, 138.41 (CH, C-aromatic), 139.59, 143.26 (C, C-aromatic), 152.34 (CH, C-aromatic), 154.26, 162.84 (C, C-aromatic).

2-(1-(2-(6-Ethylthieno[2,3-*d*]pyrimidin-4-*yl*)hydrazono)ethyl)phenol (453)

($C_{16}H_{16}N_4OS$; M.W.= 312.4)



451 **202** **453**

General procedure 20;

Reagent: 6-ethyl-4-hydrazinylthieno[2,3-*d*]pyrimidine (**451**) (0.10 g, 0.5 mmol);

Pale yellow solid;

Yield: 0.06 g (44%)

Melting Point: 123-127°C

MS (ESI^+): 313.0 $[M+H]^+$

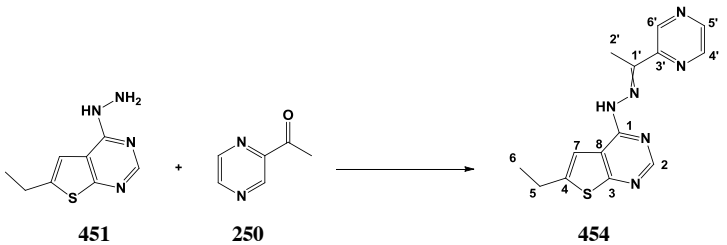
Single species observed.

^{1}H-NMR (DMSO-d$_6$), δ: 1.35 (t, J= 7.5 Hz, 3H, H-6), 2.53 (s, 3H, H-2'), 2.95 (q, J= 7.5 Hz, 2H, H-5), 6.89-6.94 (m, 2H, H-aromatic), 7.27-7.31 (m, 2H, H-aromatic), 7.62 (dd, J_1= 7.5 Hz, J_2= 1.5 Hz, 1H, H-aromatic), 7.66 (s, 1H, H-7), 8.54 (s, 1H, H-2), 10.63 (bs, 1H, NH), 13.01 (bs, 1H, OH).

^{13}C-NMR (DMSO-d$_6$), δ: 14.02, 14.93 (CH_3, C-6, 2'), 23.59 (CH_2, C-5), 115.61 (CH, C-aromatic), 115.79 (C, C-aromatic), 117.13, 118.49 (CH, C-aromatic), 121.24, 124.32 (C, C-aromatic), 128.12, 130.64 (CH, C-aromatic), 144.33 (C, C-aromatic), 152.82 (CH, C-aromatic), 154.27, 158.07, 165.25 (C, C-aromatic).

6-Ethyl-4-(2-(1-(pyrazin-2-*yl*)ethylidene)hydrazinyl)thieno[2,3-*d*]pyrimidine (454)

($C_{14}H_{14}N_6S$; M.W.= 298.4)



451 **250** **454**

General procedure 20;

Reagent: 6-ethyl-4-hydrazinylthieno[2,3-*d*]pyrimidine (**451**) (0.10 g, 0.5 mmol);

Pale yellow solid;

Yield: 0.11 g (71%)

Melting Point: 149-152°C

MS (ESI^+): 299.0 $[M+H]^+$

Two species observed. Major/minor species ratio: 5/1.

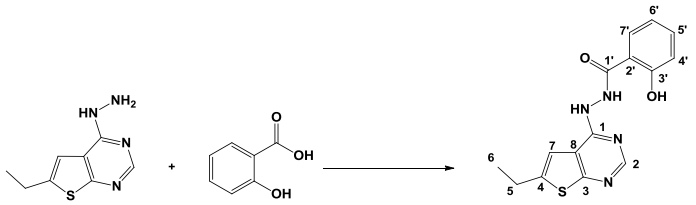
^{1}H-NMR (DMSO-d_6), δ: (major species) 1.33 (t, J= 7.5 Hz, 3H, H-6), 2.47 (s, 3H, H-2'), 2.93 (q, J= 7.5 Hz, 2H, H-5), 7.77-7.78 (m, 1H, H-aromatic), 8.51 (s, 1H, H-2), 8.61-8.63 (m, 1H, H-aromatic), 8.66 (dd, J_1= 2.5 Hz, J_2= 1.6 Hz, 1H, H-aromatic), 9.23 (d, J= 1.6 Hz, 1H, H-aromatic), 10.99 (bs, 1H, NH).

^{1}H-NMR (DMSO-d_6), δ: (minor species) 1.29 (t, J= 7.5 Hz, 3H, H-6), 2.48 (s, 3H, H-2'), 2.88 (q, J= 7.5 Hz, 2H, H-5), 7.16-7.17 (m, 1H, H-aromatic), 7.92 (d, J= 3.7 Hz, 1H, H-aromatic), 8.58 (d, J= 2.5Hz, 1H, H-aromatic), 8.61-8.63 (m, 1H, H-aromatic), 9.79 (d, J= 1.6 Hz, 1H, H-aromatic), 12.00 (bs, 1H, NH).

^{13}C-NMR (DMSO-d_6), δ: (major and minor species) 11.75, 12.24, 14.49, 15.35 (CH_3, C-6, 2'), 23.21, 23.50 (CH_2, C-5), 115.69 (C, C-aromatic), 117.04, 117.68, 141.79, 143.19, 143.27, 143.43, 143.49, 143.66 (CH, C-aromatic), 144.00, 147.56, 150.73 (C, C-aromatic), 152.06 (CH, C-aromatic), 155.02, 168.55 (C, C-aromatic).

6.4.16.2 *N*'-(6-Ethylthieno[2,3-*d*]pyrimidin-4-*yl*)-2-arylcarbohydrazides (455-456)

_N_'-(6-Ethylthieno[2,3-*d*]pyrimidin-4-*yl*)-2-hydroxybenzohydrazide (455)
($C_{15}H_{14}N_4O_2S$; M.W.= 314.4)



451 **275** **455**

General procedure 22;

Reagent: 6-ethyl-4-hydrazinylthieno[2,3-*d*]pyrimidine (**451**) (0.2 g, 1 mmol);

Purification: re-crystallisation from EtOH;

White solid;

Yield: 0.10 g (28%)

Melting Point: 176-179°C

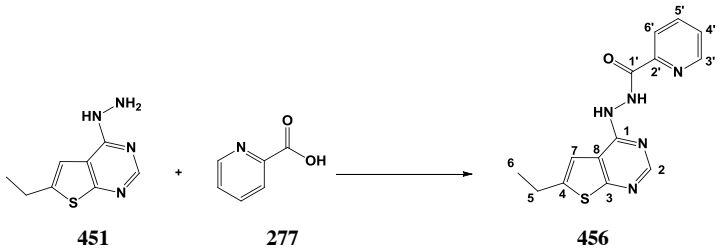
MS (ESI^+): 314.9 $[M+H]^+$

^{1}H-NMR (DMSO-d_6), δ: 1.32 (t, J= 7.2 Hz, 3H, H-6), 2.93 (q, J= 7.2 Hz, 2H, H-5), 6.96-7.01 (m, 2H, H-aromatic), 7.43 (s, 1H, H-7), 7.46-7.50 (m, 1H, H-aromatic), 7.98 (d, J= 7.9 Hz, 1H, H-aromatic), 8.39 (s, 1H, H-2), 10.09 (bs, 1H, NH), 10.83 (bs, 1H, NH), 11.87 (bs 1H, OH).

^{13}C-NMR (DMSO-d_6), δ: 14.97 (CH_3, C-6), 23.53 (CH_2, C-5), 114.64 (CH, C-aromatic), 114.77, 115.23, 116.99 (C, C-aromatic), 117.35, 119.06, 128.39 (CH, C-aromatic), 132.27 (C, C-aromatic), 134.13 (CH, C-aromatic), 144.57 (C, C-aromatic), 152.51 (CH, C-aromatic), 159.13 (C, C-aromatic), 168.00 (C, C-1').

***N*'-(6-Ethylthieno[2,3-*d*]pyrimidin-4-*yl*)picolinohydrazide (456)**

($C_{14}H_{13}N_5OS$; M.W.= 299.35)



General procedure 22;

Reagent: 6-ethyl-4-hydrazinylthieno[2,3-*d*]pyrimidine (**451**) (0.2 g, 1 mmol);

Purification: re-crystallisation from EtOH;

Pale yellow solid;

Yield: 0.08 g (26%)

Melting Point: 149-152°C

MS (ESI^+): 299.9 $[M+H]^+$

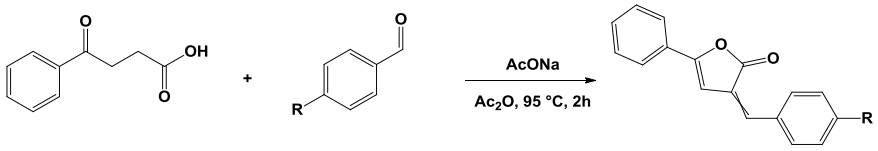
^{1}H-NMR (DMSO-d$_6$), δ: 1.30 (t, J= 7.2 Hz, 3H, H-6), 2.91 (q, J= 7.2 Hz, 2H, H-5), 7.41 (s, 1H, H-7), 7.66-7.71 (m, 1H, H-aromatic), 8.02-8.08 (m, 2H, H-aromatic), 8.33 (s, 1H, H-2), 8.74 (d, J= 4.2 Hz, 1H, H-aromatic), 9.90 (bs, 1H, NH), 10.83 (bs, 1H, NH).

^{13}C-NMR (DMSO-d$_6$), δ: 14.95 (CH_3, C-6), 23.50 (CH_2, C-5), 114.78 (CH, C-aromatic), 115.19, 116.33 (C, C-aromatic), 122.44, 127.04 (CH, C-aromatic), 132.84 (C, C-aromatic), 137.87 (CH, C-aromatic), 144.13 (C, C-aromatic), 148.71 (CH, C-aromatic), 149.26 (C, C-aromatic), 152.81 (CH, C-aromatic), 163.47 (C, C-1').

6.5 Synthesis of pyrrolone structures

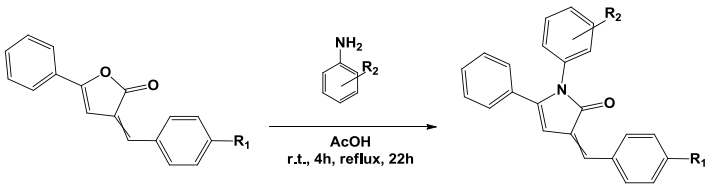
6.5.1 General procedures 24-25

General procedure 24: synthesis of 3-arylidene-5-phenylfuran-2(3*H*)-ones



A stirred solution of 3-benzoyl propanoic acid (**460**) (1 g, 5.6 mmol), benzaldehyde (6.6 mmol) and sodium acetate (0.47 g, 5.6 mmol) in 7 mL of acetic anhydride was heated at 95°C for 2 h under N_2 atmosphere . The reaction mixture was then cooled to r.t., diluted with MeOH and left in an ice-bath for 2 h. The resulting precipitate was filtered and washed with a cold 50% solution of MeOH in water to give 3-arylidene-5-phenylfuran-2(3*H*)-ones, that were used for the following step without further purification.

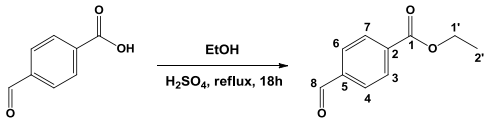
General procedure 25: synthesis of 1-aryl-3-arylidene-5-phenyl-1*H*-pyrrol-2(3*H*)-ones



A mixture of 3-arylidene-5-phenylfuran-2(3H)-one (2.0 mmol) and differently substituted aniline (1.9 mmol) in 18 mL of glacial acetic acid was stirred at r.t. under N_2 atmosphere for 4 h, then heated to reflux for 22 h. The solvent was then evaporated at reduced pressure and the crude residue was purified by flash column chromatography to afford pure 1-aryl-3-arylidene-5-phenyl-1*H*-pyrrol-2(3*H*)-ones.

6.5.2 Ethyl 4-formylbenzoate (461)[70]

($C_{10}H_{10}O_3$; M.W.= 178.2)



483 **461**

4-Formyl-benzoic acid (**483**) (2 g, 13.4 mmol), H_2SO_4 (0.6 mL) and EtOH (62 mL) were mixed and heated under reflux for 18 h. The reaction mixture was then cooled to r.t. and the organic solvent was removed under vacuum. The residue was dissolved in EtOAc (40 mL), washed with saturated $NaHCO_3$ solution (2 x40 mL), brine (2 x40 mL), and distilled water (2 x40 mL), and finally dried over $MgSO_4$. The organic solvent was then removed under vacuum to afford the title compound as a brown oil, that was used for the following step without further purification.

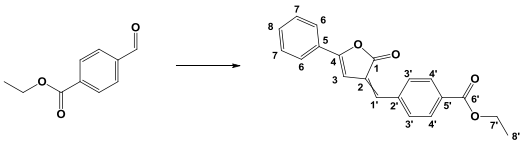
Yield: 12.0 g (84%)

^{1}H-NMR ($CDCl_3$), δ: 1.43 (t, J= 7.1 Hz, 3H, H-2'), 4.43 (q, J= 7.1 Hz, 2H, H-1'), 7.96 (d, J= 8.5 Hz, 2H, H-aromatic), 8.21 (d, J= 8.5 HZ, 2H, H-aromatic), 10.11 (s, 1H, H-8).

^{13}C-NMR ($CDCl_3$), δ: 14.26 (CH_3, C-2'), 61.38 (CH_2, C-1'), 129.46, 130.14 (CH, C-aromatic), 135.48, 139.10 (C, C-2, 5), 165.56 (C, C-1), 191.62 (CH, C-8).

6.5.3 3-Arylidene-5-phenylfuran-2(3*H*)-ones (466-470)

4-(2-Oxo-5-phenyl-furan-3-ylidenemethyl)-benzoic acid ethyl ester (466)
($C_{20}H_{16}O_4$; M.W.= 320.3)



461 **466**

General procedure 24;

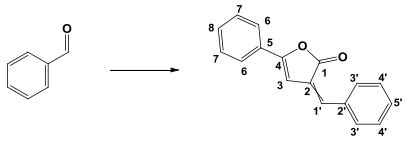
Yellow solid;

Yield: 0.55 g (31%)

^{1}H-NMR (DMSO-d_6), δ: 1.34 (t, J= 7.0 Hz, 3H, H-8'), 4.34 (q, J= 7.0 Hz, 2H, H-7'), 7.40 (s, 1H, H-alkenylic), 7.56-7.60 (m, 3H, H-aromatic), 7.63 (s, 1H, H-alkenylic), 7.88-7.92 (m, 2H, H-aromatic), 7.97 (d, J= 7.8 Hz, 2H, H-aromatic), 8.01 (d, J= 8.3 Hz, 2H, H-aromatic).

^{13}C-NMR (DMSO-d_6), δ: 14.07 (CH_3, C-8'), 60.97 (CH_2, C-7'), 101.07, 125.47 (CH, C-alkenylic, aromatic), 126.57, 127.50 (C, C-aromatic), 129.02, 129.52, 130.58 (CH, C-aromatic), 130.65 (C, C-aromatic), 130.91, 133.12 (CH, C-aromatic), 138.68, 157.08 (C, C-aromatic), 165.10, 168.37 (C, C-1, 6').

3-Benzylidene-5-phenyl-3*H*-furan-2-one (467)[71]
($C_{17}H_{12}O_2$; M.W.= 248.3)



462 **467**

General procedure 24;

Yellow solid;

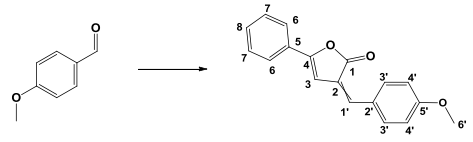
Yield: 0.49 g (35%)

^{1}H-NMR (DMSO-d_6), δ: 7.41 (s, 1H, H-alkenylic), 7.49-7.55 (m, 6H, H-aromatic), 7.63 (s, 1H, H-alkenylic), 7.88-7.92 (m, 4H, H-aromatic).

^{13}C-NMR (DMSO-d$_6$), δ: 101.11 (CH, C-alkenylic), 124.61 (C, C-aromatic), 125.30 (CH, C-aromatic), 127.74 (C, C-aromatic), 128.86, 129.11, 129.29, 130.55, 130.59 (CH, C-aromatic), 134.42 (C, C-aromatic), 135.14 (CH, C-aromatic), 155.99 (C, C-aromatic), 168.74 (C, C-1).

3-(4-Methoxy-benzylidene)-5-phenyl-3*H*-furan-2-one (468)[72]

(C$_{18}$H$_{14}$O$_3$; M.W.= 278.3)



463 **468**

General procedure 24;

Yellow solid;

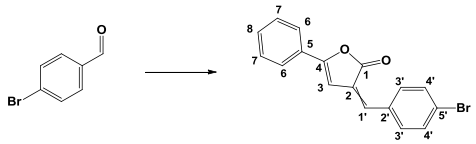
Yield: 0.51 g (33%)

^{1}H-NMR (DMSO-d$_6$), δ: 3.85 (s, 3H, H-6'), 7.06 (d, J= 8.8 Hz, 2H, H-aromatic), 7.36 (s, 1H, H-3), 7.45-7.53 (m, 3H, H-aromatic), 7.60 (d, J= 0.7 Hz, 1H, H-1'), 7.86-7.89 (m, 4H, H-aromatic).

^{13}C-NMR (DMSO-d$_6$), δ: 55.44 (CH_2, C-6'), 101.14, 114.71 (CH, C-alkenylic, aromatic), 121.82 (C, C-aromatic), 125.04 (CH, C-aromatic), 127.19, 127.97 (C, C-aromatic), 128.92, 130.19, 132.82, 135.32 (CH, C-aromatic), 154.84, 161.34 (C, C-aromatic), 169.08 (C, C-1).

3-(4-Bromo-benzylidene)-5-phenyl-3*H*-furan-2-one (469)

(C$_{17}$H$_{11}$BrO$_2$; M.W.= 327.2)



464 **469**

General procedure 24;

Yellow solid;

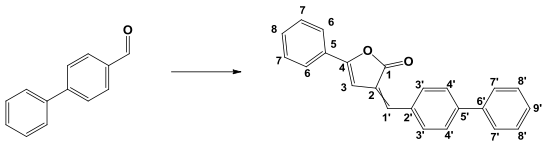
Yield: 0.93 g (51%)

^{1}H-NMR ($CDCl_3$), δ: 7.28 (s, 1H, H-alkenylic), 7.38 (s, 1H, H-alkenylic), 7.46-7.50 (m, 3H, H-aromatic), 7.52 (d, J= 8.4 Hz, 2H, H-aromatic), 7.63 (d, J= 7.6 Hz, 2H, H-aromatic), 7.78-7.83 (m, 2H, H-aromatic).

^{13}C-NMR ($CDCl_3$), δ: 99.52 (CH, C-alkenylic), 121.12 (C, C-aromatic), 125.46 (CH, C-aromatic), 127.01, 127.54 (C, C-aromatic), 128.95, 130.76, 131.32, 132.41, 133.71 (CH, C-aromatic), 152.34, 159.53 (C, C-aromatic), 168.89 (C, C-1).

3-(4-Bromo-benzylidene)-5-phenyl-3*H*-furan-2-one (470)

($C_{23}H_{16}O_2$; M.W.= 324.4)



465 **470**

General procedure 24;

Yellow solid;

Yield: 0.74 g (41%)

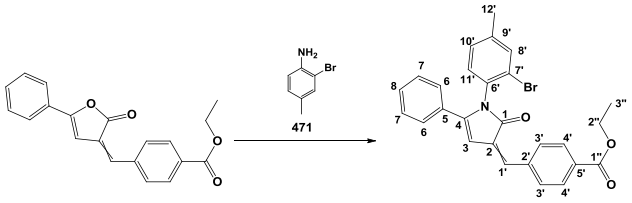
^{1}H-NMR (DMSO-d_6), δ: 7.40-7.45 (m, 2H, H-aromatic), 7.47-7.56 (m, 5H, H-aromatic), 7.67 (s, 1H, H-alkenylic), 7.74-7.79 (m, 2H, H-aromatic), 7.80 (d, J= 8.3 Hz, 2H, H-aromatic), 7.89-7.93 (m, 2H, H-aromatic), 7.98 (d, J= 8.3 Hz, 2H, H-aromatic).

^{13}C-NMR (DMSO-d_6), δ: 101.24 (CH, C-alkenylic), 124.35 (C, C-aromatic), 125.30, 126.74, 127.19 (CH, C-aromatic), 127.77 (C, C-aromatic), 128.16, 128.98, 129.07, 130.54, 131.34 (CH, C-aromatic), 133.57 (C, C-aromatic), 134.59 (CH, C-aromatic), 138.98, 141.93, 155.94 (C, C-aromatic), 168.89 (C, C-1).

6.5.4 1-Aryl-3-arylidene-5-phenyl-1*H*-pyrrol-2(3*H*)-ones (459, 475-482)

4-[1-(2-Bromo-4-methyl-phenyl)-2-oxo-5-phenyl-1,2-dihydro-pyrrol-3-ylidene methyl]-benzoic acid ethyl ester (459)

($C_{27}H_{22}BrNO_3$; M.W.= 488.4)



General procedure 25;

Orange solid;

T.L.C. System: *n*hexane:EtOAc 8:2 v/v, Rf: 0.36.

Purification: flash column chromatography (*n*hexane:EtOAc 100:0 v/v increasing to *n*hexane:EtOAc 90:10 v/v).

Yield: 0.41 g (44%)

Melting Point: 166-168°C

MS (ESI^+): 488.1, 490.1 $[M+H]^+$

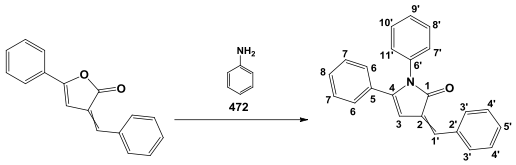
Microanalysis: Calculated for $C_{27}H_{22}BrNO_3$ (488.4); Theoretical: %C = 66.40, %H = 4.54, %N = 2.87; Found: %C = 66.29, %H = 4.25, %N = 2.71.

^{1}H-NMR ($CDCl_3$), δ: 1.44 (t, J= 7.1 Hz, 3H, H-3''), 2.36 (s, 3H, H-12'), 4.43 (q, J= 7.1 Hz, 2H, H-2''), 6.50 (d, J= 0.8 Hz, 1H, H-1'), 7.06-7.17 (m, 2H, H-aromatic), 7.27-7.30 (m, 4H, H-aromatic), 7.30-7.35 (m, 1H, H-aromatic), 7.46-7.47 (m, 1H, H-aromatic), 7.55 (s, 1H, H-3), 7.76 (d, J= 8.3 Hz, 2H, H-aromatic), 8.13 (d, J= 8.3 Hz, 2H, H-aromatic).

^{13}C-NMR ($CDCl_3$), δ: 14.34 (CH_3, C-3''), 20.90 (CH_3, C-12'), 61.19 (CH_2, C-2''), 100.82 (CH, C-1'), 123.44 (C, C-aromatic), 127.56, 128.41, 129.06, 129.42, 129.97, 130.01, 130.45 (CH, C-aromatic), 130.63, 130.80, 131.06 (C, C-aromatic), 131.43 (CH, C-aromatic), 132.69 (C, C-aromatic), 134.04 (CH, C-aromatic), 140.10, 140.36, 149.85 (C, C-aromatic), 166.04, 169.38 (C, C-1'', 1).

3-Benzylidene-1,5-diphenyl-1,3-dihydro-pyrrol-2-one (475)[73]

($C_{23}H_{17}NO$; M.W.= 323.4)



462 **475**

General procedure 25;

Orange solid;

T.L.C. System: *n*hexane-EtOAc 8:2 v/v, Rf: 0.53.

Purification: flash column chromatography (*n*hexane:EtOAc 100:0 v/v increasing to *n*hexane:EtOAc 70:30 v/v).

Yield: 0.21 g (34%)

Melting Point: 165-167°C

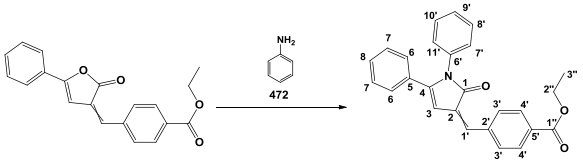
MS (ESI^+): 324.1 $[M+H]^+$

^{1}H-NMR (DMSO-d$_6$), δ: 6.82 (s, 1H, H-alkenylic), 7.13 (d, J= 7.7 Hz, 2H, H-aromatic), 7.24-7.35 (m, 6H, H-aromatic), 7.36-7.40 (m, 2H, H-aromatic), 7.42 (s, 1H, H-alkenylic), 7.43-7.48 (m, 1H, H-aromatic), 7.48-7.55 (m, 2H, H-aromatic), 7.88 (d, J= 7.5 Hz, 2H, H-aromatic).

^{13}C-NMR (DMSO-d$_6$), δ: 101.92, 127.20, 127.47, 128.28, 128.63, 128.74 (CH, C-aromatic), 128.92 (C, C-aromatic), 129.07, 129.13, 129.17 (CH, C-aromatic), 129.93 (C, C-aromatic), 130.65, 132.28 (CH, C-aromatic), 135.10, 135.66, 147.95 (C, C-aromatic), 169.03 (C, C-1).

4-(2-Oxo-1,5-diphenyl-1,2-dihydro-pyrrol-3-ylidenemethyl)-benzoic acid ethyl ester (476)

($C_{26}H_{21}NO_3$; M.W.= 395.5)



461 **476**

General procedure 25;

Orange solid;

T.L.C. System: *n*hexane:EtOAc 7:3 v/v, Rf: 0.66.

Purification: flash column chromatography (*n*hexane:EtOAc 100:0 v/v increasing to *n*hexane:EtOAc 80:20 v/v).

Yield: 0.42 g (57%)

Melting Point: 133-135°C

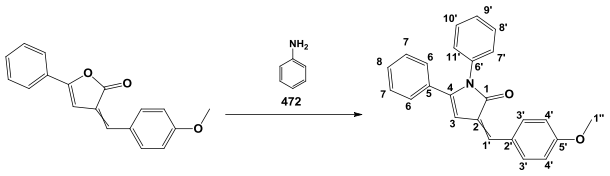
MS (ESI^+): 396.1 $[M+H]^+$

^{1}H-NMR (DMSO-d$_6$), δ: 1.34 (t, J= 7.1 Hz, 3H, H-3''), 4.34 (q, J= 7.1 Hz, 2H, H-2''), 6.84 (s, 1H, H-alkenylic), 7.12-7.15 (m, 2H, H-aromatic), 7.29-7.35 (m, 6H, H-aromatic), 7.36-7.43 (m, 3H, H-aromatic), 7.99 (d, J= 8.5 Hz, 2H, H-aromatic), 8.03 (d, J= 8.5 Hz, 2H, H-aromatic).

^{13}C-NMR (DMSO-d$_6$), δ: 14.10 (CH_3, C-3''), 60.89 (CH_2, C-2''), 101.78, 127.13, 127.32, 127.72, 128.50, 128.78, 129.44, 129.57 (CH, C-aromatic), 130.16 (C, C-aromatic), 130.34, 130.54 (CH, C-aromatic), 130.73, 135.49, 139.49, 149.32 (C, C-aromatic), 165.20, 168.89 (C, C-1'', 1).

3-(4-Methoxy-benzylidene)-1,5-diphenyl-1,3-dihydro-pyrrol-2-one (477)

($C_{24}H_{19}NO_2$; M.W.= 353.4)



463 **477**

General procedure 25;

Yellow solid;

T.L.C. System: *n*hexane:EtOAc 7:3 v/v, Rf: 0.63.

Purification: flash column chromatography (*n*hexane:EtOAc 100:0 v/v increasing to *n*hexane:EtOAc 80:20 v/v).

Yield: 0.26 g (39%)

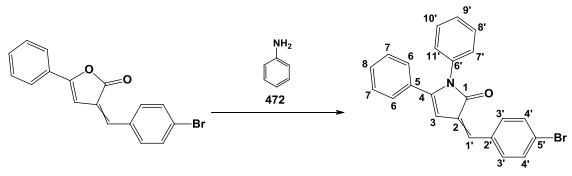
Melting Point: 150-152°C

MS (ESI^+): 354.1 $[M+H]^+$

^{1}H-NMR ($CDCl_3$), δ: 6.50 (s, 3H, H-1''), 6.49 (s, 1H, H-alkenylic), 7.00 (d, J= 8.7 Hz, 2H, H-aromatic), 7.14-7.17 (m, 2H, H-aromatic), 7.22-7.31 (m, 6H, H-aromatic), 7.32-7.33 (m, 2H, H-aromatic), 7.53 (s, 1H, H-alkenylic), 7.70 (d, J= 8.7 Hz, 2H, H-aromatic).

^{13}C-NMR ($CDCl_3$), δ: 55.42 (CH_3, C-1''), 102.11, 114.54, 126.71, 127.11 (CH, C-aromatic), 127.56 (C, C-aromatic), 127.68, 128.28, 128.55, 128.69 (CH, C-aromatic), 128.84, 131.16 (C, C-aromatic), 132.17, 133.24 (CH, C-aromatic), 135.89, 147.16, 160.96 (C, C-aromatic), 169.90 (C, C-1).

3-(4-Bromo-benzylidene)-1,5-diphenyl-1,3-dihydro-pyrrol-2-one (478)
($C_{23}H_{16}BrNO$; M.W.= 402.3)



464 **478**

General procedure 25;

Orange solid;

T.L.C. System: *n*hexane:EtOAc 7:3 v/v, Rf: 0.66.

Purification: flash column chromatography (*n*hexane:EtOAc 100:0 v/v increasing to *n*hexane:EtOAc 80:20 v/v).

Yield: 0.22 g (29%)

Melting Point: 155-157°C

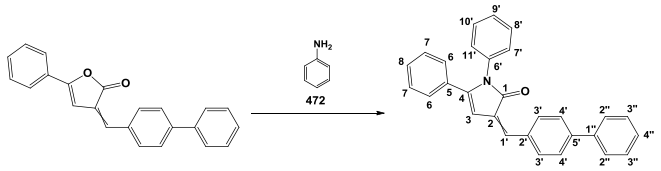
MS (ESI^+): 402.0, 404.0 $[M+H]^+$

^{1}H-NMR ($CDCl_3$), δ: 6.44 (s, 1H, H-alkenylic), 7.13-7.16 (m, 2H, H-aromatic), 7.23-7.25 (m, 2H, H-aromatic), 7.26-7.30 (m, 3H, H-aromatic), 7.31-7.38 (m, 3H, H-aromatic), 7.47 (s, 1H, H-alkenylic), 7.56 (d, J= 8.6 Hz, 2H, H-aromatic), 7.60 (d, J= 8.6 Hz, 2H, H-aromatic).

^{13}C-NMR ($CDCl_3$), δ: 101.60 (CH, C-aromatic), 123.90 (C, C-aromatic), 126.95, 127.09, 127.29, 127.74, 128.36, 128.78, 129.25 (CH, C-aromatic), 130.16, 130.79 (C, C-aromatic), 131.52, 131.55 (CH, C-aromatic), 134.81, 135.59, 148.94 (C, C-aromatic), 169.57 (C, C-1).

3-Biphenyl-4-ylmethylene-1,5-diphenyl-1,3-dihydro-pyrrol-2-one (479)

($C_{29}H_{21}NO$; M.W.= 399.5)



465 **479**

General procedure 25;

Orange solid;

T.L.C. System: *n*hexane:EtOAc 7:3 v/v, Rf: 0.70.

Purification: flash column chromatography (*n*hexane:EtOAc 100:0 v/v increasing to *n*hexane:EtOAc 80:20 v/v).

Yield: 0.20 g (27%)

Melting Point: 187-189°C

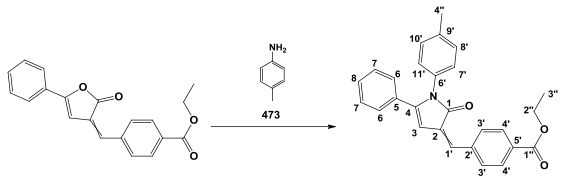
MS (ESI^+): 400.1 $[M+H]^+$

^{1}H-NMR ($CDCl_3$), δ: 6.57 (d, J= 0.8 Hz, 1H, H-1'), 7.16-7.19 (m, 2H, H-aromatic), 7.25-7.34 (m, 6H, H-aromatic), 7.35-7.39 (m, 2H, H-aromatic), 7.42 (tt, J_1= 7.3 Hz, J_2= 1.8 Hz, 1H, H-aromatic), 7.49-7.53 (m, 2H, H-aromatic), 7.61 (s, 1H, H-3), 7.66-7.70 (m, 2H, H-aromatic), 7.72 (d, J= 8.3 Hz, 2H, H-aromatic), 7.81 (d, J= 8.3 Hz, 2H, H-aromatic).

^{13}C-NMR ($CDCl_3$), δ: 101.12 (CH, C-1'), 126.87, 127.07, 127.14, 127.91, 128.10, 128.34, 128.63, 128.87, 128.96, 129.09 (CH, C-aromatic), 129.55, 130.07 (C, C-aromatic), 131.22, 132.82 (CH, C-aromatic), 134.91, 135.74, 140.16, 142.34, 148.26 (C, C-aromatic), 169.80 (C, C-1).

4-(2-Oxo-5-phenyl-1-p-tolyl-1,2-dihydro-pyrrol-3-ylidenemethyl)-benzoic acid ethyl ester (480)

($C_{27}H_{23}NO_3$; M.W.= 409.5)



461 **480**

General procedure 25;

Orange solid;

T.L.C. System: *n*hexane:EtOAc 8:2 v/v, Rf: 0.43.

Purification: flash column chromatography (*n*hexane:EtOAc 100:0 v/v increasing to *n*hexane:EtOAc 85:15 v/v).

Yield: 0.25 g (32%)

Melting Point: 161-163°C

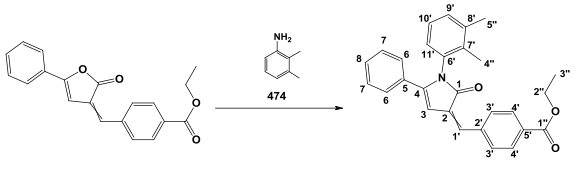
MS (ESI^+): 410.2$[M+H]^+$

^{1}H-NMR ($CDCl_3$), δ: 1.44 (t, J= 7.1 Hz, 3H, H-3''), 2.36 (s, 3H, H-12'), 4.43 (q, J= 7.1 Hz, 2H, H-2''), 6.47 (d, J= 0.8 Hz, 1H, H-1'), 7.03 (d, J= 8.2 Hz, 2H, H-aromatic), 7.15 (d, J= 8.2 Hz, 2H, H-aromatic), 7.25-7.35 (m, 5H, H-aromatic), 7.54 (s, 1H, H-3), 7.75 (d, J= 8.3 Hz, 2H, H-aromatic), 8.13 (d, J= 8.3 Hz, 2H, H-aromatic).

^{13}C-NMR ($CDCl_3$), δ: 14.34 (CH_3, C-3''), 21.13 (CH_3, C-12'), 61.18 (CH_2, C-2''), 101.39 (CH, C-1'), 126.92, 127.81, 128.35, 129.32, 129.49, 129.92, 130.01 (CH, C-aromatic), 130.75 (C, C-aromatic), 131.16 (CH, C-aromatic), 131.42, 132.91, 136.89, 140.18, 149.79 (C, C-aromatic), 166.04, 169.66 (C, C-1'', 1).

4-[1-(2,3-Dimethyl-phenyl)-2-oxo-5-phenyl-1,2-dihydro-pyrrol-3-ylidenemethyl]-benzoic acid ethyl ester (481)

($C_{28}H_{25}NO_3$; M.W.= 423.5)



461 **481**

General procedure 25;

Orange solid;

T.L.C. System: *n*hexane:EtOAc 8:2 v/v, Rf: 0.39.

Purification: flash column chromatography (*n*hexane:EtOAc 100:0 v/v increasing to *n*hexane:EtOAc 85:15 v/v).

Yield: 0.45 g (56%)

Melting Point: 64-66°C

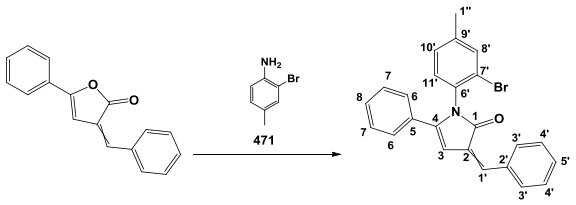
MS (ESI^+): 424.2 $[M+H]^+$

^{1}H-NMR ($CDCl_3$), δ: 1.44 (t, J= 7.1 Hz, 3H, H-3''), 2.13 (s, 3H, C<u>H</u>$_3$), 2.32 (s, 3H, C<u>H</u>$_3$), 4.43 (q, J= 7.1 Hz, 2H, H-2''), 6.52 (d, J= 0.8 Hz, 1H, H-1'), 6.89 (d, J= 7.7 Hz, 1H, H-aromatic), 7.05-7.09 (m, 1H, H-aromatic), 7.15 (d, J= 7.5 Hz, 1H, H-aromatic), 7.21-7.27 (m, 4H, H-aromatic), 7.28-7.32 (m, 1H, H-10'), 7.54 (s, 1H, H-3), 7.77 (d, J= 8.3 Hz, 2H, H-aromatic), 8.13 (d, J= 8.3 Hz, 2H, H-aromatic).

^{13}C-NMR ($CDCl_3$), δ: 14.34 (CH_3, C-3''), 14.66, 20.42 (CH_3, C-12', 13'), 61.18 (CH_2, C-2''), 100.57 (CH, C-1'), 125.95, 126.35, 127.41, 128.36, 129.35, 129.88, 129.94, 130.02 (CH, C-aromatic), 130.74 (C, C-aromatic), 131.14 (CH, C-aromatic), 131.40, 134.83, 135.17, 138.26, 140.22, 150.41 (C, C-aromatic), 166.05, 169.59 (C, C-1'', 1).

3-Benzylidene-1-(2-bromo-4-methyl-phenyl)-5-phenyl-1,3-dihydro-pyrrol-2-one (482)

($C_{24}H_{18}BrNO$; M.W.= 416.3)



462 **482**

General procedure 25;

Yellow solid;

T.L.C. System: *n*hexane:EtOAc 8:2 v/v, Rf: 0.38.

Purification: flash column chromatography (*n*hexane:EtOAc 100:0 v/v increasing to *n*hexane:EtOAc 85:15 v/v).

Yield: 0.39 g (50%)

Melting Point: 175-177°C

MS (ESI^+): 416.0, 418.0 $[M+H]^+$

^{1}H-NMR ($CDCl_3$), δ: 2.36 (s, 3H, H-12'), 6.53 (d, J= 0.8 Hz, 1H, H-1'), 7.11-7.15 (m, 2H, H-aromatic), 7.25-7.33 (m, 5H, H-aromatic), 7.41 (tt, J_1= 7.3 Hz, J_2= 2.2 Hz, 1H, H-aromatic), 7.45-7.49 (m, 3H, H-aromatic), 7.58 (s, 1H, H-3), 7.72 (d, J= 7.5 Hz, 2H, H-aromatic).

^{13}C-NMR ($CDCl_3$), δ: 20.90 (CH_3, C-12'), 101.10 (CH, C-1'), 123.49 (C, C-aromatic), 127.52, 128.34, 128.93, 129.02, 129.12, 129.47 (CH, C-aromatic), 129.61 (C, C-aromatic), 130.31, 130.49 (CH, C-aromatic), 131.78, 132.92 (C, C-aromatic), 133.34, 134.00 (CH, C-aromatic), 135.90, 140.20, 148.51 (C, C-aromatic), 169.60 (C, 1).

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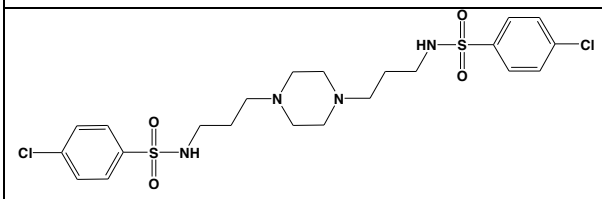
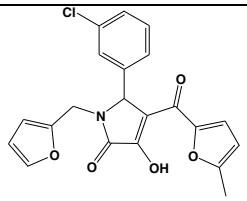
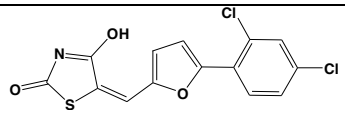
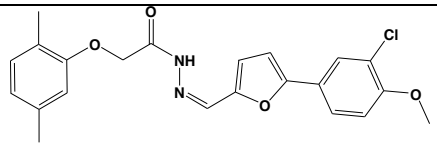
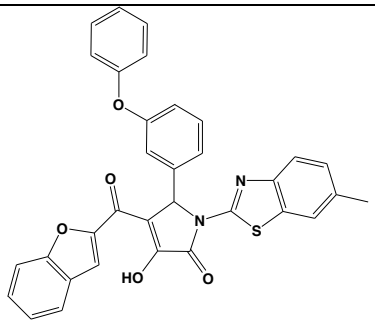
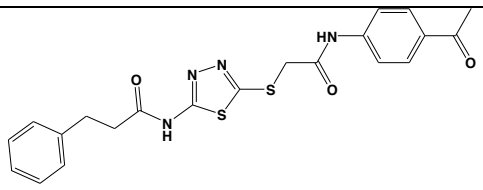
Appendix I

Structure and biological evaluation of purchased compounds

All the structures selected with virtual screening studies and purchased from the SPECS company were tested for their potential antiviral activity in the subgenomic replicon and cytostatic assays. Their structures and biological results are shown in tables 1-3.

Structure-based virtual screening on the enzyme closed conformation (3KQN)

Structure	Compound	EC_{50}(μM)	CC_{50}(μM)	EC_{90}(μM)	SI
	**12**	8	>90	89.4% inhibition at 90	>6.9
	**483**	30.6	>125.6	-	>4.1
	**484**	13.2	33.4	-	2.5
	**485**	26.4	>121.1	>121.1	>4.5
	**486**	41.8	>89.5	>89.5	>2.1
	**487**	>113.4	>113.4	>113.4	-

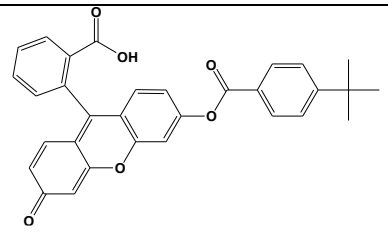
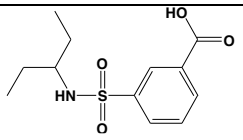
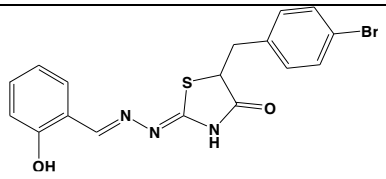
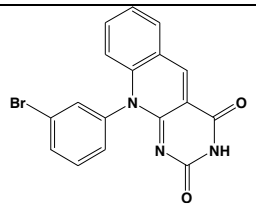
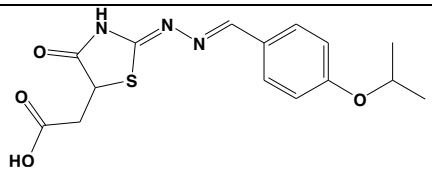
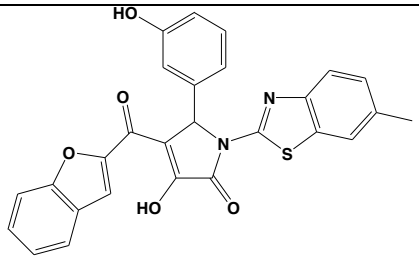
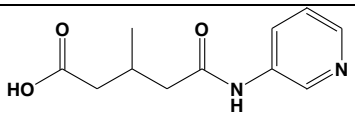
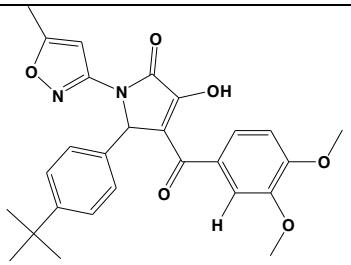
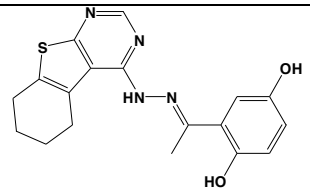
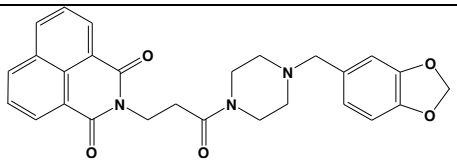
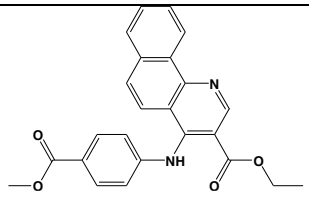
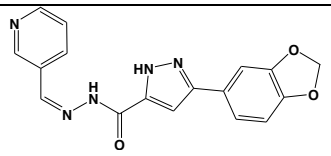
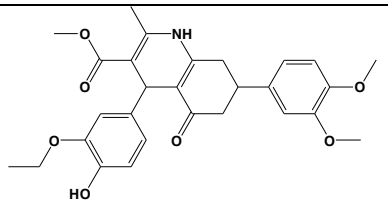
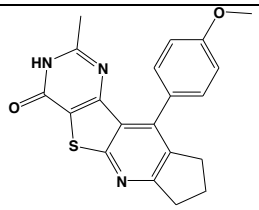
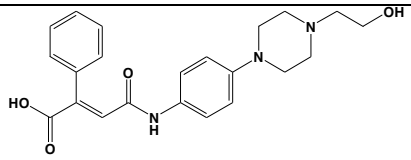
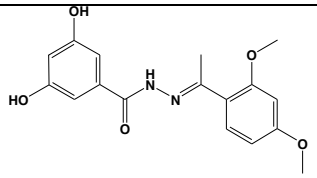
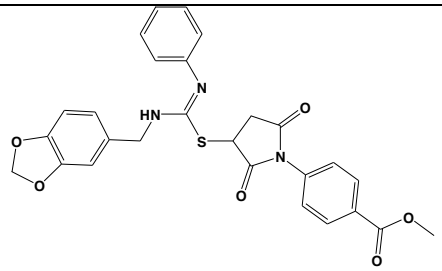
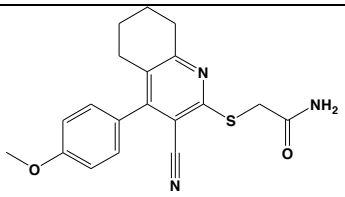
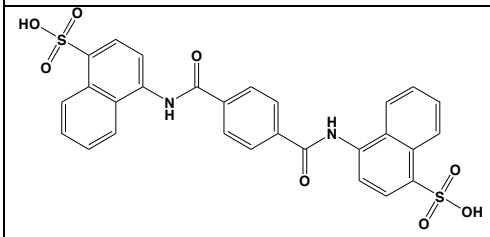
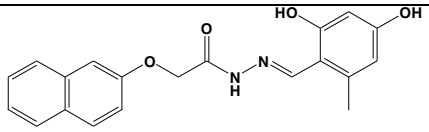
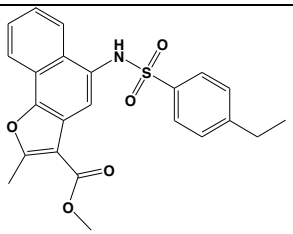
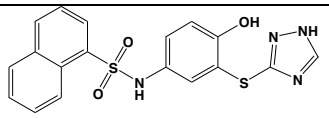
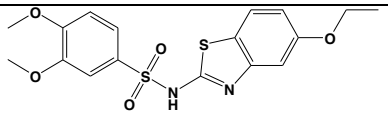
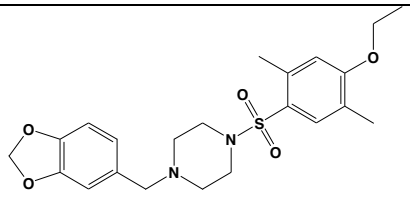
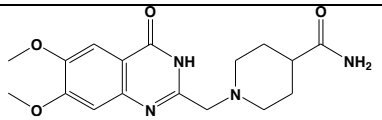
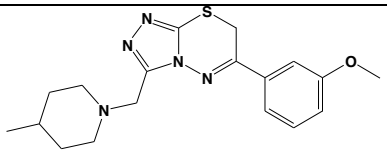
	**488**	>101.5	>101.5	>101.5	-
	**489**	>184.2	>184.2	>184.2	-
	**490**	10.4	15.7	-	1.5
	**491**	81.7	>135.8	-	>1.6
	**492**	126.7	>149	-	>1.1
	**493**	35.8	>103.6	-	>2.8
	**494**	>224.9	>224.9	>224.9	-
	**495**	57.2	>104.9	-	>1.8

Table 1: Antiviral and cytotoxicity data for compounds **12**, **483-495**

Structure-based virtual screening on the enzyme open conformation (3KQH)

Structure	Compound	EC_{50}(μM)	CC_{50}(μM)	EC_{90}(μM)	SI
	**187**	<1	>141	>116	>141
	**496**	32	>106	-	>3.3
	**497**	10.9	56.2	-	5.2
	**498**	>149	>149	>149	-
	**499**	36	>101	-	>2.8
	**500**	21	128	-	6.1
	**501**	65	>126	-	>.19
	**502**	>151	>151	>151	-

	**503**	12.8	62.6	-	4.9
	**504**	129.5	>141.5	>141.5	>1.1
	**505**	>86	>86	>86	-
	**506**	1.9	51.9	-	27.3
	**507**	1.1	4.7	-	4.3
	**508**	42.2	>125.5	-	>2.9
	**509**	>126.8	>126.8	>126.8	-
	**510**	34.5	>116	-	>3.4
	**511**	>144	>144	>144	-
	**512**	129.5	>139	>139	>1.1

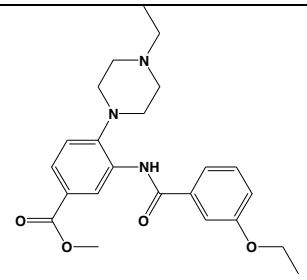
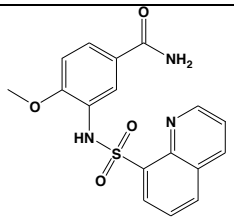
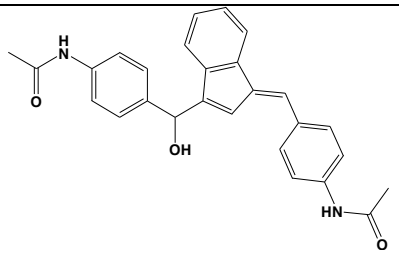
	**513**	38.9	61.7	-	1.6
	**514**	>139.9	>139.9	>139.9	-
	**515**	21.5	48.8	-	2.3

Table 2: Antiviral and cytotoxicity data for compounds **187**, **596-515**

Ligand-based virtual screening on triphenylmethane derivative 458

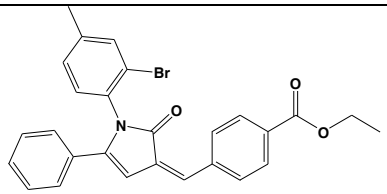
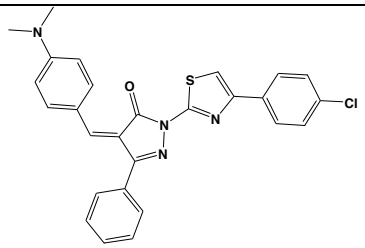
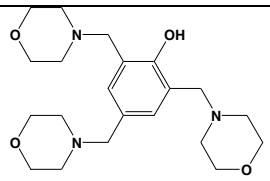
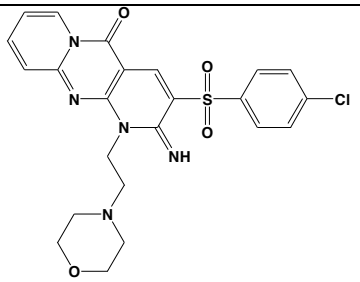
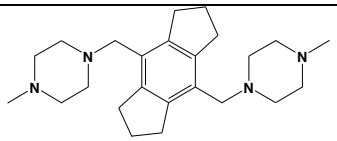
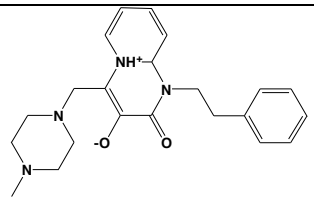
Structure	Compound	$EC_{50}(\mu M)$	$CC_{50}(\mu M)$	$EC_{90}(\mu M)$	SI
	**459**	38.3	>204.8	149.1	>5.3
	**516**	146.8	>206.2	>206.2	>1.4
	**517**	>255.4	>255.4	>255.4	-
	**518**	28	150.6	97.6	5.4
	**519**	7.7	36.9	-	4.8
	**520**	234.6	>264.2	>264.2	-

Table 3: Antiviral and cytotoxicity data for compounds **459**, **516-520**