



Azole chroman-4-one, tetralone and chromen-4-one derivatives: synthesis, *Mycobacterium tuberculosis* CYP121A1 enzyme inhibition and antimycobacterial activity

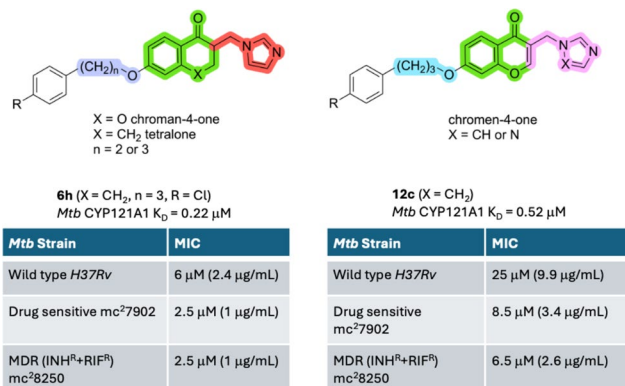
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Abstract

Tuberculosis (TB) remains a major global health challenge, driven by the emergence of drug-resistant *Mycobacterium tuberculosis* (*Mtb*) strains and the limitations of existing treatment regimens. Twenty CYP enzymes unique to *Mtb* represent an underexploited class of targets, offering opportunities for selective inhibition. Among these, CYP121A1 enzyme has been identified as a promising target. Three series of azole derivatives employing either a chroman-4-one, tetralone or chromen-4-one pharmacophore were synthesised and evaluated against *Mtb* CYP121A1 (binding affinity, K_D) and against wild type and resistant *Mtb* strains. From these three series, the tetralones with a propyloxy linked to a 4-F (**6g**) and 4-Cl (**6h**) benzene ring showed the most promising activity overall considering binding affinity (CYP121A1 K_D **6g** $0.18 \pm 0.1 \mu\text{M}$; **6h** $0.22 \pm 0.12 \mu\text{M}$, cf. cYY $12.3 \pm 1.1 \mu\text{M}$) and antimycobacterial activity against wild-type *H37Rv* strain (MIC **6g** $12.5 \mu\text{M}$, $2.7 \mu\text{g/mL}$; **6h** $6 \mu\text{M}$, $2.4 \mu\text{g/mL}$) and mc²7902 triple auxotrophic drug susceptible strain (MIC **6g** $2.6 \mu\text{M}$, $1 \mu\text{g/mL}$; **6h** $2.5 \mu\text{M}$, $1 \mu\text{g/mL}$) comparable with ethambutol and ethionamide. Importantly, the chroman-4-one and tetralone derivatives all retained antimycobacterial activity against isoniazid (INH), rifampicin (RIF) and INH+RIF resistant strains. Tetralone derivative **6h** and chromen-4-one **12c** displayed mycobacteriostatic properties and both compounds displayed favourable SI values in A459 cells and macrophages with the tetralone **6h** showing an improved therapeutic window with $\text{SI} > 10$. Type II direct CYP121A1 binding was generally supported by computational studies with all compounds near the haem. Generally, correlation was observed between binding affinity and antimycobacterial activity.



Keywords *Mycobacterium tuberculosis* · MtbCYP121A1 · Azoles · Drug resistance · Computational studies

Extended author information available on the last page of the article

Introduction

Recent WHO estimates of worldwide deaths by cause (2021), highlights tuberculosis (TB) as the world's tenth leading cause of death. Around one-quarter of the global population is infected with TB, and following the COVID-19 pandemic, it has reasserted itself as the leading cause of death from a single infectious agent [1, 2]. In 2024, about 10.7 million people were infected with TB and TB accounted for 1.23 million deaths [2]. In 2024, approximately 390,000 people developed rifampicin (RIF) resistant TB (RR-TB) and multidrug resistant TB (MDR-TB, defined as resistance to rifampicin and isoniazid) [2]. Long duration and complexity of TB treatment regimens are considered to be the main contributors of non-adherence and treatment related toxicity [2]. The emergence of *Mycobacterium tuberculosis* (*Mtb*) resistant strains has resulted from non-compliance with TB therapy and drug resistance poses a significant challenge in TB prevention and treatment [2]. The mechanisms of drug resistance in *Mtb* include drug target modification, prodrug inactivation, *Mtb* enzyme-mediated drug inactivation, over-expression of efflux pumps, and the cell wall permeability barrier [3, 4]. Latent and non-growing *Mtb* is also developing resistance to antibiotics [3], and most of the anti-TB agents, e.g. RIF, are unable to eradicate this dormant *Mtb* since they usually target functions related to the growth and multiplication of *Mtb*, which is missing in latent *Mtb* [3]. Therefore, there is an urgent need to conduct research on alternative *Mtb* targets for which no resistance exists in the population, to provide additional anti-TB drugs to add to the TB combination therapy approach [2, 5].

While studying the *Mtb* genome (*H37Rv*) for the purpose of finding new drug targets, 20 *Mtb* CYP enzymes were discovered [6]. The density of the CYP genes in the *Mtb* genome is about 200 times greater than in the human genome, indicating that these *Mtb* CYPs have essential biological roles [6, 7]. *Mtb* genome sequencing also demonstrated the significance of lipid metabolism in *Mtb*, as a substantial proportion of the coding capacity was devoted to enzymes involved in lipid synthesis or catabolism [7, 8]. The waxy cell wall architecture of *Mtb* is related to the highlighted significance of lipogenesis in *Mtb* [6, 7]. However, since the majority of the 20 *Mtb* CYP genes are not found in operons or gene clusters with known functions, their genomic dispersion and chromosomal location do not reveal anything about their biochemical roles.

One of the *Mtb* CYP enzymes of interest in drug design is CYP121A1 encoded by *Rv2276* and, although the function is unknown, it has been proposed that either inhibition of CYP121A1 may result in an accumulation of the natural substrate dicycloytyrosine (cYY) (Fig. 1A) resulting in

toxicity in *Mtb* or the metabolite, mycocylosin (Fig. 1A), may be involved in cell growth or structural stability of *Mtb* [9].

The CYP121A1 crystal structure of the ligand-free form was solved at high atomic resolution of 1.06 Å (PDB 1N40), and this was the first breakthrough in the enzyme characterisation [10, 11]. The second breakthrough was the identification of the enzyme substrate cYY [9, 10, 12]. The substrate binding site is large (1350 Å³) and saturated with water molecules, with a network of H-bonded amino acids [11]. The proximal 5th axial ligand, Cys345, binds to the haem Fe with a Fe-S bond distance of 2.30 Å, while the distance for the Fe-O (molecular oxygen in crystal structure PDB 1N40) bond is 2.21 Å. In the enzyme resting state, the 6th water ligand forms a H-bond with Ser237, while Ser237 binds to Ala233 and Arg386 [11]. The presence of the large and polar Arg386 residue at a close distance to the haem iron is a distinct feature of CYP121A1, which makes it a key residue [11]. Apart from Gln385 and Arg386, most of the residues in the CYP121A1 binding site cavity are hydrophobic in nature [11].

CYP121A1 secondary structure showed two main domains; helices and sheet rich domains; and the haem is buried in between (Fig. 1B). The loops above the catalytic cavity form an active site dome, which renders the cavity rigid and squeezed; however, a considerable part of the cavity is found protruding between the F and G helices (Fig. 1B), which was found to be the access channel to the enzyme active site [11]. In the ligand-free state, there is no clear visible access of the substrate to the buried active site [11]. Accordingly, the CYP121A1 structure has been proposed to oscillate between open and closed conformations, which are so-called “breathing motions” in between the F/G helices [10] (Fig. 1B). Amit et al. proposed that dimerisation of CYP121A1 is essential for enzyme function [13]. By engineering a monomer by double mutation of Ile180 in the G helix and Ile166 in the F helix by alanine residues, they found that the catalytic function decreased by 75% compared with the dimer, with accumulation of cYY and reduction in mycocylosin production [13].

The crystal structure of cYY-bound CYP121A1 was determined at 1.4 Å resolution (PDB 3G5H) [9] and in this crystal structure the proximal 5th axial ligand, Cys345, binds to the haem Fe with a Fe-S bond distance of 2.35 Å, while the distance between the 6th axial water ligand and Fe- is 2.44 Å. cYY was found to only partially occupy the catalytic cavity, remaining surrounded by water molecules (Fig. 1B/C). Structurally, cYY comprises a diketopiperazine (DKP) ring that is tethered between two tyrosyl rings. One tyrosine ring is orientated between helices F and G, whilst the other is directed toward the I helix, coordinating with the haem iron from the side [9] (Fig. 1B). The 6th axial water

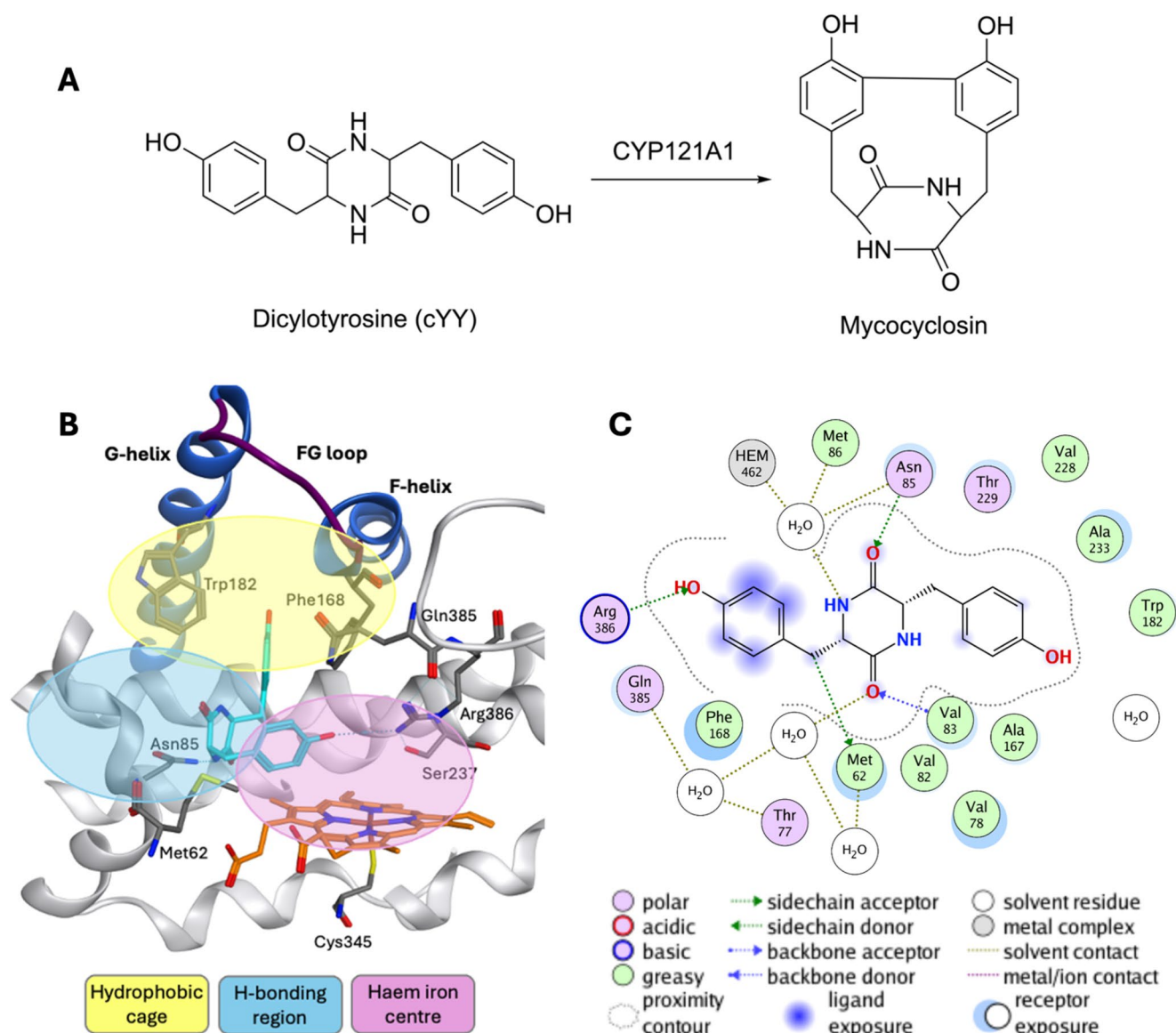


Fig. 1 **A** The oxidative coupling reaction of the two tyrosine rings of cYY catalysed by CYP121A1 [36] **B** The three main binding areas in *Mtb* CYP121A1 active site and the F and G α -helices are labelled

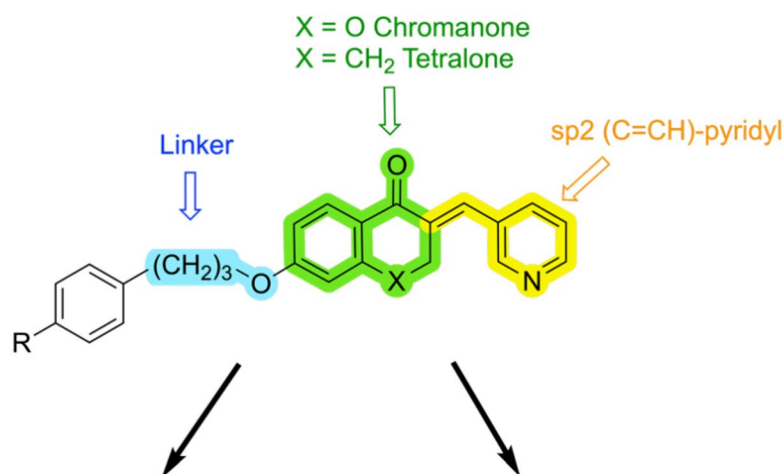
[haem in orange and cYY in cyan] **C** 2D image of cYY-bound CYP121A1 (PDB 3G5H), showing binding interactions with the key amino acid residues

ligand is not displaced upon substrate binding and cYY induces a blue shift in the Soret band from 416 nm to 395 nm upon binding to CYP121A1 (type I spectral response) [14].

The azole antifungal drugs clotrimazole, miconazole, and econazole were initially evaluated against CYP121A1 and displayed MIC values of 11.0 $\mu\text{g/mL}$ for clotrimazole and 8.0 $\mu\text{g/mL}$ for miconazole and econazole [8]. This led to the design of other CYP121A1 inhibitors containing an azole group including pyrazole, imidazole and triazole [15–20] or a haem-binding N-containing heterocycle e.g. pyridine [21, 22]. We recently reported the design and biological evaluation of 3-(pyridine-3-ylmethylene)chroman-4-one

and tetralone derivatives with a propyloxy linker (Fig. 2A) optimal for activity [22]. However, a clear distinction was observed between the chromanone and tetralone derivatives (Fig. 2A) with the chromanone derivatives ($X = \text{O}$, Fig. 2A) showing potent CYP121A1 binding affinity ($R = \text{Cl}$, K_D 0.5 ± 0.01 M; $R = \text{OCH}_3$, K_D 0.32 ± 0.04 μM) compared with the natural substrate cYY (K_D 16.9 ± 0.1 μM) while the tetralone derivatives displayed negligible binding affinity. In contrast, the tetralone derivatives ($X = \text{CH}_2$, Fig. 2A) displayed improved antimycobacterial activity ($R = \text{Cl}$ or OCH_3 , MIC 3 μM , 1.2 $\mu\text{g/mL}$) compared with the chromanone derivatives ($R = \text{Cl}$, MIC 50 μM , 20.3 $\mu\text{g/mL}$; $R =$

Pyridine derivatives

A

Imidazole and Triazole derivatives

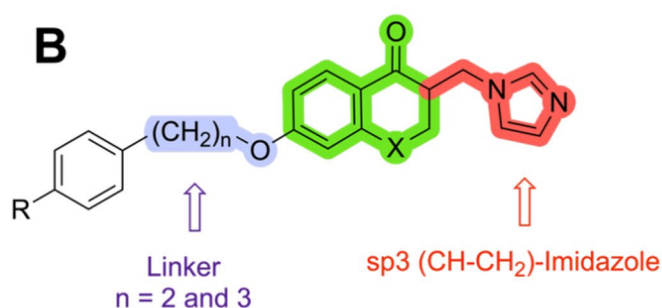
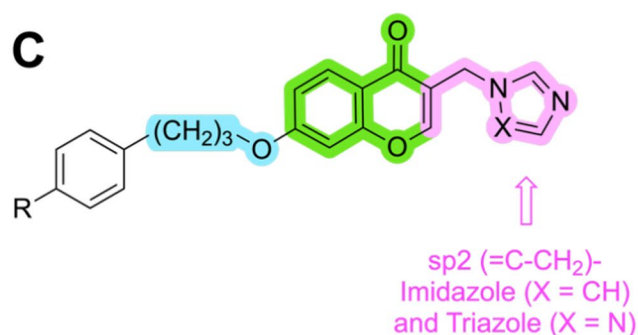
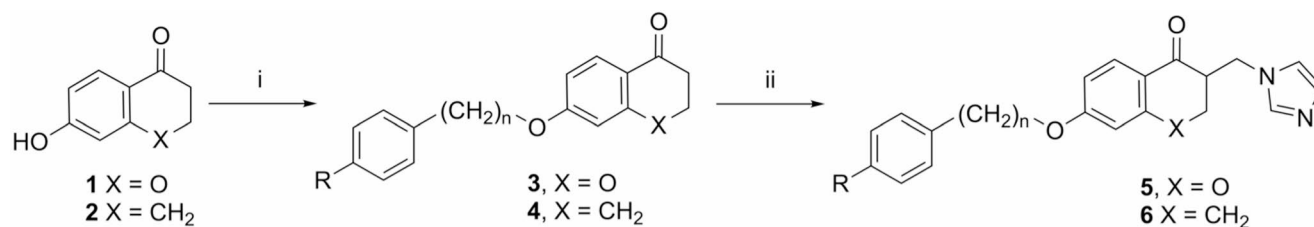
B**C**

Fig. 2 Modification of lead pyridine derivatives (**A**) to evaluate effect of linker, azole (imidazole and triazole) and hybridisation (restriction vs. free rotation) (**B** and **C**) on CYP121A1 binding and antimycobacterial activity



Scheme 1 Synthesis of chroman-4-one (**5**) and tetralone (**6**) derivatives. *Reagents and conditions:* (i) phenethyl/phenylpropyl bromide, acetone, K₂CO₃, 60 °C, overnight, 29–95%; (ii) L-proline, (HCHO)_n, HCl, imidazole, DMSO, 85 °C, overnight, 22–46%

OCH₃, MIC 25 μM, 10.0 μg/mL) against the *H37Rv Mtb* reference strain.

To explore the differences observed, the pyridine derivative structure was modified to first investigate the effect of changing the restricted sp² hybridised (C=CH)-pyridine with a more flexible sp³ hybridised (CH-CH₂)-imidazole linker and also prepare compounds with both the ethyloxy (*n*=2) and propyloxy (*n*=3) linker (Fig. 2B). Following this, a more direct comparison with the restricted sp² hybridised (=C-CH₂)-imidazole derivatives (Fig. 2C) will allow determination on the effect of hybridisation on binding interactions (*K_D* and MIC) with the triazoles added (Fig. 2C) to allow comparison between pyridine, imidazole and triazole.

Results and discussion

Synthesis

General procedure for the preparation of imidazole chroman-4-one (**5**) and tetralone (**6**) derivatives

The phenylalkoxy derivatives (**3** and **4**) were prepared by alkylation of 7-hydroxychroman-4-one (**1**) and 7-hydroxytetralone (**2**) as previously described [22] (Scheme 1). The imidazoles (**5** and **6**) were prepared by Mannich reaction, with the Mannich salt first formed on reaction of L-proline [23] and paraformaldehyde with the addition of 0.1 M

equivalent of HCl as catalyst in dry DMSO and heating at 80 °C for 1 h, followed by reaction of the resulting Mannich salt with the phenylalkoxy derivatives (**3** and **4**) for a further 1 h. Imidazole was then added and the reaction stirred at 60 °C overnight to give the required products (**5** and **6**) in low to moderate yields (Scheme 1, Table S1) after purification by gradient column chromatography as the reaction did not go to completion.

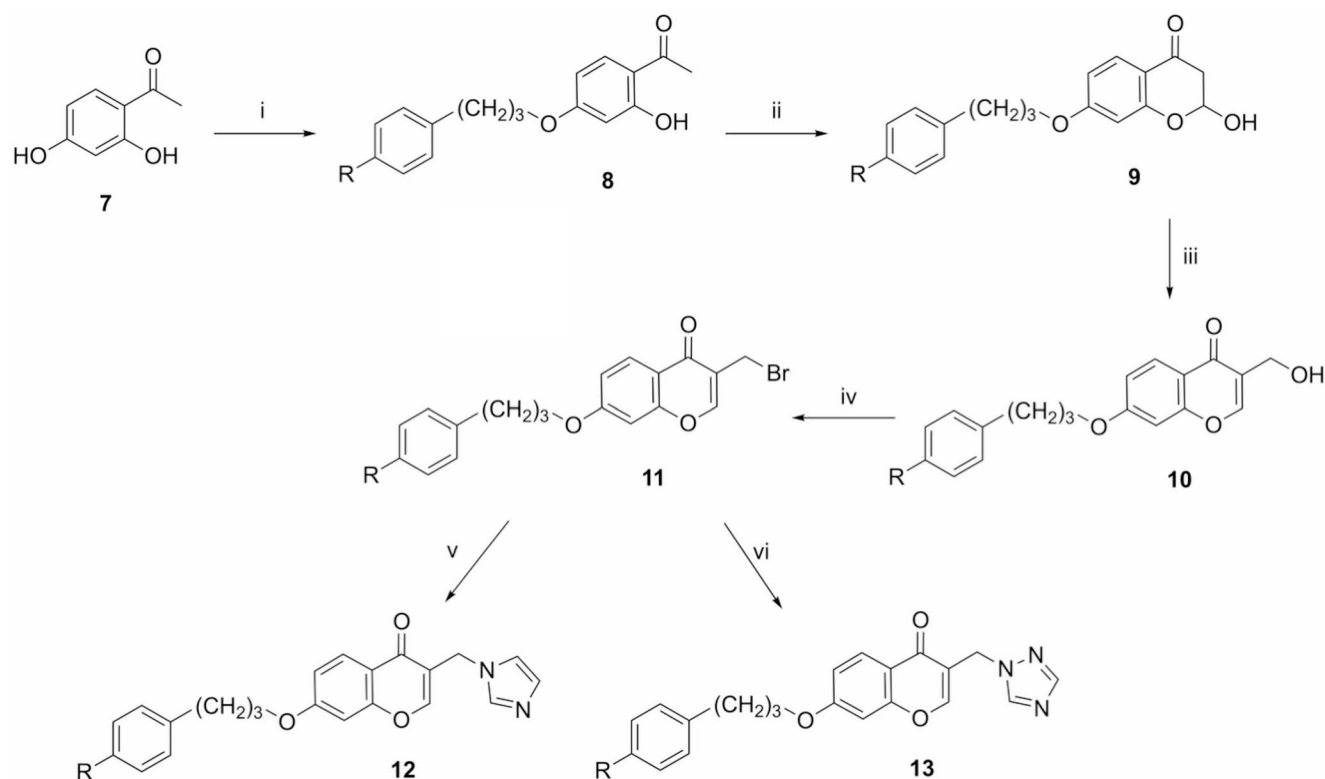
General procedure for the preparation of imidazole chromen-4-one (**12**) and triazole chromen-4-one (**13**) derivatives

The 3-(imidazole/triazole)-7-alkoxy-4*H*-chromen-4-one derivatives (**12**, **13**) were prepared in five steps starting from 2,4-dihydroxyacetophenone (**7**) (Scheme 2). Reaction of **7** with phenylpropyl bromides and K₂CO₃ as base gave the alkylated derivatives (**8**) then the 2-hydroxy-chroman-4-one derivatives (**9**) were obtained by Claisen condensation of **8** with ethyl formate. Reaction of the 2-hydroxy-chroman-4-ones (**9**) with formaldehyde and NaOAc followed by a dehydration reaction with HCl gave the 3-hydroxymethyl derivatives (**10**) [24, 25]. Bromination of the hydroxy group with PBr₃ gave the bromides (**11**), which on treatment with

either the imidazole or triazole anion, generated *in situ* by reaction of imidazole/triazole with K₂CO₃, gave the final imidazole (**12**) and triazole (**13**) products in high yield (Scheme 2, Table S2).

UV-Vis spectral CYP121A1 binding

CYP121A1 binding affinity was measured using a UV-Vis spectral assay [26]. All imidazole chroman-4-one derivatives (**5**) induced a clear shift of the Soret band, with the largest shift observed with the chloro (**5h**) and bromo (**5i**) propyloxy derivatives, which moved from 414 to 422 nm (Table 1). This behaviour is characteristic of a Type II spectral response, consistent with displacement of the 6th axial water ligand and direct coordination of the imidazole nitrogen to the haem iron. All imidazole chroman-4-one derivatives exhibited stronger binding to CYP121A1 compared with the natural substrate cYY (K_D = 12.3 ± 1.1 μM), with K_D values ranging from 0.76 to 5.9 μM (Table 1). In general, the propyloxy derivatives exhibited better binding compared with the ethoxy derivatives, indicating the importance of extending the linker for improving hydrophobic interactions and positioning the substituted aryl in the hydrophobic cage between the F and G helices. Among the



Scheme 2 Synthesis of chromen-4-one derivatives (**12** and **13**). **Reagents and conditions:** (i) phenylpropyl bromides, acetone, K₂CO₃, 60 °C, overnight, 40–72% (ii) (a) NaOMe, ethyl formate, THF, r.t. 30 min (b) H₂O, AcOH, r.t., 10 min (iii) (a) NaOAc, 37% aq. HCHO,

acetone, r.t. 2 h (b) c. HCl, r.t. overnight, 56–69% over 2 steps (iv) PBr₃, CH₂Cl₂, r.t. 1 h, 90–100% (v) (a) imidazole, K₂CO₃, CH₃CN, 45 °C, 1 h (b) **11**, CH₃CN, 70 °C, overnight, 76–96% (vi) (a) triazole, K₂CO₃, CH₃CN, 45 °C, 1 h (b) **11**, CH₃CN, 70 °C, overnight, 84–96%

Table 1 Soret band shift and binding affinity (K_D) values of final compounds **5**, **6**, **12** and **13**

Cmpd	<i>n</i>	<i>R</i>	X	Soret shift (nm)	K_D (μ M)
5a	2	H	O	415 to 419	5.9 ± 0.4
5b	2	F	O	414 to 420	3.2 ± 0.04
5c	2	Cl	O	414 to 420	2.2 ± 0.04
5d	2	Br	O	415 to 420	0.76 ± 0.4
5e	2	OMe	O	414 to 418	4.0 ± 0.05
5f	3	H	O	414 to 420	4.8 ± 0.05
5g	3	F	O	414 to 420	1.9 ± 0.05
5h	3	Cl	O	414 to 422	2.2 ± 0.05
5i	3	Br	O	414 to 422	1.0 ± 0.04
5j	3	OMe	O	414 to 421	2.6 ± 0.05
6a	2	H	CH ₂	415 to 419	0.58 ± 0.12
6b	2	F	CH ₂	415 to 420	0.44 ± 0.02
6c	2	Cl	CH ₂	415 to 420	0.40 ± 0.1
6d	2	Br	CH ₂	414 to 420	0.75 ± 0.04
6e	2	OMe	CH ₂	415 to 418	0.17 ± 0.03
6f	3	H	CH ₂	415 to 420	0.35 ± 0.07
6g	3	F	CH ₂	415 to 420	0.18 ± 0.1
6h	3	Cl	CH ₂	415 to 421	0.22 ± 0.12
6i	3	Br	CH ₂	414 to 422	0.87 ± 0.04
6j	3	OMe	CH ₂	415 to 422	0.09 ± 0.0
12a	-	H	-	416 to 420	1.33 ± 0.06
12b	-	F	-	416 to 420	0.94 ± 0.27
12c	-	Cl	-	416 to 420	0.52 ± 0.4
12d	-	Br	-	416 to 421	0.08 ± 0.06
12e	-	OMe	-	415 to 417	0.37 ± 0.13
13a	-	H	-	415 to 417	5.1 ± 0.38
13b	-	F	-	415 to 418	0.1 ± 0.07
13c	-	Cl	-	416 to 418	0.22 ± 0.04
13d	-	Br	-	415 to 418	1.80 ± 0.43
13e	-	OMe	-	415 to 420	1.77 ± 0.06
cYY	-	-	-	417 to 395	12.3 ± 1.1

chroman-4-one series, the bromo derivatives (**5d**, $K_D = 0.76 \pm 0.4 \mu\text{M}$) and (**5i**, $K_D = 1.0 \pm 0.04 \mu\text{M}$) showed the tightest binding, followed by the fluoro (**5g**, $K_D = 1.9 \pm 0.05 \mu\text{M}$) and the chloro (**5h**, $K_D = 2.2 \pm 0.05 \mu\text{M}$) derivatives. The weakest binding was observed for the unsubstituted derivatives (**5a**, $K_D = 5.9 \pm 0.4 \mu\text{M}$; **5f**, $K_D = 4.8 \pm 0.047 \mu\text{M}$), while the methoxy derivative (**5j**, $K_D = 2.6 \pm 0.045 \mu\text{M}$) moderately binds to the enzyme.

The comparable tetralone derivatives (**6**) also induced a clear shift of the Soret band, with the largest shift observed with the bromo derivative (**6i**), which moved from 414 to 422 nm (Table 1). As seen with the chroman-4-one derivatives (**5**), the propyloxy derivatives generally exhibited better binding compared with the ethoxy counterparts and the tetralones (**6**) outperformed the chroman-4-one derivatives (**5**) for binding affinity (Table 1). Optimal binding was observed for the ethoxy (**6e**, $K_D = 0.17 \pm 0.03 \mu\text{M}$) and propyloxy (**6j**, $K_D = 0.09 \pm 0.0 \mu\text{M}$) linked methoxy derivatives, followed by the propyloxy linked fluoro (**6g**, $K_D = 0.18 \pm 0.1 \mu\text{M}$) and chloro (**6h**, $K_D = 0.22 \pm 0.12 \mu\text{M}$) derivatives.

All imidazole (**12a-e**) and triazole (**13a-e**) chromen-4-one derivatives showed stronger binding to CYP121A1 compared with their corresponding chroman-4-one analogues (**5f-j**) (Table 1). With the exception of the methoxy derivative (**12e**), the imidazole derivatives (**12**) shifted the Soret band to longer wavelength (red shift), with the largest shift observed for the bromo derivative (**12d**), which moved from 416 to 421 nm, indicating the displacement of the 6th axial water molecule and a direct haem iron coordination. Among the imidazole chromen-4-one series, the tightest binding was observed for the bromo derivative (**12d**, $K_D = 0.08 \pm 0.06 \mu\text{M}$), whereas the unsubstituted phenyl derivative (**12a**, $K_D = 1.33 \pm 0.06 \mu\text{M}$) showed the weakest affinity. In contrast, the triazole derivatives (**13**) produced only slight red shifts of the Soret band (+2–3 nm), suggesting modest perturbations of the haem environment rather than strong Type II coordination. The exception was the methoxy derivative (**13e**), which exhibited a larger shift to 420 nm (+5 nm). Within this series, the fluoro derivative (**13b**, $K_D = 0.1 \pm 0.07 \mu\text{M}$) bound most tightly to CYP121A1, while the unsubstituted phenyl derivative (**13a**, $K_D = 5.1 \pm 0.38 \mu\text{M}$) again showed the weakest binding. These findings for chroman-4-one (**5**), tetralone (**6**) and chromen-4-ones (**12** and **13**) emphasise that phenyl substitution significantly influences binding strength, thereby contributing to more favourable ligand CYP121A1 interactions.

Antimycobacterial activity

All the final chroman-4-one (**5**), tetralone (**6**) and chromen-4-one (**12** and **13**) derivatives were evaluated against *Mtb* reference strain *H37Rv* using the SPOTi method [27] and also against Albert Einstein College of Medicine mc^2 ($\Delta panCD \Delta leuCD \Delta argB$ triple auxotrophic strains of *Mtb H37Rv*) drug sensitive and rifampicin resistant (RIF^R), isoniazid resistant (INH^R) and MDR (RIF^R and INH^R) strains [28] (strains detailed in Table S3) by REMA assay [29]. The standard drugs used for comparison were the first line anti-TB drugs isoniazid, rifampicin and ethambutol. The tetralone derivatives with the ethoxy or propyloxy linker showed comparable antimycobacterial activity. The ethoxy linked chloro (**6c**) and bromo (**6d**) tetralones displayed optimal antimycobacterial activity against all the mc^2 strains (drug sensitive and drug/multidrug resistant) (**6c**, MIC 2.63 μM , 1 $\mu\text{g/mL}$; **6d**, MIC 2.35 μM , 1 $\mu\text{g/mL}$ vs. mc^2 drug sensitive strain and MIC 4.70 μM , 2 $\mu\text{g/mL}$ vs. drug/multidrug resistant mc^2 strains). The propyloxy linked fluoro (**6g**, MIC 2.64 μM , 1 $\mu\text{g/mL}$), chloro (**6h**, MIC 2.53 μM , 1 $\mu\text{g/mL}$) and bromo (**6i**, MIC 2.28 μM , 1 $\mu\text{g/mL}$) tetralones were optimal with retention of antimycobacterial activity against all mc^2 drug/multidrug resistant strains (Table 2 and Table S4).

Table 2 MIC (μM) of the final imidazole chroman-4-one (**5**) and tetralone derivatives (**6**)

Cmpd	R	X	MIC ^a (μM)					
			H37Rv (wild type)	mc ² 7902 (susceptible)	mc ² 8247 (RIF ^R)	mc ² 8245 (INH ^R)	mc ² 8250 (RIF ^R & INH ^R)	mc ² 8256 (RIF ^R & INH ^R)
<i>n</i> =2								
5a	H	O	125	81.5	64.2	74.3	71.3	74.0
5b	F	O	50	21.8	21.8	21.8	21.8	21.8
5c	Cl	O	50	20.9	20.9	20.9	20.9	20.9
5d	Br	O	125	54.1	34.9	45.8	33.5	38.1
5e	OMe	O	50	42.3	42.3	42.3	42.3	42.3
6a	H	CH ₂	3	11.6	11.6	11.6	11.6	11.6
6b	F	CH ₂	6	11.0	5.5	5.5	5.5	5.5
6c	Cl	CH ₂	50	2.6	2.6	2.6	2.6	2.6
6d	Br	CH ₂	25	2.4	4.7	4.7	4.7	4.7
6e	OMe	CH ₂	25	21.3	21.3	21.3	21.3	21.3
<i>n</i> =3								
5f	H	O	50	22.1	22.1	22.1	22.1	22.1
5g	F	O	50	21.0	21.0	21.0	21.0	21.0
5h	Cl	O	50	10.1	10.1	10.1	10.1	10.1
5i	Br	O	50	9.1	9.1	9.1	9.1	9.1
5j	OMe	O	50	20.4	20.4	20.4	20.4	20.4
6f	H	CH ₂	25	5.6	5.6	5.6	5.6	5.6
6g	F	CH ₂	12.5	2.6	2.6	2.6	2.6	2.6
6h	Cl	CH ₂	6	2.5	2.5	2.5	2.5	2.5
6i	Br	CH ₂	12.5	2.3	2.3	2.3	2.3	2.3
6j	OMe	CH ₂	25	5.1	5.1	5.1	5.1	5.1
Isoniazid			1.8	0.7	0.7	NA	NA	NA
Rifampicin			0.3	0.1	NA	0.1	NA	NA
Ethambutol			2.45	2.5	4.9	4.9	4.9	2.5

^a Results are the average of two independent experiments. NA: no activity

Table 3 MIC (μM) of the final imidazole (**12**) and triazole (**13**) chromen-4-one derivatives

Cmpd	R	MIC ^a (μM)					
		H37Rv (wild type)	mc ² 7902 (susceptible)	mc ² 8247 (RIF ^R)	mc ² 8245 (INH ^R)	mc ² 8250 (RIF ^R & INH ^R)	mc ² 8256 (RIF ^R & INH ^R)
12a	H	25	27.6±5.1	29.9±9.4	61.3±21.9	29.3±8.2	30.3±7.5
12b	F	12.5	18.8±3.1	15.0±1.5	45.9±12.8	14.3±1.3	17.9±3.3
12c	Cl	25	8.5±1.9	7.4±0.8	30.5±11.1	6.5±1.0	7.4±1.4
12d	Br	25	24.4±6.0	22.0±2.6	42.5±9.9	21.5±2.4	21.0±4.6
12e	OMe	25	28.6±6.0	20.6±4.0	32.8±1.2	19.8±2.9	29.5±9.9
13a	H	>200	NA	NA	NA	NA	NA
13b	F	>200	NA	NA	NA	NA	NA
13c	Cl	>200	NA	NA	NA	NA	NA
13d	Br	>200	NA	NA	NA	NA	NA
13r	OMe	>200	NA	NA	NA	NA	NA
Isoniazid		1.8	1.2±0.0	1.0±0.0	-	-	-
Rifampicin		0.3	0.1±0.0	-	0.1±0.0	-	-
Ethambutol		2.45	10.3±0.4	13.9±0.4	12.0±1.1	11.6±0.2	12.0±0.3

[a] H37Rv results are the average of two independent experiments, while assays for the mc² strains were performed in triplicate. NA: no activity

The imidazole chromen-4-one derivatives (**12**) showed antimycobacterial activity against mc²7902 drug sensitive strain comparable with their equivalent chroman-4-ones (**5**) with the chloro derivative (**12c**, MIC 8.47 μM , 3.35 $\mu\text{g}/\text{mL}$) showing optimal activity in this series (Table 3 and Table S5). All the imidazole chromen-4-ones (**12**) retained

activity against the rifampicin resistant (RIF^R) and MDR (RIF^R and INH^R) strains, however a reduction in antimycobacterial activity was observed against the isoniazid (INH^R) strain. None of the triazole chromen-4-one derivatives (**13**) showed any antimycobacterial activity against the *Mtb* strains (MIC>200 $\mu\text{g}/\text{mL}$) (Table 3 and Table S5).

Structure-activity relationships

Halide substitution (F, Cl, Br) in the phenyl ring was optimal considering both binding affinity (K_D) and antimycobacterial activity (MIC), although Br may be more sterically limited for binding affinity. The 4-methoxy substitution showed improved binding affinity for the tetralone derivatives (**6e** and **6j**) but was sub-optimal for the chroman-4-one (**5e** and **5j**) and chromen-4-one (**12e**) derivatives (Table 1). The propyloxy linker was optimal for antimycobacterial activity for the chroman-4-one (**5**) and tetralone (**6**) derivatives. The tetralones (**6**) harbouring halide substitutions consistently displayed better antimycobacterial activity against *Mtb* H37Rv wild type and mc²7902 drug susceptible strain compared with the chroman-4-ones (**5**) (Table 2 and Table S4).

Comparison of the previously described pyridine derivatives [22] with the imidazole and triazole derivatives is presented using the 4-chlorophenyl propyloxy derivatives as exemplars (Fig. 3).

Introduction of the imidazole moiety in the chroman-4-one (**5h**) and in the chromen-4-one (**12c**) in place of the pyridyl group (**Pyr1**) resulted in comparable antimycobacterial activity against *H37Rv* wild type and mc²7902 drug sensitive *Mtb* strains, however introduction of the triazole moiety in the chromen-4-one (**13c**) resulted in the loss of antimycobacterial activity (Fig. 3). Maintaining a sp² hybridised carbon, present in the pyridine derivative (**Pyr1**), resulted in retention of binding affinity with the chromen-4-one derivatives (K_D (μ M): **Pyr1** 0.5 ± 0.01 ; **12c** 0.52 ± 0.4 ; **13c** 0.22 ± 0.44), while introduction of the more flexible

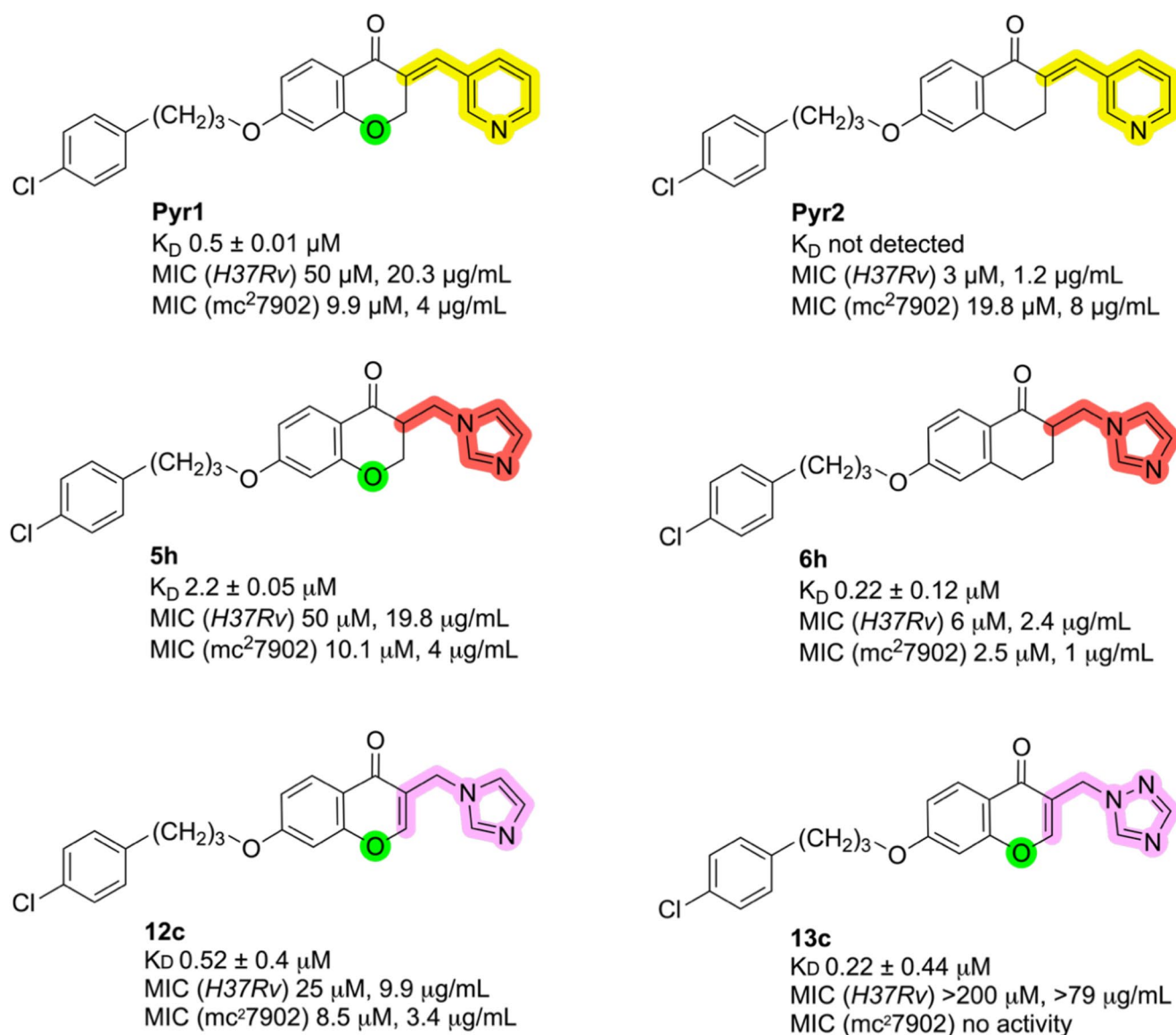


Fig. 3 Comparison of *Mtb* CYP121A1 binding affinity (K_D) and antimycobacterial activity (MIC) against *H37Rv* wild type and mc²7902 drug sensitive *Mtb* strains of exemplar compounds

Table 4 MBC₉₅ of **6h** and **12c** compared with RIF, INH and LZD

Cmpd	Antibacterial (μM)		
	MIC ₉₅	MBC ₉₅ CFU/mL	MBC ₉₅ Regrowth
6h	2.85	119.36	67.25
12c	3.43	59.44	52.53
RIF	0.04	0.32	0.22
INH	1.84	4.14	2.78
LZD	1.18	61.64	10.36

RIF – rifampicin; INH – isoniazid; LZD – linezolid

sp³ hybridised -CHCH₂-imidazole resulted in a reduction in binding affinity (**5h**, K_D = 2.2 ± 0.0 μM) (Fig. 3), however it should be noted that all the derivatives **5**, **6**, **12** and **13** displayed tighter binding compared with the natural substrate cYY (K_D = 12.3 ± 1.1 μM) (Table 1). The previously described pyridine tetralone derivatives [22] displayed the more promising antimycobacterial activity compared with the pyridyl chroman-4-one derivatives as illustrated by the exemplars **Pyr1** and **Pyr2** (Fig. 3). The imidazole tetralone derivatives (**6**) also showed improved antimycobacterial activity compared with the imidazole chroman-4-ones (**5**) and imidazole chromen-4-ones (**12**). CYP121A1 binding affinity was the most notable difference between the pyridyl tetralones (e.g. **Pyr2**), for which no detectable binding affinity was observed [22], and the imidazole tetralones (Table 3), which displayed tight binding affinity (e.g. **6h**, K_D = 0.22 ± 0.12 μM) (Fig. 3).

Minimum bactericidal concentration (MBC₉₅)

Two compounds, the propyloxy extended chloro tetralone derivative (**6h**) and the most potent of the chromen-4-one derivatives (**12c**) were evaluated for minimum bactericidal concentration (MBC₉₅) against *Mtb* mc²7902. The bactericidal activity was examined using a regrowth-based assay and a colony-forming unit (CFU/mL) based MBC determination with RIF, INH and linezolid (LZD) used as cidal, cidal, and static controls, respectively. Results from both assays showed that derivatives **6h** and **12c** displayed mycobacteriostatic properties with the calculated MBC/MIC ratio > 10-fold, which is above the threshold (≤ 4) commonly used to define bactericidal activity [30] (Table 4).

Cytotoxicity

The cytotoxicity of compounds **6h** and **12c** was evaluated against A549 alveolar type II epithelial cells and uninfected RAW264.7 macrophages, following 96 h exposure with cell viability measured using standard MTS analysis [31]. The IC₅₀ was calculated to assess potential toxicity, drug efficacy, and corresponding selectivity index (SI) (Table 5). The tetralone **6h** showed IC₅₀ values of 34.79 μM and 40.06 μM against A549 and RAW264.7 cells, respectively, while

Table 5 Antibacterial, cytotoxicity and selectivity indices of **6h** and **12c**

Cmpd	MIC ₉₅ (M)	Cytotoxicity (IC ₅₀)		S.I. (IC ₅₀ /MIC ₉₅)	
	mc ² 7902	A549	RAW264.7	A549	RAW264.7
6h	2.85	34.79	40.06	12.23	14.08
12c	3.43	27.83	30.44	8.11	8.85

chromen-4-one **12c** showed IC₅₀ values of 27.83 μM and 30.44 μM, respectively. Considering the bacterial MIC₉₅ values (**6h**, 2.85 μM; **12c**, 3.43 μM), both compounds displayed favourable SI values in A459 cells and macrophages with the tetralone **6h** showing an improved therapeutic window with SI > 10 compared with chromen-4-one **12c**.

Computational

Molecular docking analyses were performed using Molecular Operating Environment (MOE) software [32], with chroman-4-one (**5**), tetralone (**6**) and chromen-4-one (**12** and **13**) derivatives docked into the crystal structure of *Mtb* CYP121A1 co-crystallised with a piperazine azole derivative (PDB ID: 5O4K) [33] to produce the corresponding protein–ligand complexes. The optimal protein–ligand complexes, determined by ligand position, key binding interactions and energy value, were subjected to 200 ns MD simulations using the Desmond module of the Schrödinger software [34] to assess the stability and dynamic behaviour of the protein–ligand complexes.

Almost all the ethoxy linker chroman-4-one derivatives (**5a**–**5e**) displaced the 6th axial water ligand and formed a direct interaction between the imidazole nitrogen and the haem iron, with Fe–N distances ranging from 2.34 to 2.48 Å. Across the series, the ligands adopted binding orientations comparable with cYY, stabilised by hydrophobic interactions contributed by the chroman-4-one scaffold or the attached phenyl substituents. The propyloxy linker derivatives (**5f**–**5j**) exhibited an interaction profile similar to the ethoxy-linked analogues. Most compounds coordinated directly to the haem iron, displaying comparable Fe–N distances ranging from 2.34 to 2.42 Å. This direct binding was complemented by a range of stabilising interactions involving both the chroman-4-one core and the phenyl substituents through water mediated interactions and hydrophobic contacts. Ligands **5c** and **5f** (*R*- and *S*-enantiomers) are presented as exemplars for the ethoxy and propyloxy linker chroman-4-one derivatives.

The *R*-**5c** enantiomer adopted a conformation that oriented the imidazole moiety perpendicular to the haem plane, allowing the imidazole nitrogen to form a direct and stable coordination with the haem iron at a distance of 2.48 Å (Fig. 4). In this orientation, the chroman-4-one carbonyl oxygen was optimally positioned to establish a network of

Fig. 4 A 3D image of binding interactions of compound *R-5c* in *Mtb* CYP121A1 active site at the end of the 200 ns MD simulation; haem (brown), *R-5c* (cyan), amino acid residues (grey) and water molecules (red spheres), (B) 2D image of derivative *R-5c* binding interactions with *Mtb* CYP121A1 active site residues

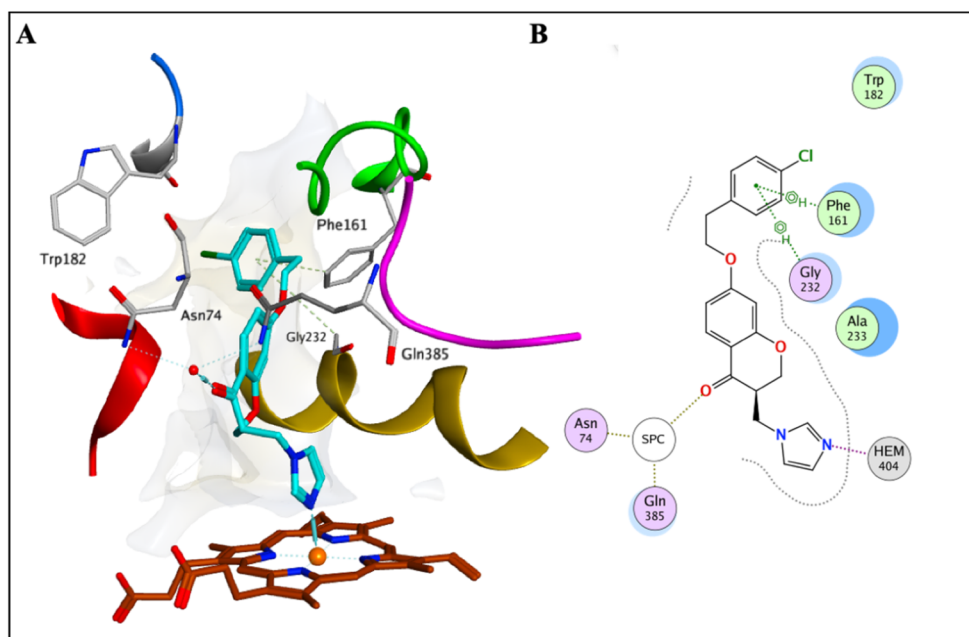
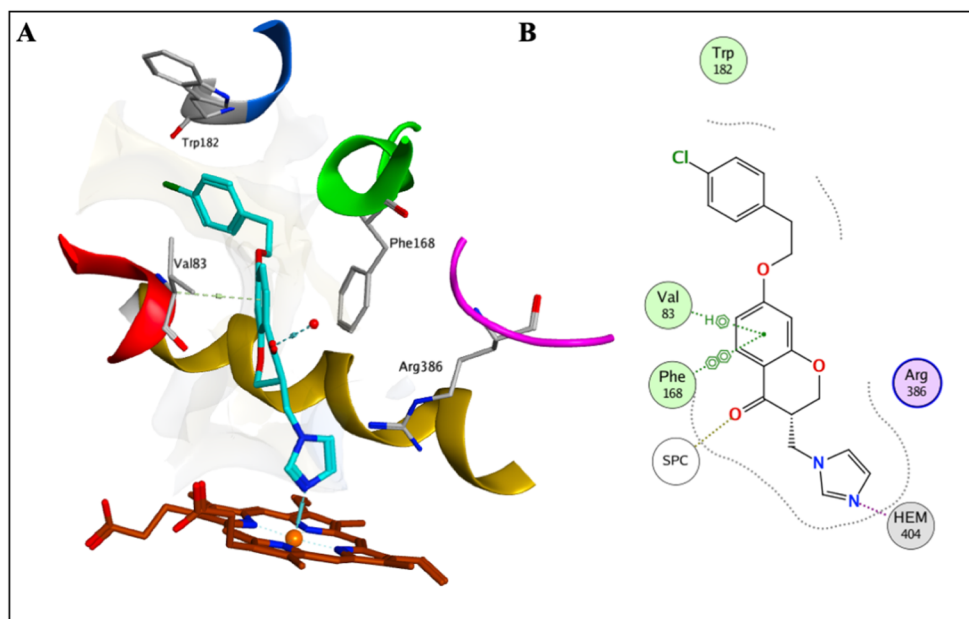


Fig. 5 A 3D image of binding interactions of compound *S-5c* in *Mtb* CYP121A1 active site at the end of the 200 ns MD simulation; haem (brown), *S-5c* (cyan), amino acid residues (grey) and water molecules (red spheres), (B) 2D image of derivative *S-5c* binding interactions with *Mtb* CYP121A1 active site residues



water mediated hydrogen bonds with two essential residues Gln385 and Asn74 both of which contribute to stabilising the CYP121A1 substrate. Moreover, the binding orientation positioned the phenyl substituent within the hydrophobic pocket. In this region, the substituted aryl ring engaged in a π -hydrogen interaction with Phe161 on the F-helix, thereby strengthening the hydrophobic packing. Additional stabilisation was provided by further π -hydrogen contacts with Gly232 on the I-helix, effectively securing the ligand within the binding site. The *S-5c* enantiomer adopted a binding orientation in which the imidazole nitrogen coordinated directly to the haem iron, forming a stable axial interaction

with an N-Fe distance of 2.38 Å (Fig. 5). However, this conformation positioned the chroman-4-one carbonyl away from key residues, preventing the water mediated H-bonding network observed for *R-5c*. Instead, *S-5c* was stabilised primarily through hydrophobic interactions: the chroman-4-one phenyl ring was sandwiched between Val83 and Phe168, forming H- π and π - π interactions, respectively. Additionally, although no strong interaction was detected, the phenyl substituent was oriented toward Trp182, placing it in proximity to the aromatic cage and potentially contributing additional hydrophobic interactions.

Fig. 6 A 3D image of binding interactions of compound *R-5f* in *Mtb* CYP121A1 active site at the end of the 200 ns MD simulation; haem (brown), *R-5f* (cyan), amino acid residues (grey) and water molecules (red spheres), (B) 2D image of derivative *R-5f* binding interactions with *Mtb* CYP121A1 active site residues

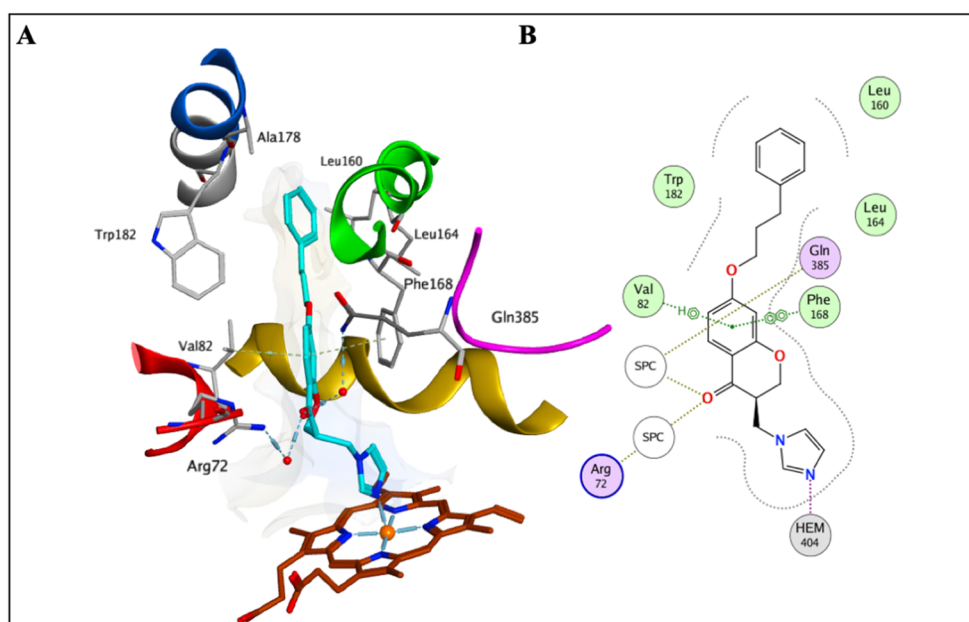
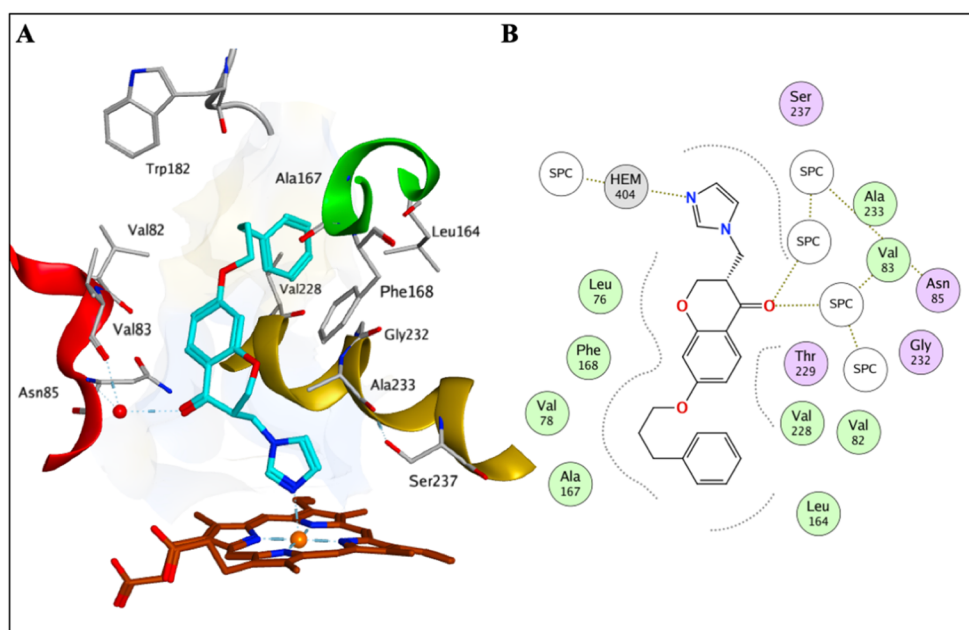


Fig. 7 A 3D image of binding interactions of compound *S-5f* in *Mtb* CYP121A1 active site at the end of the 200 ns MD simulation; haem (brown), *S-5f* (cyan), amino acid residues (grey) and water molecules (red spheres), (B) 2D image of derivative *S-5f* binding interactions with *Mtb* CYP121A1 active site residues



The propyloxy chroman-4-one exemplar *R-5f* oriented itself so that the imidazole ring was positioned perpendicularly over the haem, forming a direct coordination bond with the iron at a distance of 2.37 Å (Fig. 6). This positioning also placed the carbonyl oxygen in an optimal position to form water-mediated contacts with Arg72 and Gln385. The chroman-4-one core was tightly sandwiched between Val82 and Phe168, forming stabilising hydrophobic contacts through H- π and π - π interactions, respectively. Although the propyloxy linker positioned the terminal phenyl group favourably within the hydrophobic pocket surrounded by non-polar residues such as Leu160, Leu164, Ala178 and Trp182, no obvious interaction was observed with these residues.

In contrast to the *R*-enantiomer, the *S-5f* ligand adopted a noticeably different binding orientation while still binding directly to the haem iron, with an Fe-N distance of 2.40 Å (Fig. 7). In this pose, the chroman-4-one carbonyl was directed toward the B helix similar to the orientation observed for the cYY carbonyl allowing it to participate in a water-mediated interaction network with Val83 and Asn85. However, this arrangement positioned the chroman-4-one core away from the aromatic residues that stabilise the *R*-enantiomer, preventing the formation of hydrophobic contacts. Likewise, although the propyloxy linker placed the terminal phenyl ring within the hydrophobic pocket, its orientation did not show additional hydrophobic contacts.

The tetralones (**6**) displayed a more mixed binding profile. The imidazole moiety of the tetralone derivatives (**6**) did not bind directly with the haem but were orientated to the side of the haem binding site (Fig. 8A), replicating the position of the natural substrate cYY, resulting in either indirect (water mediated) binding with the haem (e.g. *S-6h*, Fig. 8C) or H-bonding interactions between the imidazole nitrogen and two important cYY binding residues: Arg386 (e.g. *R-6b*, Fig. 8B) or Asn74 (e.g. *R-6j*, Fig. 8D).

The terminal phenyl rings of the ethoxy derivatives were positioned to allow hydrophobic or π - π interactions with Phe168 in the F-helix, while the propyloxy derivatives

extended further into the access channel with the terminal phenyl rings positioned between the F- and G-helices and in close proximity to both Phe168 and Trp182.

The chromen-4-one imidazole/triazole derivatives (**12/13**) exhibited direct coordination to the haem iron, with Fe-N distances ranging from 2.39 to 2.46 Å. Across both series, the ligands were involved in a range of stabilising interactions, including water-mediated interactions involving the chromen-4-one carbonyl and diverse hydrophobic contacts arising from the aromatic side of the ligand. The imidazole derivative **12c** and the triazole derivative **13e** are presented as exemplars. The imidazole derivative **12c**

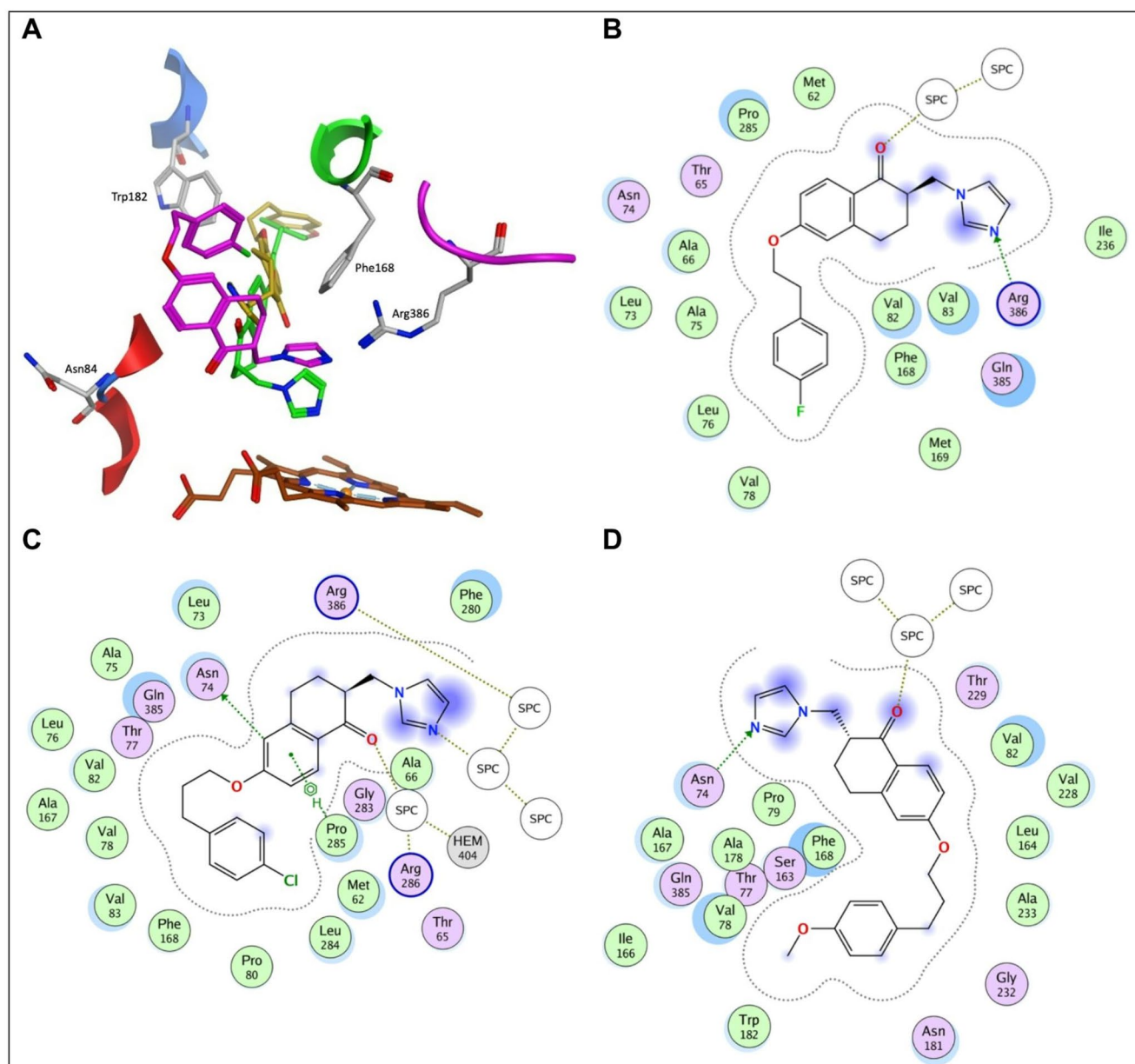


Fig. 8 A 3D image illustrating positioning of exemplar compounds *R-6b* (magenta), *S-6h* (green) and *R-6j* (gold), in the *Mtb* CYP121A1 active site after 200 ns MD simulation; haem (brown), amino acid resi-

dues (grey). 2D ligplot images of derivatives (**B**) *R-6b* (**C**) *S-6h* and (**D**) *R-6j* showing binding interactions with *Mtb* CYP121A1 active site and solvation residues

Fig. 9 A 3D image of binding interactions of compound **12c** in *Mtb* CYP121A1 active site at the end of the 200 ns MD simulation; haem (brown), **12c** (cyan), amino acid residues (grey) and water molecules (red spheres), (B) 2D image of derivative **12c** binding interactions with *Mtb* CYP121A1 active site residues

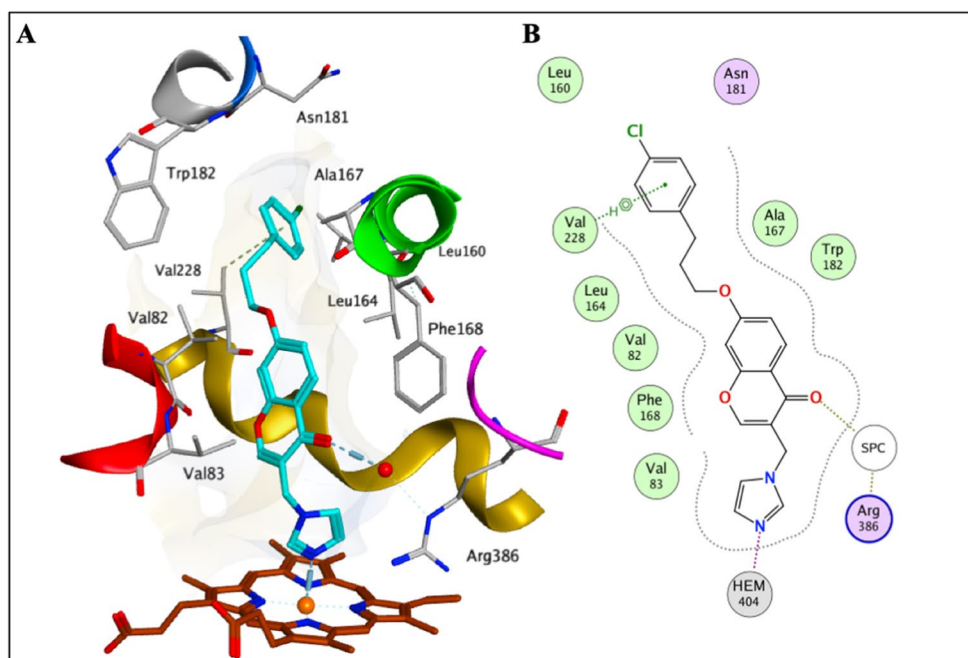
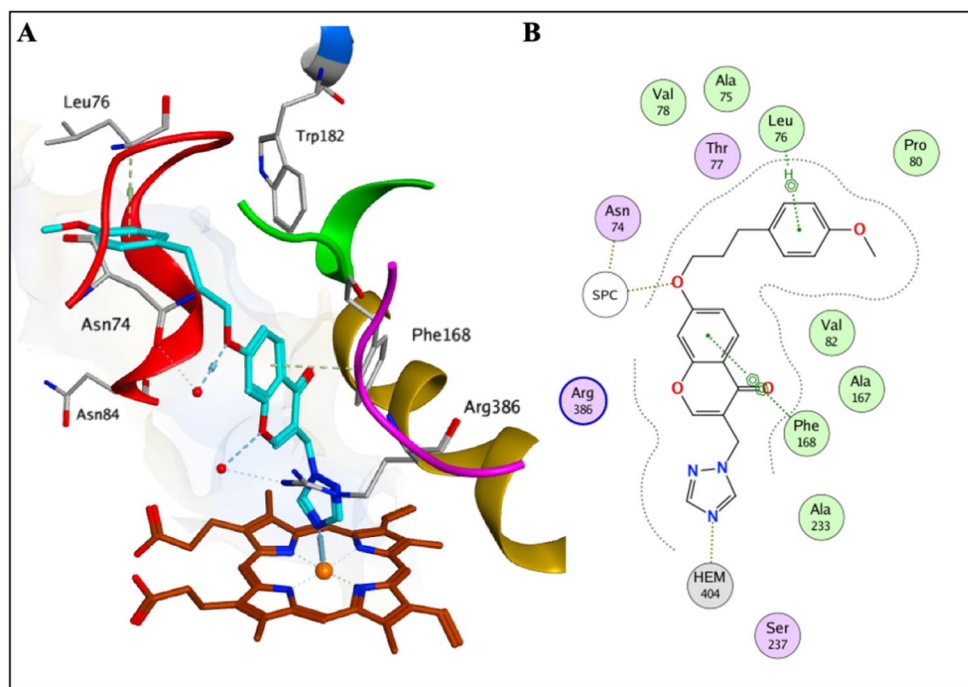


Fig. 10 A 3D image of binding interactions of compound **13e** in *Mtb* CYP121A1 active site at the end of the 200 ns MD simulation; haem (brown), **13e** (cyan), amino acid residues (grey) and water molecules (red spheres), (B) 2D image of derivative **13e** binding interactions with *Mtb* CYP121A1 active site residues



binds directly to the haem iron, displaying an Fe-N distance of 2.41 Å (Fig. 9). In its binding conformation, the ligand positions the chromen-4-one carbonyl so that it can form a water-mediated interaction with Arg386, helping to stabilise the orientation of the chromen-4-one backbone. Due to the rigid planar nature of the chromen-4-one backbone, the pendant phenyl substituent is directed toward the lower part of the hydrophobic cavity, where it engages in a H- π interaction with Val228 on the I-helix.

The triazole derivative **13e** also exhibited direct coordination to the haem iron, binding through the triazole N4 atom with an Fe-N distance of 2.31 Å, indicating a strong axial interaction (Fig. 10). The ligand adopted a binding orientation in which the entire ligand lay perpendicular to the haem plane, a conformation that facilitated multiple stabilising contacts within the active site. In this pose, the chromen-4-one oxygen formed a water-mediated interaction with Arg386, while the propoxy linker oxygen engaged in an additional water-bridged interaction with Asn74, further

stabilising the ligand. The perpendicular orientation also positioned the chromen-4-one ring toward Phe168, enabling a π - π stacking interaction that contributed to stabilisation of the aromatic core. Moreover, the increased flexibility provided by the propyloxy linker allowed the tail phenyl substituent to rotate toward the B-C loop, where it formed a hydrophobic H- π interaction with Leu76.

Conclusions

The preparation of the imidazole chroman-4-one (**5**) and tetralones (**6**) derivatives was achieved in two steps from 7-hydroxychroman-4-one (**1**) and 7-hydroxytetralone (**2**), respectively, while the preparation of the imidazole and triazole chromen-4-ones (**12** and **13**) required a five step synthesis starting from 2,4-dihydroxyacetophenone (**7**). All the reactions were efficient except for the second step in the preparation of chroman-4-one (**5**) and tetralone (**6**) imidazole derivatives. The second step involved a Mannich reaction resulting in low to moderate yields (22–46%). Different solvents and temperatures were investigated (MeCN, DMF, DMSO and temperature 80–110 °C) to optimise the Mannich reaction, with DMSO and 80 °C found to be optimal. The initial reaction of L-proline/formaldehyde/chroman-4-one (**3**) or tetralone (**4**) with catalytic HCl to form the Mannich adduct, was optimised with 1 equivalent of each reagent found to be effective for reaction completion. The final nucleophilic reaction of imidazole with the Mannich adduct was the limiting reaction. Four equivalents of imidazole (a 3 equivalent excess) were optimal with the addition of further equivalents failing to push the reaction any further with a more time consuming aqueous work-up to remove the increasing quantities of unreacted imidazole and subsequently reduced yields.

Binding affinity (K_D) for chroman-4-one (**5**) derivatives containing the imidazole or pyridine haem binding group and chromen-4-one (**12**) with the imidazole haem binding group was comparable, while the derivatives containing a triazole haem binding group (**13**) showed a smaller shift in the Soret band. Tetralone imidazoles (**6**) clearly outperformed tetralone pyridines (**Pyr2**), which did not show any binding (Fig. 3). Antimycobacterial activity (MIC) of chroman-4-ones (**5**), tetralones (**6**) and chromen-4-ones (**12**) containing either imidazole or pyridine haem binding group was comparable, while the triazole (**13**) derivatives were inactive. Although having either sp² hybridised (C=CH)-(**Pyr1**, **Pyr2**, **12**) or sp³ hybridised (CH-CH₂) (**5**, **6**) linker between the haem binding motif and the chroman-4-one/tetralone/chromen-4-one pharmacophore did not result in any significant difference in antimycobacterial activity (MIC) vs. *H37Rv* wild type of mc²7902 drug-sensitive

strain (Fig. 3), sp² hybridised chromen-4-one derivatives (**12**) showed reduced antimycobacterial activity against the INH^R strain mc²8245. For chroman-4-one (**5**) and tetralone derivatives (**6**), the C3 linker length between the pharmacophore and the unsubstituted/substituted phenyl was preferred.

Type II direct binding was generally supported by computational studies, with the exception of the tetralones (**6**), however computational studies of the tetralones would indicate close positioning to the haem with either water-mediated interaction between the imidazole and the haem Fe or direct binding between the imidazole and either Arg386 or Asn74 with displacement of the 6th axial water molecule. Generally, correlation was observed between binding affinity and antimycobacterial activity. The triazole derivatives (**13**) were a clear exception, with good binding affinity but negligible antimycobacterial activity, which was consistent with previous studies on triazole derivatives [19, 20].

With the exception of tetralone **6i** that had one violation (cLogP 5.25), all the final compounds (**5**, **6**, **12**, **13**) adhered to Lipinski's rule of five across all four criteria: molecular weight < 500 (MW 346.43–441.325); LogP < 5 (cLogP Crippens fragmentation [35] 2.98–4.98); H-bond acceptors < 10 (HB-accept 3–6); < 5 H-bond donors (HB-donor 0) (Table S6). The tetralones (**6**) were the most promising derivatives and showed improved *Mtb* CYP121A1 binding affinity compared with the parent tetralone pyridyl derivatives (e.g. **Pyr2**, Fig. 10). Although both exemplar compounds (**6h** and **12c**) displayed favourable SI values in A459 cells and macrophages with the tetralone **6h** showing an improved therapeutic window with SI > 10, further SAR is needed to improve antimycobacterial activity further and enhance selectivity.

Experimental

General

All chemicals, reagents and solvents were purchased from Sigma-Aldrich, Alfa Aesar, VWR, Acros and Fluka and dried prior to use over molecular sieves (4 Å). For column chromatography silica gel (Fluka Kieselgel 60) was used and TLC was performed on pre-coated silica plates (dimension 20 × 20 cm) (ALUGRAM[®] SIL G/UV₂₅₄) with visualisation via UV light (254 nm). Melting points were determined on an electrothermal instrument (Gallenkamp) and are uncorrected. ¹H, ¹³C (DEPT), and ¹⁹F NMR spectra were recorded on a Bruker Advance DP500 spectrometer operating at 500, 125 and 470 MHz, respectively using either CDCl₃ or DMSO-*d*₆. Chemical shifts are given in parts per million (ppm) relative to the internal standard

tetramethylsilane (Me₄Si); coupling constants (*J* values) were given in Hertz (Hz). HPLC-HRMS spectra were performed at the University of Bath, UK on a Zorbax Eclipse Plus C18 Rapid Resolution 2.1 × 50 mm, 1.8 µm particle size using a 7.5-minute gradient method 5:95 v/v water: methanol with 0.1% formic acid as additive. Microanalysis was performed by MEDAC Ltd (Chobham, UK). Intermediate compounds **3** and **4** were prepared as previously described [22]. Experimental methods for all intermediate compounds (**8**, **9**, **10** and **11**), antimycobacterial minimum inhibition concentration (MIC) – SPOTi and REMA methods, CYP121A1 binding affinity (*K_D*) and computational methods can be found in the supporting information.

Chemistry

General method for the preparation of imidazole chroman-4-one (**5**) and tetralone (**6**) derivatives

A mixture of L-proline (1 equiv.), paraformaldehyde (1 equiv.), chroman-4-one (**3**) or tetralone (**4**) (1 equiv.), and c. HCl (0.1 equiv) in dry DMSO (3 mL/mmol) was stirred at 85 °C for 1 h, then imidazole (4 equiv.) was added, and the reaction stirred overnight. The reaction mixture was poured into iced H₂O (30 mL/mmol) and extracted with CH₂Cl₂ (50 mL/mmol). The organic layer was washed with H₂O (5 × 30 mL), dried (MgSO₄) and evaporated under reduced pressure. The crude product was purified by gradient column chromatography (petroleum ether - EtOAc 50:50 v/v) to remove unreacted starting material then the pure product was eluted with CH₂Cl₂-MeOH 90:10 v/v for chroman-4-ones **5** or CH₂Cl₂-MeOH 98:2 v/v for tetralones **6**.

3-((1*H*-imidazol-1-yl)methyl)-7-phenethoxychroman-4-one (**5a**)

Prepared from 7-phenethoxychroman-4-one (**3a**) (0.25 g, 0.93 mmol). The pure compound was obtained as a colourless syrup, yield: 0.09 g (28%); TLC (CH₂Cl₂-MeOH 95:5 v/v) *R_f* 0.68; ¹H NMR (CDCl₃): δ 8.04 (s, 1H, imidazole), 7.84 (d, *J*=8.9 Hz, 1H, Ar), 7.34 (d, *J*=8.9 Hz, 2 H, Ar), 7.28 (m, 3 H, Ar), 7.15 (s, 1H, imidazole), 7.06 (s, 1H, imidazole), 6.62 (dd, *J*=2.4, 8.9 Hz, 1H, Ar), 6.41 (d, *J*=2.3 Hz, 1H, Ar), 4.53 (dd, *J_{A,X}* = 4.6 Hz, *J_{A,B}* = 11.5 Hz, 1H, CH_x-CH_aCH_b-imidazole), 4.48 (dd, *J_{A,X}* = 5.1 Hz, *J_{A,B}* = 14.5 Hz, 1H, CH_xCH_aH_b), 4.43 (dd, *J_{B,X}* = 7.5 Hz, *J_{B,A}* = 14.5 Hz, 1H, CH_xCH_aH_b), 4.22 (t, *J*=7.0 Hz, 2 H, CH₂), 4.09 (dd, *J_{B,X}* = 9.6 Hz, *J_{A,B}* = 11.5 Hz, 1H, CH_xCH_aCH_b-imid), 3.12 (t, *J*=7.0 Hz, 2 H, CH₂), 3.10 (m, 1H, CH_xCH_aCH_b); ¹³C NMR (CDCl₃): δ 189.54 (C=O), 165.76 (C, Ar), 163.59 (C, Ar), 137.54 (C, Ar), 130.88 (CH, imidazole), 129.15 (CH, Ar), 128.95 (2 x CH, Ar), 128.79 (CH, imidazole), 128.59

(2 x CH, Ar), 127.85 (CH, imidazole), 126.73 (CH, Ar), 114.14 (C, Ar), 111.05 (CH, Ar), 101.33 (CH, Ar), 69.14 (CH₂), 68.46 (CH₂), 46.52 (CH), 43.89 (CH₂-imidazole), 35.43 (CH₂); HPLC: 100%, RT=3.98 min; HRMS (ESI, *m/z*): Theoretical mass: 349.1547 [M+H]⁺, observed mass: 349.1552 [M+H]⁺.

3-((1*H*-imidazol-1-yl)methyl)-7-(4-fluorophenethoxy)chroman-4-one (**5b**)

Prepared from 7-(4-fluorophenethoxy)chroman-4-one (**3b**) (0.325 g, 1.13 mmol). The pure compound was obtained as a brown oil, yield: 0.18 g (43%); TLC (CH₂Cl₂-MeOH 95:5 v/v) *R_f* 0.66; ¹H NMR (CDCl₃): δ 8.01 (s, 1H, imidazole), 7.83 (d, *J*=8.9 Hz, 1H, Ar), 7.24 (m, 2H, Ar), 7.14 (s, 1H, imidazole), 7.05 (s, 1H, imidazole), 7.02 (m, 2H, Ar), 6.61 (dd, *J*=2.4, 8.9 Hz, 1H, Ar), 6.40 (d, *J*=2.4 Hz, 1H, Ar), 4.52 (dd, *J_{A,X}* = 4.6 Hz, *J_{A,B}* = 11.5 Hz, 1H, CH_xCH_aCH_b-imidazole), 4.47 (dd, *J_{A,X}* = 5.1 Hz, *J_{A,B}* = 14.5 Hz, 1H, CH_xCH_aH_b), 4.41 (dd, *J_{B,X}* = 7.5 Hz, *J_{B,A}* = 14.5 Hz, 1H, CH_xCH_aH_b), 4.18 (t, *J*=7 Hz, 2H, CH₂), 4.08 (dd, *J_{B,X}* = 9.7 Hz, *J_{A,B}* = 11.5 Hz, 1H, CH_xCH_aCH_b-imidazole), 3.11 (m, 1H, CH_xCH_aH_b), 3.09 (t, *J*=7 Hz, 2H, CH₂); ¹⁸F NMR: δ -116.29 (Ar-F); ¹³C NMR (CDCl₃): δ 189.49 (C=O), 165.63 (C, Ar), 163.65 (C, Ar), 161.77 (d, ¹*J_{CF}* = 244.5 Hz, C-F, Ar), 133.43 (d, ⁴*J_{CF}* = 3.3 Hz, C, Ar), 130.39 (d, ³*J_{CF}* = 8.1 Hz, 2 x CH, Ar), 129.92 (CH, Ar), 128.75 (CH, imidazole), 127.96 (CH, imidazole), 127.49 (CH, imidazole), 115.38 (d, ²*J_{CF}* = 21.6 Hz, 2 x CH, Ar), 114.22 (C, Ar), 110.98 (CH, Ar), 101.33 (CH, Ar), 69.04 (CH₂), 68.64 (CH₂), 46.58 (CH), 43.69 (CH₂-imidazole), 34.62 (CH₂); HPLC: 100%, RT=4.27 min; HRMS (ESI, *m/z*): Theoretical mass: 366.138 [M+H]⁺, observed mass: 366.139 [M+H]⁺.

3-((1*H*-imidazol-1-yl)methyl)-7-(4-chlorophenethoxy)chroman-4-one (**5c**)

Prepared from 7-(4-chlorophenethoxy)chroman-4-one (**3c**) (0.2 g, 0.66 mmol). The pure compound was obtained as a white wax, yield: 0.064 g (25%); TLC (CH₂Cl₂-MeOH 95:5 v/v) *R_f* 0.66; ¹H NMR (CDCl₃): δ 8.31 (s, 1H, imidazole), 7.83 (d, *J*=8.8 Hz, 1H, Ar), 7.29 (d, *J*=6.4 Hz, 2 H, Ar), 7.21 (d, *J*=8.0 Hz, 2 H, Ar), 7.17 (s, 1H, imidazole), 7.10 (s, 1H, imidazole), 6.60 (d, *J*=8.9 Hz, 1H, Ar), 6.39 (s, 1H, Ar), 4.59 (dd, *J_{A,X}* = 4.6 Hz, *J_{A,B}* = 11.4 Hz, 1H, CH_xCH_aCH_b-imidazole), 4.55 (dd, *J_{A,X}* = 4.9 Hz, *J_{A,B}* = 14.0 Hz, 1H, CH_xCH_aH_b), 4.51 (dd, *J_{B,X}* = 5.9 Hz, *J_{B,A}* = 14.0 Hz, 1H, CH_xCH_aH_b), 4.19 (t, *J*=6.7 Hz, 2 H, CH₂), 4.10 (t, *J*=10.7 Hz, 1H, CH_xCH_aCH_b-imidazole), 3.13 (m, 1H, CH_xCH_aH_b), 3.08 (t, *J*=6.7 Hz, 2 H, CH₂); ¹³C NMR (CDCl₃): δ 189.69 (C=O), 165.67 (C, Ar), 163.70

(C, Ar), 136.11 (C, Ar), 132.65 (C, Ar), 130.88 (CH, imidazole), 130.28 (2 x CH, Ar), 129.11 (CH, Ar), 128.69 (2 x CH, Ar), 123.8 (CH, imidazole), 123.22 (CH, imidazole), 114.17 (C, Ar), 111 (CH, Ar), 101.34 (CH, Ar), 68.79 (CH₂), 68.57 (CH₂), 46.15 (CH), 44.71 (CH₂-imidazole), 34.76 (CH₂); HPLC: 100%, RT=4.37 min; HRMS (ESI, m/z): Theoretical mass (³⁵Cl): 383.1157 [M+H]⁺, observed mass: 383.1167 [M+H]⁺; theoretical mass (³⁷Cl): 385.1127 [M+H]⁺, observed mass: 385.1137 [M+H]⁺.

3-((1*H*-imidazol-1-yl)methyl)-7-(4-bromophenethoxy)chroman-4-one (5d)

Prepared from 7-(4-bromophenethoxy)chroman-4-one (**3d**) (0.3 g, 0.86 mmol). The pure compound was obtained as a white solid, yield: 0.08 g (22%); m.p. 108–110 °C; TLC (CH₂Cl₂-MeOH 95:5 v/v) R_f 0.66; ¹H NMR (CDCl₃): δ 8.15 (s, 1H, imidazole), 7.84 (d, *J*=8.9 Hz, 1H, Ar), 7.46 (d, *J*=8.4 Hz, 2 H, Ar), 7.17 (m, 3 H, Ar x 2 and imidazole), 7.07 (s, 1H, imidazole), 6.61 (dd, *J*=2.4, 8.9 Hz, 1H, Ar), 6.39 (d, *J*=2.4 Hz, 1H, Ar), 4.56 (dd, *J*_{A,X} = 4.6 Hz, *J*_{A,B} = 11.5 Hz, 1H, CH_xCH_aCH_b-imidazole), 4.50 (dd, *J*_{A,X} = 5.2 Hz, *J*_{A,B} = 14.5 Hz, 1H, CH_xCH_aH_b), 4.45 (dd, *J*_{B,X} = 7.2 Hz, *J*_{B,A} = 14.5 Hz, 1H, CH_xCH_aH_b), 4.19 (t, *J*=6.8 Hz, 2 H, CH₂), 4.09 (dd, *J*_{B,X} = 9.8 Hz, *J*_{A,B} = 11.5 Hz, 1H, CH_xCH_aCH_b-imidazole), 3.12 (m, 1H, CH_xCH_aH_b) 3.08 (t, *J*=6.7 Hz, 2 H, CH₂); ¹³C NMR (CDCl₃): δ 189.42 (C=O), 165.49 (C, Ar), 163.48 (C, Ar), 137.74 (CH, imidazole), 136.67 (C, Ar), 131.64 (2 x CH, Ar), 130.70 (2 x CH, Ar), 129.97 (CH, imidazole), 129.24 (CH, Ar), 120.60 (C, Ar), 119.40 (CH, imidazole), 114.28 (C, Ar), 110.93 (CH, Ar), 101.32 (CH, Ar), 68.68 (CH₂), 68.42 (CH₂), 46.76 (CH), 43.21 (CH₂-imidazole), 34.83 (CH₂); HPLC: 100%, RT=4.14 min; HRMS (ESI, m/z): Theoretical mass (⁷⁹Br): 427.0652 [M+H]⁺, observed mass: 427.0653 [M+H]⁺; theoretical mass (⁸¹Br): 429.0632 [M+H]⁺, observed mass: 429.0636 [M+H]⁺.

3-((1*H*-imidazol-1-yl)methyl)-7-(4-methoxyphenethoxy)chroman-4-one (5e)

Prepared from 7-(4-methoxyphenethoxy)chroman-4-one (**3e**) (0.25 g, 0.84 mmol). The pure compound was obtained as a light brown oil, yield: 0.08 g (24%); TLC (CH₂Cl₂-MeOH 95:5 v/v) R_f 0.68; ¹H NMR (CDCl₃): δ 7.91 (s, 1H, imidazole), 7.83 (d, *J*=8.8 Hz, 1H, Ar), 7.20 (d, *J*=8 Hz, 2 H, Ar), 7.13 (s, 1H, imidazole), 7.04 (s, 1H, imidazole), 6.87 (d, *J*=7.9 Hz, 2 H, Ar), 6.61 (d, *J*=8.9 Hz, *J*=2.3 Hz, 1H, Ar), 6.40 (d, *J*=2.3 Hz, 1H, Ar), 4.50 (dd, *J*_{A,X} = 4.6 Hz, *J*_{A,B} = 11.5 Hz, 1H, CH_xCH_aCH_b-imidazole), 4.45 (dd, *J*_{A,X} = 5.0 Hz, *J*_{A,B} = 14.5 Hz, 1H, CH_xCH_aH_b), 4.39 (dd, *J*_{B,X} = 7.6 Hz, *J*_{B,A} = 14.5 Hz, 1H, CH_xCH_aH_b), 4.17 (t, *J*=7 Hz,

2 H, CH₂), 4.08 (dd, *J*_{B,X} = 9.6 Hz, *J*_{A,B} = 11.5 Hz, 1H, CH_xCH_aCH_b-imidazole), 3.1 (s, 3 H, CH₃), 3.09 (m, 1H, CH_xCH_aH_b), 3.05 (t, *J*=6.9 Hz, 2 H, CH₂); ¹³C NMR (CDCl₃): δ 189.50 (C=O), 165.78 (C, Ar), 163.55 (C, Ar), 158.43 (C, Ar), 129.92 (2 x CH, Ar), 129.55 (CH, Ar), 129.50 (CH, imidazole), 129.16 (2 x CH, Ar), 128.90 (CH, imidazole), 128.35 (CH, imidazole), 114.13 (C, Ar), 114.01 (CH, Ar), 111.04 (CH, Ar), 101.32 (CH, Ar), 69.40 (CH₂), 68.44 (CH₂), 55.27 (CH₃), 46.62 (CH), 43.61 (CH₂-imidazole), 34.54 (CH₂); HPLC: 100%, RT=4.25 min; HRMS (ESI, m/z): Theoretical mass: 379.1653 [M+H]⁺, observed mass: 379.1587 [M+H]⁺.

3-((1*H*-imidazol-1-yl)methyl)-7-(3-phenylpropoxy)chroman-4-one (5f)

Prepared from 7-(3-phenylpropoxy)chroman-4-one (**3f**) (0.5 g, 1.77 mmol). The pure compound was obtained as a beige solid, yield: 0.27 g (43%); m.p. 91–93 °C; TLC (CH₂Cl₂-MeOH 95:5 v/v) R_f 0.70; ¹H NMR (CDCl₃): δ 8.19 (s, 1H, imidazole), 7.84 (d, *J*=8.8 Hz, 1H, Ar), 7.30 (m, 2 H, Ar), 7.22 (m, 3 H, Ar), 7.16 (s, 1H, imidazole), 7.09 (s, 1H, imidazole), 6.62 (d, *J*=8.9 Hz, 1H, Ar), 6.39 (s, 1H, Ar), 4.57 (dd, *J*_{A,X} = 4.5 Hz, *J*_{A,X} = 11.4 Hz, 1H, CH_xCH_aCH_b-imidazole), 4.51 (dd, *J*_{A,X} = 5.6 Hz, *J*_{A,B} = 14.8 Hz, 1H, CH_xCH_aH_b), 4.46 (dd, *J*_{B,X} = 6.7 Hz, *J*_{B,A} = 14.0 Hz, 1H, CH_xCH_aH_b), 4.10 (t, *J*=10.6 Hz, 1H, CH_xCH_aCH_b-imidazole), 4.00 (t, *J*=6.2 Hz, 2 H, CH₂), 3.12 (m, 1H, CH_xCH_aH_b), 2.82 (t, *J*=7.7 Hz, 2 H, CH₂), 2.14 (quintet, *J*=6.8 Hz, 2 H, CH₂); ¹³C NMR (CDCl₃): δ 189.42 (C=O), 165.99 (C, Ar), 163.55 (C, Ar), 140.98 (C, Ar), 129.71 (CH, imidazole), 129.23 (CH, imidazole), 129.18 (CH, Ar), 128.91 (CH, imidazole), 128.50 (2 x CH, Ar), 128.46 (2 x CH, Ar), 126.11 (CH, Ar), 114.09 (C, Ar), 111.05 (CH, Ar), 101.24 (CH, Ar), 68.42 (CH₂), 67.43 (CH₂), 46.71 (CH), 43.54 (CH₂-imidazole), 31.95 (CH₂), 30.42 (CH₂); Anal. Calcd for C₂₂H₂₂N₂O₃ (362.4290): C 72.91%, H 6.12%, N 7.73%. Found: C 72.68%, H 5.97%, N 7.89%.

3-((1*H*-imidazol-1-yl)methyl)-7-(3-(4-fluorophenyl)propoxy)chroman-4-one (5g)

Prepared from 7-(3-(4-fluorophenyl)propoxy)chroman-4-one (**3g**) (0.3 g, 1.0 mmol). The pure compound was obtained as a white solid, yield: 0.11 g (29%); m.p. 108–110 °C; TLC (CH₂Cl₂-MeOH 95:5 v/v) R_f 0.70; ¹H NMR (CDCl₃): δ 7.85 (d, *J*=8.9 Hz, 1H, Ar), 7.79 (s, 1H, imidazole), 7.16 (dd, *J*=5.4, 8.7 Hz, 2H, Ar), 7.13 (s, 1H, imidazole), 7.02 (s, 1H, imidazole), 6.99 (m, 2H, Ar), 6.62 (dd, *J*=2.4, 8.9 Hz, 1H, Ar), 6.38 (d, *J*=2.4 Hz, 1H, Ar), 4.48 (dd, *J*_{A,X} = 4.5 Hz, *J*_{A,B} = 11.5 Hz, 1H, CH_xCH_aCH_b-imidazole), 4.44 (dd, *J*_{A,X} = 4.9 Hz, *J*_{A,B} = 14.5 Hz, 1H,

$\text{CH}_x\text{CH}_a\text{H}_b$), 4.38 (dd, $J_{B,X} = 7.9$ Hz, $J_{B,A} = 14.5$ Hz, 1H, $\text{CH}_x\text{CH}_a\text{H}_b$), 4.08 (dd, $J_{B,X} = 9.4$ Hz, $J_{B,A} = 11.5$ Hz, 1H, $\text{CH}_x\text{CH}_a\text{CH}_b$ -imidazole), 3.98 (t, $J = 6.2$ Hz, 2H, CH_2), 3.08 (m, 1H, $\text{CH}_x\text{CH}_a\text{H}_b$), 2.79 (t, $J = 7.6$ Hz, 2H, CH_2), 2.10 (m, 2H, CH_2); ^{19}F NMR: δ -117.23 (Ar-F); ^{13}C NMR (CDCl_3): δ 189.42 (C=O), 165.87 (C, Ar), 163.54 (C, Ar), 161.38 (d, $^1J_{\text{CF}} = 244.0$ Hz, C-F, Ar), 136.57 (d, $^4J_{\text{CF}} = 3.5$ Hz, C, Ar), 130.09 (CH, imidazole), 129.80 (d, $^3J_{\text{CF}} = 8.0$ Hz, 2 x CH, Ar), 129.44 (CH, imidazole), 129.21 (CH, Ar), 128.46 (CH, imidazole), 115.24 (d, $^2J_{\text{CF}} = 20.9$ Hz, 2 x CH, Ar), 114.16 (C, Ar), 111.0 (CH, Ar), 101.22 (CH, Ar), 68.44 (CH_2), 67.22 (CH_2), 46.72 (CH), 43.39 (CH_2 -imidazole), 31.15 (CH_2), 30.54 (CH_2); HPLC: 100%, RT=4.36 min; HRMS (ESI, m/z): Theoretical mass: 381.1618 $[\text{M}+\text{H}]^+$, observed mass: 381.1618 $[\text{M}+\text{H}]^+$.

3-((1*H*-imidazol-1-yl)methyl)-7-(3-(4-chlorophenyl)propoxy)chroman-4-one (5h)

Prepared from 7-(3-(4-chlorophenyl)propoxy)chroman-4-one (**3h**) (0.5 g, 1.58 mmol). The pure compound was obtained as a white solid, yield: 0.28 g (45%); m.p. 76–78 °C; TLC (CH_2Cl_2 -MeOH 9:1 v/v) R_f 0.46; ^1H NMR (CDCl_3): δ 7.86 (d, $J = 8.9$ Hz, 1H, Ar), 7.84 (s, 1H, imidazole), 7.27 (d, $J = 9.6$ Hz, 2 H, Ar), 7.14 (d, $J = 8.5$ Hz, 2 H, Ar and 1H, imidazole), 7.03 (s, 1H, imidazole), 6.62 (dd, $J = 1.6$, 8.7 Hz, 1H, Ar), 6.38 (d, $J = 1.3$ Hz, 1H, Ar), 4.49 (dd, $J_{A,X} = 4.3$ Hz, $J_{A,B} = 11.5$ Hz, 1H, $\text{CH}_x\text{CH}_a\text{CH}_b$ -imidazole), 4.45 (dd, $J_{A,X} = 4.9$ Hz, $J_{A,B} = 14.8$ Hz, 1H, $\text{CH}_x\text{CH}_a\text{H}_b$), 4.38 (dd, $J_{B,X} = 7.8$ Hz, $J_{B,A} = 14.3$ Hz, 1H, $\text{CH}_x\text{CH}_a\text{H}_b$), 4.08 (dd, $J_{B,X} = 10.9$ Hz, $J_{B,A} = 10.0$ Hz, 1H, $\text{CH}_x\text{CH}_a\text{CH}_b$ -imidazole), 3.98 (t, $J = 6.0$ Hz, 2 H, CH_2), 3.07 (m, 1H, $\text{CH}_x\text{CH}_a\text{H}_b$), 2.79 (t, $J = 7.4$ Hz, 2 H, CH_2), 2.11 (q, $J = 7.0$ Hz, 2 H, CH_2); ^{13}C NMR (CDCl_3): δ 189.42 (C=O), 165.81 (C, Ar), 163.52 (C, Ar), 139.40 (C, Ar), 137.77 (CH, imidazole), 131.85 (C, Ar), 130.14 (CH, imidazole), 129.83 (2 x CH, Ar), 129.23 (CH, Ar), 128.60 (2 x CH, Ar), 119.37 (CH, imidazole), 114.19 (C, Ar), 110.99 (CH, Ar), 101.21 (CH, Ar), 68.42 (CH_2), 68.14 (CH_2), 46.82 (CH), 43.21 (CH_2 -imidazole), 31.33 (CH_2), 30.34 (CH_2); HPLC: 100%, RT=4.54 min; HRMS (ESI, m/z): Theoretical mass (^{35}Cl): 397.1313 $[\text{M}+\text{H}]^+$, observed mass: 397.1324 $[\text{M}+\text{H}]^+$; theoretical mass (^{37}Cl): 399.1283 $[\text{M}+\text{H}]^+$, observed mass: 399.1299 $[\text{M}+\text{H}]^+$.

3-((1*H*-imidazol-1-yl)methyl)-7-(3-(4-bromophenyl)propoxy)chroman-4-one (5i)

Prepared from 7-(3-(4-bromophenyl)propoxy)chroman-4-one (**3i**) (0.3 g, 0.83 mmol). The pure compound was obtained as a white solid, yield: 0.12 g (32%); m.p. 117–118 °C; TLC (CH_2Cl_2 -MeOH 95:5 v/v) R_f 0.70; ^1H NMR

(CDCl_3): δ 7.86 (d, $J = 8.8$ Hz, 1H, Ar), 7.54 (s, 1H, imidazole), 7.42 (d, $J = 8.4$ Hz, 2 H, Ar), 7.10 (s, 1H, imidazole), 7.08 (d, $J = 8.5$ Hz, 2 H, Ar), 6.97 (s, 1H, imidazole), 6.62 (dd, $J = 2.4$, 8.8 Hz, 1H, Ar), 6.38 (d, $J = 2.3$ Hz, 1H, Ar), 4.43 (dd, $J_{A,X} = 4.5$ Hz, $J_{A,B} = 11.5$ Hz, 1H, $\text{CH}_x\text{CH}_a\text{CH}_b$ -imidazole), 4.40 (dd, $J_{A,X} = 4.8$ Hz, $J_{A,B} = 14.6$ Hz, 1H, $\text{CH}_x\text{CH}_a\text{H}_b$), 4.32 (dd, $J_{B,X} = 8.4$ Hz, $J_{B,A} = 14.6$ Hz, 1H, $\text{CH}_x\text{CH}_a\text{H}_b$), 4.08 (dd, $J = 9.1$, 11.5 Hz, 1H, CH_aCH_b -imidazole), 3.98 (t, $J = 6.2$ Hz, 2 H, CH_2), 3.05 (m, 1H, $\text{CH}_x\text{CH}_a\text{H}_b$), 2.77 (t, $J = 7.6$ Hz, 2 H, CH_2), 2.10 (m, 2 H, CH_2); ^{13}C NMR (CDCl_3): δ 189.41 (C=O), 165.81 (C, Ar), 163.53 (C, Ar), 139.92 (C, Ar), 131.56 (2 x CH, Ar), 130.23 (2 x CH, Ar), 129.85 (CH, imidazole), 129.23 (C, Ar), 128.50 (CH, imidazole), 119.87 (C, Ar), 119.48 (CH, imidazole), 114.19 (C, Ar), 110.99 (CH, Ar), 101.23 (CH, Ar), 68.44 (CH_2), 67.14 (CH_2), 46.79 (CH), 43.30 (CH_2 -imidazole), 31.40 (CH_2), 30.27 (CH_2); HPLC: 100%, RT=4.53 min; HRMS (ESI, m/z): Theoretical mass (^{79}Br): 441.0808 $[\text{M}+\text{H}]^+$, observed mass: 441.0819 $[\text{M}+\text{H}]^+$; theoretical mass (^{81}Br): 443.0788 $[\text{M}+\text{H}]^+$, observed mass: 443.0801 $[\text{M}+\text{H}]^+$.

3-((1*H*-imidazol-1-yl)methyl)-7-(3-(4-methoxyphenyl)propoxy)chroman-4-one (5j)

Prepared from 7-(3-(4-methoxyphenyl)propoxy)chroman-4-one (**3j**) (0.5 g, 1.6 mmol). The pure compound was obtained as a beige solid, yield: 0.29 g (46%); m.p. 120–122 °C; TLC (CH_2Cl_2 -MeOH 9:1 v/v) R_f 0.50. ^1H NMR (CDCl_3): δ 8.12 (s, 1H, imidazole), 7.84 (d, $J = 8.8$ Hz, 1H, Ar), 7.16 (s, 1H, imidazole), 7.12 (d, $J = 8.6$ Hz, 2 H, Ar), 7.08 (s, 1H, imidazole), 6.85 (d, $J = 8.6$ Hz, 2 H, Ar), 6.62 (dd, $J = 2.4$, 8.9 Hz, 1H, Ar), 6.38 (d, $J = 2.3$ Hz, 1H, Ar), 4.55 (dd, $J_{A,X} = 4.6$ Hz, $J_{A,B} = 11.5$ Hz, 1H, $\text{CH}_x\text{CH}_a\text{CH}_b$ -imidazole), 4.50 (dd, $J_{A,X} = 5.2$ Hz, $J_{A,B} = 14.4$ Hz, 1H, $\text{CH}_x\text{CH}_a\text{H}_b$), 4.45 (dd, $J_{B,X} = 7.2$ Hz, $J_{B,A} = 14.5$ Hz, 1H, $\text{CH}_x\text{CH}_a\text{H}_b$), 4.10 (dd, $J_{B,X} = 9.8$ Hz, $J_{B,A} = 11.4$ Hz, 1H, $\text{CH}_x\text{CH}_a\text{CH}_b$ -imidazole), 3.98 (t, $J = 6.3$ Hz, 2 H, CH_2), 3.80 (s, 3 H, CH_3), 3.11 (m, 1H, $\text{CH}_x\text{CH}_a\text{H}_b$), 2.75 (t, $J = 7.5$ Hz, 2 H, CH_2), 2.09 (m, 2 H, CH_2); ^{13}C NMR (CDCl_3): δ 189.49 (C=O), 166.04 (C, Ar), 163.60 (C, Ar), 157.96 (C, Ar), 132.98 (C, Ar), 129.69 (CH, imidazole), 129.37 (2 x CH, Ar), 129.13 (2 x CH, Ar), 127.57 (CH, imidazole), 120.07 (CH, imidazole), 114.05 (C, Ar), 113.90 (C, Ar), 111.09 (CH, Ar), 101.24 (CH, Ar), 68.45 (CH_2), 67.41 (CH_2), 55.27 (CH_3), 46.57 (CH), 43.80 (CH_2 -imidazole), 31.00 (CH_2), 30.62 (CH_2). Anal. Calcd for $\text{C}_{23}\text{H}_{24}\text{N}_2\text{O}_4$ (392.4550): C 70.39%, H 6.16%, N 7.13%. Found: C 70.69%, H 6.05%, N 6.89.

2-((1*H*-imidazol-1-yl)methyl)-6-phenethoxy-3,4-dihydronaphthalen-1(2*H*)-one (6a)

Prepared from 6-phenethoxy-3,4-dihydronaphthalen-1(2*H*)-one (**4a**) (0.3 g, 1.13 mmol). The pure compound was obtained as a brown oil, yield: 0.13 g (33%); TLC (CH₂Cl₂-MeOH 9:1 v/v) *R_f* 0.62; ¹H NMR (CDCl₃): δ 8.04 (d, *J*=9.0 Hz, 1H, Ar), 7.52 (s, 1H, imidazole), 7.30 (m, 5 H, Ar), 7.06 (s, 1H, imidazole), 6.96 (s, 1H, Ar), 6.86 (d, *J*=9.0 Hz, 1H, Ar), 6.68 (s, 1H, imidazole), 4.50 (dd, *J*=4.0, 14.0 Hz, 1H, CH_aCH_b-imidazole), 4.39 (dd, *J*=6.5, 14.0 Hz, 1H, CH_aCH_b-imidazole), 4.24 (t, *J*=7.0 Hz, 2 H, CH₂), 3.13 (t, *J*=7.0 Hz, 2 H, CH₂), 2.98 (m, 1H, CH₂), 2.92 (m, 1H, CH₂), 2.80 (m, 1H, CH), 2.06 (m, 1H, CH₂), 1.72 (m, 1H, CH₂); ¹³C NMR (CDCl₃): δ 195.47 (C=O), 163.24 (C, Ar), 146.27 (C, Ar), 137.99 (CH, imidazole), 137.74 (C, Ar), 130.08 (CH, Ar), 129.56 (CH, imidazole), 128.97 (2 x CH, Ar), 128.59 (2 x CH, Ar), 126.70 (CH, Ar), 125.63 (C, Ar), 119.71 (CH, imidazole), 113.93 (CH, Ar), 113.14 (CH, Ar), 68.89 (CH₂), 48.58 (CH), 46.68 (CH₂-imidazole), 35.60 (CH₂), 29.06 (CH₂), 26.67 (CH₂); HPLC: 100%, RT=4.36 min; HRMS (ESI, *m/z*): Theoretical mass: 347.1759 [M+H]⁺, observed mass: 347.1754 [M+H]⁺.

2-((1*H*-imidazol-1-yl)methyl)-6-(4-fluorophenethoxy)-3,4-dihydronaphthalen-1(2*H*)-one (6b)

Prepared from 6-(4-fluorophenethoxy)-3,4-dihydronaphthalen-1(2*H*)-one (**4b**) (0.4 g, 1.4 mmol). The pure compound was obtained as a beige solid, yield: 0.18 g (35%); m.p. 96–98 °C; TLC (CH₂Cl₂-MeOH 9:1 v/v) *R_f* 0.68; ¹H NMR (CDCl₃): δ 8.03 (d, *J*=8.5 Hz, 1H, Ar), 7.52 (s, 1H, imidazole), 7.25 (t, *J*=7.5 Hz, 2H, Ar), 7.06 (s, 1H, imidazole), 7.04 (t, *J*=8.5 Hz, 2H, Ar), 6.96 (s, 1H, Ar), 6.85 (d, *J*=8.5 Hz, 1H, Ar), 6.67 (s, 1H, imidazole), 4.50 (dd, *J*=4.0, 14.0 Hz, 1H, CH_aCH_b-imidazole), 4.39 (dd, *J*=6.5, 14.0 Hz, 1H, CH_aCH_b-imidazole), 4.21 (t, *J*=7.0 Hz, 2H, CH₂), 3.10 (t, *J*=7.0 Hz, 2H, CH₂), 2.99 (m, 1H, CH₂), 2.92 (m, 1H, CH₂), 2.81 (m, 1H, CH), 2.07 (m, 1H, CH₂), 1.72 (m, 1H, CH₂); ¹⁹F NMR (CDCl₃): δ -116.38 (Ar-F); ¹³C NMR (CDCl₃): δ 195.47 (C=O), 163.12 (C, Ar), 162.75 and 160.80 (d, ¹*J*_{CF}=245.2 Hz, C-F, Ar), 146.29 (C, Ar), 138.00 (CH, imidazole), 133.49 (d, ⁴*J*_{CF}=3.3 Hz, C, Ar), 130.43 (d, ³*J*_{CF}=7.8 Hz, 2 x CH, Ar), 130.11 (CH, Ar), 129.56 (CH, imidazole), 125.71 (C, Ar), 119.71 (CH, imidazole), 115.46 (d, ²*J*_{CF}=21.4 Hz, 2 x CH, Ar), 113.86 (CH, Ar), 113.14 (CH, Ar), 68.79 (CH₂), 48.58 (CH), 46.67 (CH₂-imidazole), 34.79 (CH₂), 29.06 (CH₂), 26.66 (CH₂); Anal. Calcd. for C₂₂H₂₁FN₂O₂•0.2H₂O (368.0234): C 71.80%, H 5.86%, N 7.61%. Found: C 71.69%, H 5.71%, N 7.57%.

2-((1*H*-imidazol-1-yl)methyl)-6-(4-chlorophenethoxy)-3,4-dihydronaphthalen-1(2*H*)-one (6c)

Prepared from 6-(4-chlorophenethoxy)-3,4-dihydronaphthalen-1(2*H*)-one (**4c**) (0.3 g, 1.0 mmol). The pure compound was obtained as a brown oil, yield: 0.156 g (41%); TLC (CH₂Cl₂-MeOH 9:1 v/v) *R_f* 0.63; ¹H NMR (CDCl₃): δ 8.22 (s, 1H, imidazole), 8.01 (d, *J*=9.0 Hz, 1H, Ar), 7.29 (d, *J*=8.0 Hz, 2 H, Ar), 7.23 (d, *J*=8.0 Hz, 2 H, Ar), 7.14 (s, 1H, imidazole), 7.09 (s, 1H, Ar), 6.84 (d, *J*=9.0 Hz, 1H, Ar), 6.66 (s, 1H, imidazole), 4.55 (dd, *J*=4.0, 14.0 Hz, 1H, CH_aCH_b-imidazole), 4.39 (dd, *J*=6.5, 14.0 Hz, 1H, CH_aCH_b-imidazole), 4.21 (t, *J*=7.0 Hz, 2 H, CH₂), 3.10 (t, *J*=7.0 Hz, 2 H, CH₂), 2.99 (m, 1H, CH₂), 2.92 (m, 1H, CH₂), 2.81 (m, 1H, CH), 2.07 (m, 1H, CH₂), 1.72 (m, 1H, CH₂); ¹³C NMR (CDCl₃): δ 195.50 (C=O), 163.17 (C, Ar), 146.37 (C, Ar), 136.30 (C, Ar), 135.96 (CH, imidazole), 132.55 (C, Ar), 130.31 (2 x CH, Ar), 130.10 (CH, Ar), 128.69 (2 x CH, Ar), 126.32 (CH, imidazole), 125.61 (C, Ar), 120.54 (CH, imidazole), 113.92 (CH, Ar), 113.16 (CH, Ar), 68.54 (CH₂), 48.40 (CH), 47.36 (CH₂-imidazole), 34.92 (CH₂), 29.13 (CH₂), 26.70 (CH₂); HPLC: 100%, RT=4.45 min; HRMS (ESI, *m/z*): Theoretical mass (³⁵Cl): 381.1370 [M+H]⁺, observed mass: 381.1363 [M+H]⁺; theoretical mass (³⁷Cl): 383.1341 [M+H]⁺, observed mass: 383.1352 [M+H]⁺.

2-((1*H*-imidazol-1-yl)methyl)-6-(4-bromophenethoxy)-3,4-dihydronaphthalen-1(2*H*)-one (6d)

Prepared from 6-(4-bromophenethoxy)-3,4-dihydronaphthalen-1(2*H*)-one (**4d**) (0.29 g, 0.84 mmol). The pure compound was obtained as a beige oil, yield: 0.15 g (42%); TLC (CH₂Cl₂-MeOH 9:1 v/v) *R_f* 0.57; ¹H NMR (CDCl₃): δ 8.03 (d, *J*=9.0 Hz, 1H, Ar), 7.53 (s, 1H, imidazole), 7.46 (d, *J*=8.5 Hz, 2 H, Ar), 7.17 (d, *J*=8.0 Hz, 2 H, Ar), 7.06 (s, 1H, imidazole), 6.96 (s, 1H, Ar), 6.84 (d, *J*=8.5 Hz, 1H, Ar), 6.66 (s, 1H, imidazole), 4.50 (dd, *J*=4.5, 14.5 Hz, 1H, CH_aCH_b-imidazole), 4.39 (dd, *J*=6.5, 14.0 Hz, 1H, CH_aCH_b-imidazole), 4.21 (t, *J*=7.0 Hz, 2 H, CH₂), 3.07 (t, *J*=7.0 Hz, 2 H, CH₂), 2.98 (m, 1H, CH₂), 2.91 (m, 1H, CH₂), 2.81 (m, 1H, CH), 2.07 (m, 1H, CH₂), 1.74 (m, 1H, CH₂); ¹³C NMR (CDCl₃): δ 195.49 (C=O), 163.06 (C, Ar), 146.30 (C, Ar), 136.84 (C, Ar), 131.64 (2 x CH, Ar), 130.72 (2 x CH, Ar), 130.12 (CH, Ar), 129.37 (CH, imidazole), 129.04 (CH, imidazole), 125.72 (C, Ar), 120.58 (C, Ar), 119.77 (CH, imidazole), 113.84 (CH, Ar), 113.14 (CH, Ar), 68.44 (CH₂), 48.55 (CH), 47.70 (CH₂-imidazole), 35.00 (CH₂), 29.06 (CH₂), 26.65 (CH₂); HPLC: 100%, RT=4.44 min; HRMS (ESI, *m/z*): Theoretical mass (⁷⁹Br): 425.0864 [M+H]⁺, observed mass: 425.0859 [M+H]⁺; theoretical mass (⁸¹Br): 427.0844 [M+H]⁺, observed mass: 427.0842 [M+H]⁺.

2-((1*H*-imidazol-1-yl)methyl)-6-(4-methoxyphenethoxy)-3,4-dihydronaphthalen-1(2*H*)-one (6e)

Prepared from 6-(4-methoxyphenethoxy)-3,4-dihydronaphthalen-1(2*H*)-one (**4e**) (0.22 g, 0.74 mmol). The pure compound was obtained as a brown oil, yield: 0.11 g (39%); TLC (CH₂Cl₂-MeOH 9:1 v/v) *R_f* 0.52; ¹H NMR (CDCl₃): δ 8.03 (d, *J*=9.0 Hz, 1H, Ar), 7.52 (s, 1H, imidazole), 7.20 (d, *J*=8.0 Hz, 2 H, Ar), 7.06 (s, 1H, imidazole), 6.96 (s, 1H, Ar), 6.89 (d, *J*=8.0 Hz, 2 H, Ar), 6.86 (d, *J*=9.0 Hz, 1H, Ar), 6.67 (s, 1H, imidazole), 4.51 (dd, *J*=4.5, 14.5 Hz, 1H, CH_aCH_b-imidazole), 4.37 (dd, *J*=6.5, 14.5 Hz, 1H, CH_aCH_b-imidazole), 4.19 (t, *J*=7.0 Hz, 2 H, CH₂), 3.82 (s, 3 H, CH₃), 3.08 (t, *J*=7.0 Hz, 2 H, CH₂), 2.98 (m, 1H, CH₂), 2.92 (m, 1H, CH₂), 2.81 (m, 1H, CH), 2.08 (m, 1H, CH₂), 1.74 (m, 1H, CH₂); ¹³C NMR (CDCl₃): δ 195.47 (C=O), 163.28 (C, Ar), 158.44 (C, Ar), 146.26 (C, Ar), 137.99 (CH, imidazole), 130.07 (CH, Ar), 129.93 (2 x CH, Ar), 129.74 (C, Ar), 129.54 (CH, imidazole), 125.60 (C, Ar), 119.71 (CH, imidazole), 114.02 (2 x CH, Ar), 113.94 (CH, Ar), 113.14 (CH, Ar), 69.15 (CH₂), 55.28 (CH₃), 48.58 (CH), 46.69 (CH₂-imidazole), 34.71 (CH₂), 29.06 (CH₂), 26.67 (CH₂); HPLC: 100%, RT=4.34 min; HRMS (ESI, *m/z*): Theoretical mass: 377.1865 [M+H]⁺, observed mass: 377.1864 [M+H]⁺.

2-((1*H*-imidazol-1-yl)methyl)-6-(3-phenylpropoxy)-3,4-dihydronaphthalen-1(2*H*)-one (6f)

Prepared from 6-phenylpropoxy-3,4-dihydronaphthalen-1(2*H*)-one (**4f**) (0.5 g, 1.78 mmol). The pure compound was obtained as a beige solid, yield: 0.15 g (23%); m.p. 110–112 °C; TLC (CH₂Cl₂-MeOH 9:1 v/v) *R_f* 0.49; ¹H NMR (CDCl₃): δ 8.04 (d, *J*=9.0 Hz, 1H, Ar), 7.57 (s, 1H, imidazole), 7.31 (m, 2 H, Ar), 7.23 (m, 3 H, Ar), 7.07 (s, 1H, imidazole), 6.98 (s, 1H, Ar), 6.85 (d, *J*=9.0 Hz, 1H, Ar), 6.66 (s, 1H, imidazole), 4.51 (dd, *J*=4.5, 14.5 Hz, 1H, CH_aCH_b-imidazole), 4.41 (dd, *J*=6.5, 14.5 Hz, 1H, CH_aCH_b-imidazole), 4.02 (t, *J*=6.5 Hz, 2 H, CH₂), 3.0 (m, 1H, CH₂), 2.93 (m, 1H, CH₂), 2.83 (m, 1H, CH), 2.80 (t, *J*=7.5 Hz, 2 H, CH₂), 2.16 (quintet, *J*=6.5 Hz, 2 H, CH₂), 2.09 (m, 1H, CH₂), 1.76 (m, 1H, CH₂); ¹³C NMR (CDCl₃): δ 195.50 (C=O), 163.58 (C, Ar), 146.36 (C, Ar), 141.10 (C, Ar), 130.07 (CH, imidazole), 128.49 (5 x CH, Ar), 126.13 (CH, imidazole), 126.09 (CH, Ar), 125.42 (C, Ar), 120.64 (CH, imidazole), 114.01 (CH, Ar), 113.09 (CH, Ar), 67.11 (CH₂), 48.40 (CH), 47.42 (CH₂-imidazole), 31.98 (CH₂), 30.54 (CH₂), 29.16 (CH₂), 26.73 (CH₂); Anal. Calcd for C₂₃H₂₄N₂O₂ (360.4570): C 76.64%, H 6.71%, N 7.77%. Found: C 76.37%, H 6.71%, N 7.73%.

2-((1*H*-imidazol-1-yl)methyl)-6-(3-(4-fluorophenyl)propoxy)-3,4-dihydronaphthalen-1(2*H*)-one (6g)

Prepared from 6-(3-(4-fluorophenyl)propoxy)-3,4-dihydronaphthalen-1(2*H*)-one (**4g**) (1.0 g, 3.35 mmol). The pure compound was obtained as a beige solid, yield: 0.32 g (25%); m.p. 108–110 °C; TLC (CH₂Cl₂-MeOH 9:1 v/v) *R_f* 0.63; ¹H NMR (CDCl₃): δ 8.12 (s, 1H, imidazole), 8.02 (d, *J*=7.0 Hz, 1H, Ar), 7.17 (dd, *J*=5.50, 8.50 Hz, 2H, Ar), 7.13 (s, 1H, imidazole), 7.08 (s, 1H, Ar), 6.99 (t, *J*=9.0 Hz, 2H, Ar), 6.83 (d, *J*=9.0 Hz, 1H, Ar), 6.66 (s, 1H, imidazole), 4.54 (t, *J*=6.0 Hz, 2H, CH₂-imidazole), 4.01 (t, *J*=6.0 Hz, 2H, CH₂), 3.04 (m, 1H, CH), 2.89 (m, 2H, CH₂), 2.80 (t, *J*=7.0 Hz, 2H, CH₂), 2.19 (m, 1H, CH₂), 2.11 (quintet, *J*=6.5 Hz, 2H, CH₂), 1.74 (m, 1H, CH₂); ¹⁹F NMR (CDCl₃): δ -117.31 (Ar-F); ¹³C NMR (CDCl₃): δ 195.51 (C=O), 163.45 (C, Ar), 162.35 and 160.41 (d, ¹*J*_{CF}=244.0 Hz, C-F, Ar), 146.37 (C, Ar), 136.70 (d, ⁴*J*_{CF}=2.5 Hz, C, Ar), 130.09 (CH, Ar), 129.86 (d, ³*J*_{CF}=7.5 Hz, 2 x CH, Ar), 128.50 (CH, imidazole), 127.38 (CH, imidazole), 125.51 (C, Ar), 120.36 (CH, imidazole), 115.32 (d, ²*J*_{CF}=20.1 Hz, 2 x CH, Ar), 113.94 (CH, Ar), 113.05 (CH, Ar), 66.90 (CH₂), 48.45 (CH), 47.15 (CH₂-imidazole), 31.19 (CH₂), 30.68 (CH₂), 29.13 (CH₂), 26.70 (CH₂); Anal. Calcd for C₂₃H₂₃FN₂O₂ (378.4474): C 73.00%, H 6.13%, N 7.40%. Found: C 72.65%, H 6.13%, N 7.36%.

2-((1*H*-imidazol-1-yl)methyl)-6-(3-(4-chlorophenyl)propoxy)-3,4-dihydronaphthalen-1(2*H*)-one (6h)

Prepared from 6-(3-(4-chlorophenyl)propoxy)-3,4-dihydronaphthalen-1(2*H*)-one (**4h**) (0.5 g, 1.6 mmol). The pure compound was obtained as a white solid, yield: 0.23 g (37%); m.p. 95–97 °C; TLC (CH₂Cl₂-MeOH 9:1 v/v) *R_f* 0.59; ¹H NMR (CDCl₃): δ 8.04 (d, *J*=9.0 Hz, 1H, Ar), 7.61 (s, 1H, imidazole), 7.28 (d, *J*=8.5 Hz, 2 H, Ar), 7.15 (d, *J*=8.5 Hz, 2 H, Ar), 7.08 (s, 1H, imidazole), 6.98 (s, 1H, Ar), 6.85 (d, *J*=8.5 Hz, 1H, Ar), 6.65 (s, 1H, imidazole), 4.51 (dd, *J*=4.5, 14.5 Hz, 1H, CH_aCH_b-imidazole), 4.43 (dd, *J*=6.5, 14.5 Hz, 1H, CH_aCH_b-imidazole), 4.0 (t, *J*=6.5 Hz, 2 H, CH₂), 3.0 (m, 1H, CH₂), 2.93 (m, 1H, CH₂), 2.84 (m, 1H, CH), 2.80 (t, *J*=7.5 Hz, 2 H, CH₂), 2.18 (m, 1H, CH₂), 2.11 (quintet, *J*=6.5 Hz, 2 H, CH₂), 1.75 (m, 1H, CH₂); ¹³C NMR (CDCl₃): δ 195.54 (C=O), 163.50 (C, Ar), 146.44 (C, Ar), 139.52 (C, Ar), 137.14 (CH, imidazole), 131.84 (C, Ar), 130.09 (CH, Ar), 129.85 (2 x CH, Ar), 128.60 (2 x CH, Ar), 125.44 (C, Ar), 124.53 (CH, imidazole), 121.03 (CH, imidazole), 113.99 (CH, Ar), 113.06 (CH, Ar), 66.85 (CH₂), 48.32 (CH), 47.71 (CH₂-imidazole), 31.36 (CH₂), 30.46 (CH₂), 29.20 (CH₂), 26.74 (CH₂); Anal. Calcd for C₂₃H₂₃ClN₂O₂ (394.8990): C 69.96%, H 5.87%, N 7.09%. Found: C 70.05%, H 5.87%, N 7.00%.

2-((1*H*-imidazol-1-yl)methyl)-6-(3-(4-bromophenyl)propoxy)-3,4-dihydronaphthalen-1(2*H*)-one (6i)

Prepared from 6-(3-(4-bromophenyl)propoxy)-3,4-dihydronaphthalen-1(2*H*)-one (**4i**) (0.48 g, 1.34 mmol). The pure compound was obtained as a beige solid, yield: 0.19 g (32%); m.p. 88–90 °C; TLC (CH₂Cl₂-MeOH 9:1 v/v) *R_f* 0.49; ¹H NMR (CDCl₃): δ 8.12 (s, 1H, imidazole), 8.02 (d, *J*=9.0 Hz, 1H, Ar), 7.43 (d, *J*=8.5 Hz, 2 H, Ar), 7.14 (s, 1H, imidazole), 7.09 (s, 1H, Ar), 7.08 (d, *J*=8.5 Hz, 2 H, Ar), 6.85 (d, *J*=8.5 Hz, 1H, Ar), 6.65 (s, 1H, imidazole), 4.54 (t, *J*=5.5 Hz, 2 H, CH₂-imidazole), 4.0 (t, *J*=6.0 Hz, 2 H, CH₂), 3.04 (m, 1H, CH), 2.91 (m, 2 H, CH₂), 2.78 (t, *J*=8.0 Hz, 2 H, CH₂), 2.20 (m, 1H, CH₂), 2.11 (quintet, *J*=7.0 Hz, 2 H, CH₂), 1.74 (m, 1H, CH₂); ¹³C NMR (CDCl₃): δ 195.50 (C=O), 163.44 (C, Ar), 146.40 (C, Ar), 140.05 (C, Ar), 131.55 (2 x CH, Ar), 130.27 (2 x CH, Ar), 130.10 (CH, Ar), 130.07 (CH, imidazole), 126.17 (CH, imidazole), 125.49 (C, Ar), 120.67 (CH, imidazole), 119.85 (C, Ar), 113.96 (CH, Ar), 113.05 (CH, Ar), 66.82 (CH₂), 48.39 (CH), 47.41 (CH₂-imidazole), 31.43 (CH₂), 30.39 (CH₂), 29.16 (CH₂), 26.72 (CH₂); Anal. Calcd for C₂₃H₂₃BrN₂O₂ (439.3530): C 62.88%, H 5.28%, N 6.38%. Found: C 63.13%, H 5.21%, N 6.34%.

2-((1*H*-imidazol-1-yl)methyl)-6-(3-(4-methoxyphenyl)propoxy)-3,4-dihydronaphthalen-1(2*H*)-one (6j)

Prepared from 6-(3-(4-methoxyphenyl)propoxy)-3,4-dihydronaphthalen-1(2*H*)-one (**4j**) (0.5 g, 1.6 mmol). The pure compound was obtained as a beige solid, yield: 0.18 g (29%); m.p. 94–96 °C; TLC (CH₂Cl₂-MeOH 9:1 v/v) *R_f* 0.49; ¹H NMR (CDCl₃): δ 8.26 (s, 1H, imidazole), 8.02 (d, *J*=9.0 Hz, 1H, Ar), 7.15 (s, 1H, imidazole), 7.11 (d, *J*=8.0 Hz, 2 H, Ar), 7.11 (s, 1H, Ar), 6.85 (d, *J*=7.5 Hz, 2 H, Ar), 6.85 (m, 1H, Ar), 6.66 (s, 1H, imidazole), 4.56 (m, 2 H, CH₂-imidazole), 4.01 (t, *J*=6.0 Hz, 2 H, CH₂), 3.81 (s, 3 H, CH₃), 3.04 (m, 1H, CH), 2.94 (m, 2 H, CH₂), 2.76 (t, *J*=7.0 Hz, 2 H, CH₂), 2.23 (m, 1H, CH₂), 2.10 (quintet, *J*=6.5 Hz, 2 H, CH₂), 1.74 (m, 1H, CH₂); ¹³C NMR (CDCl₃): δ 195.50 (C=O), 163.61 (C, Ar), 157.98 (C, Ar), 146.36 (C, Ar), 137.54 (CH, imidazole), 133.10 (C, Ar), 130.06 (CH, Ar), 129.39 (2 x CH, Ar), 126.17 (CH, imidazole), 125.40 (C, Ar), 120.74 (CH, imidazole), 114.00 (2 x CH, Ar), 113.91 (CH, Ar), 113.09 (CH, Ar), 67.08 (CH₂), 55.28 (CH₃), 48.40 (CH), 47.42 (CH₂-imidazole), 31.03 (CH₂), 30.75 (CH₂), 29.16 (CH₂), 26.73 (CH₂); Anal. Calcd for C₂₄H₂₆N₂O₃ (390.4830): C 73.82%, H 6.71%, N 7.17%. Found: C 73.64%, H 6.64%, N 7.10%.

General method for the preparation of 3-(imidazole/triazole)-7-alkoxy-4*H*-chromen-4-one derivatives (12 and 13)

A mixture of imidazole/triazole (3 equiv), K₂CO₃ (3 equiv) and dry CH₃CN (2 mL/mmol) were heated at 45 °C for 1 h. After cooling to room temperature 3-(bromomethyl)-7-alkoxy-4*H*-chromen-4-one derivatives (**11**) (1 equiv) was added and the reaction mixture heated at 70 °C overnight. The solvent was evaporated under reduced pressure and the resulting mixture diluted with EtOAc (20 mL) and washed with brine (3 × 10 mL) and H₂O (3 × 10 mL). The organic layer was dried (MgSO₄) and evaporated under reduced pressure to give the crude product, which was purified by gradient column chromatography (CH₂Cl₂-MeOH 95:5 v/v) to give the product.

3-((1*H*-imidazol-1-yl)methyl)-7-(3-phenylpropoxy)-4*H*-chromen-4-one (12a)

Prepared from 3-(bromomethyl)-7-(3-phenylpropoxy)-4*H*-chromen-4-one (**11a**) (0.125 g, 0.335 mmol). The pure compound was obtained as a cream solid, yield: 0.11 g (96%); m.p. 137–139 °C; TLC (CH₂Cl₂-MeOH 95:5 v/v) *R_f* 0.3; ¹H NMR (CDCl₃): δ 8.33 (s, 1H, imidazole), 8.12 (d, *J*=8.9 Hz, 1H, Ar), 8.03 (s, 1H, CH), 7.31 (t, *J*=7.7 Hz, 2 H, Ar), 7.23 (t, *J*=7.4 Hz, 4 H, Ar and imidazole), 7.14 (s, 1H, imidazole), 7.01 (dd, *J*=2.4, 8.9 Hz, 1H, Ar), 6.81 (d, *J*=2.3 Hz, 1H, Ar), 5.16 (s, 2 H, CH₂), 4.05 (t, *J*=6.3 Hz, 2 H, CH₂), 2.85 (t, *J*=7.5 Hz, 2 H, CH₂), 2.17 (m, 2 H, CH₂); ¹³C NMR (CDCl₃): δ 175.90 (C=O), 163.92 (C, Ar), 158.34 (C, Ar), 154.29 (CH), 140.93 (C, Ar), 128.53 (2 x CH, Ar), 128.47 (2 x CH, Ar), 127.17 (CH, imidazole), 127.13 (CH, Ar), 126.83 (CH, imidazole), 126.14 (CH, Ar), 120.34 (CH, imidazole), 120.12 (C, Ar), 117.50 (C, Ar), 115.45 (CH, Ar), 100.88 (CH, Ar), 67.64 (CH₂), 42.77 (CH₂-imidazole), 31.97 (CH₂), 30.41 (CH₂); Anal. Calcd for C₂₂H₂₀N₂O₃•0.2H₂O (364.0146): C 72.59%, H 5.65%, N 7.70%. Found: C 72.50%, H 5.57%, N 7.66%.

3-((1*H*-imidazol-1-yl)methyl)-7-(3-(4-fluorophenyl)propoxy)-4*H*-chromen-4-one (12b)

Prepared from 3-(bromomethyl)-7-(3-(4-fluorophenyl)propoxy)-4*H*-chromen-4-one (**11b**) (0.12 g, 0.312 mmol). The pure compound was obtained as a cream solid, yield: 0.11 g (90%); m.p. 98–100 °C; TLC (CH₂Cl₂-MeOH 95:5 v/v) *R_f* 0.3; ¹H NMR (DMSO-*d*₆): δ 8.46 (s, 1H, CH), 7.95 (d, *J*=8.9 Hz, 1H, Ar), 7.70 (s, 1H, imidazole), 7.26 (m, 2H, Ar), 7.19 (s, 1H, imidazole), 7.13 (d, *J*=2.3 Hz, 1H, Ar), 7.10 (m, 3H, Ar), 6.85 (s, 1H, imidazole), 4.95 (s, 2H, CH₂), 4.10 (t, *J*=6.3 Hz, 2H, CH₂), 2.74 (t, *J*=7.7 Hz, 2H,

CH₂), 2.04 (m, 2H, CH₂); ¹⁹F NMR (DMSO-d₆): -117.45; ¹³C NMR (DMSO-d₆): δ 175.50 (C=O), 163.72 (C, Ar), 161.11 (d, ¹J_{CF} = 240.9 Hz, Ar), 158.31 (C, Ar), 156.15 (CH), 138.00 (CH, imidazole), 137.74 (d, ⁴J_{CF} = 3.0 Hz, C, Ar), 137.73 (C, Ar), 130.55 (d, ³J_{CF} = 7.4 Hz, 2 x CH, Ar), 128.81 (CH, imidazole), 128.70 (CH, Ar), 126.93 (CH, Ar), 120.39 (C, Ar), 119.96 (CH, imidazole), 117.55 (C, Ar), 115.71 (s, 1 C), 115.62 (d, ²J_{CF} = 20.2 Hz, 2 x CH, Ar), 101.73 (CH, Ar), 68.13 (CH₂), 41.61 (CH₂), 30.94 (CH₂), 30.53 (CH₂); Anal. Calcd for C₂₂H₁₉FN₂O₃ (378.4034): C 69.83%, H 5.06%, N 7.40%. Found: C 69.73%, H 5.31%, N 7.25%.

3-((1*H*-imidazol-1-yl)methyl)-7-(3-(4-chlorophenyl)propoxy)-4*H*-chromen-4-one (12c)

Prepared from 3-(bromomethyl)-7-(3-(4-chlorophenyl)propoxy)-4*H*-chromen-4-one (**11c**) (0.15 g, 0.37 mmol). The pure compound was obtained as a cream solid, yield: 0.12 g (85%); m.p. 126–128 °C; TLC (CH₂Cl₂-MeOH 95:5 v/v) R_f 0.3; ¹H NMR (DMSO-d₆): δ 8.46 (s, 1H, CH), 7.95 (d, *J* = 8.9 Hz, 1H, Ar), 7.70 (s, 1H, imidazole), 7.33 (d, *J* = 8.5 Hz, 2 H, Ar), 7.26 (d, *J* = 8.5 Hz, 2 H, Ar), 7.19 (s, 1H, imidazole), 7.14 (d, *J* = 2.4 Hz, 1H, Ar), 7.07 (dd, *J* = 2.4, 8.9 Hz, 1H, Ar), 6.85 (s, 1H, imidazole), 4.95 (s, 2 H, CH₂), 4.10 (t, *J* = 6.3 Hz, 2 H, CH₂), 2.74 (t, *J* = 7.7 Hz, 2 H, CH₂), 2.04 (m, 2 H, CH₂); ¹³C NMR (DMSO-d₆): δ 175.50 (C=O), 163.70 (C, Ar), 158.30 (C, Ar), 156.14 (CH), 140.67 (C, Ar), 138.02 (CH, imidazole), 130.95 (C, Ar), 130.71 (2 x CH, Ar), 128.72 (2 x CH, Ar), 126.92 (C, Ar), 120.39 (C, Ar), 119.97 (CH, imidazole), 117.56 (C, Ar), 115.70 (CH, Ar), 101.74 (CH, Ar), 68.09 (CH₂), 41.61 (CH₂), 31.09 (CH₂), 30.28 (CH₂); Anal. Calcd for C₂₂H₁₉ClN₂O₃ (394.8550): C 66.92%, H 4.85%, N 7.09%. Found: C 66.73%, H 5.02%, N 6.99%.

3-((1*H*-imidazol-1-yl)methyl)-7-(3-(4-bromophenyl)propoxy)-4*H*-chromen-4-one (12d)

Prepared from 3-(bromomethyl)-7-(3-(4-bromophenyl)propoxy)-4*H*-chromen-4-one (**11d**) (0.12 g, 0.265 mmol). The pure compound was obtained as a cream solid, yield: 0.10 g (89%); m.p. 122–123 °C; TLC (CH₂Cl₂-MeOH 95:5 v/v) R_f 0.33; ¹H NMR (DMSO-d₆): δ 8.46 (s, 1H, CH), 7.95 (d, *J* = 8.9 Hz, 1H, Ar), 7.70 (s, 1H, imidazole), 7.46 (d, *J* = 8.2 Hz, 2 H), 7.20 (d, *J* = 8.3 Hz, 3 H, Ar and imidazole), 7.13 (d, *J* = 2.2 Hz, 1H, Ar), 7.07 (dd, *J* = 2.2, 8.9 Hz, 1H, Ar), 6.85 (s, 1H, imidazole), 4.95 (s, 2 H, CH₂), 4.09 (t, *J* = 6.3 Hz, 2 H, CH₂), 2.73 (t, *J* = 7.6 Hz, 2 H, CH₂), 2.04 (quintet, *J* = 7.2 Hz, 2 H, CH₂); ¹³C NMR (DMSO-d₆): δ 175.50 (C=O), 163.70 (C, Ar), 158.30 (C, Ar), 156.14 (CH), 141.09 (C, Ar), 138.02 (CH, imidazole), 131.64 (2 x CH,

Ar), 131.13 (2 x CH, Ar), 128.70 (CH, imidazole), 126.93 (CH, Ar), 120.39 (C, Ar), 119.98 (CH, imidazole), 119.38 (C, Ar), 117.56 (C, Ar), 115.70 (CH, Ar), 101.75 (CH, Ar), 68.08 (CH₂), 41.62 (CH₂), 31.14 (CH₂), 30.22 (CH₂); Anal. Calcd for C₂₂H₁₉BrN₂O₃•0.1H₂O (441.1105): C 59.90%, H 4.39%, N 6.35%. Found: C 59.50%, H 4.42%, N 6.08%.

3-((1*H*-imidazol-1-yl)methyl)-7-(3-(4-methoxyphenyl)propoxy)-4*H*-chromen-4-one (12e)

Prepared from 3-(bromomethyl)-7-(3-(4-methoxyphenyl)propoxy)-4*H*-chromen-4-one (**11e**) (0.11 g, 0.27 mmol). The pure compound was obtained as a cream solid, yield: 0.08 g (76%); m.p. 140–142 °C; TLC (CH₂Cl₂-MeOH 95:5 v/v) R_f 0.30; ¹H NMR (CDCl₃): δ 8.13 (d, *J* = 8.9 Hz, 1H, Ar), 7.70 (s, 1H, imidazole), 7.66 (s, 1H, CH), 7.13 (d, *J* = 8.5 Hz, 2 H, Ar), 7.10 (s, 1H, imidazole), 7.08 (s, 1H, imidazole), 6.86 (d, *J* = 8.5 Hz, 2 H, Ar), 6.80 (d, *J* = 2.1 Hz, 1H, Ar), 5.03 (s, 2 H, CH₂), 4.04 (t, *J* = 6.3 Hz, 2 H, CH₂), 3.80 (s, 3 H, OCH₃), 2.79 (t, *J* = 7.5 Hz, 2 H, CH₂), 2.14 (t, *J* = 7.1 Hz, 2 H, CH₂); ¹³C NMR (CDCl₃): δ 175.73 (C=O), 163.90 (C, Ar), 158.29 (C, Ar), 157.99 (C, Ar), 153.10 (CH), 137.58 (CH, imidazole), 132.92 (C, Ar), 129.59 (CH, imidazole), 129.38 (2 x CH, Ar), 127.19 (CH, Ar), 120.70 (C, Ar), 119.52 (CH, imidazole), 117.47 (C, Ar), 115.38 (CH, Ar), 113.92 (2 x CH, Ar), 100.81 (CH, Ar), 67.58 (CH₂), 55.27 (CH₃), 42.30 (CH₂-imidazole), 31.01 (CH₂), 30.62 (CH₂); HPLC: 100%, RT = 4.18 min; HRMS (ESI, m/z): Theoretical mass: 391.1653 [M+H]⁺, observed mass: 391.1638 [M+H]⁺.

3-((1*H*-triazole-1-yl)methyl)-7-(3-phenylpropoxy)-4*H*-chromen-4-one (13a)

Prepared from 3-(bromomethyl)-7-(3-phenylpropoxy)-4*H*-chromen-4-one (**11a**) (0.10 g, 0.27 mmol). The pure compound was obtained as a white solid, yield: 0.09 g (92%); m.p. 150–152 °C; TLC (CH₂Cl₂-MeOH 95:5 v/v) R_f 0.36; ¹H NMR (CDCl₃): δ 8.46 (s, 1H, triazole), 8.11 (d, *J* = 8.9 Hz, 1H, Ar), 8.05 (s, 1H, triazole), 7.95 (s, 1H, CH), 7.32 (t, *J* = 7.5 Hz, 2 H, Ar), 7.23 (t, *J* = 7.6 Hz, 3 H, Ar), 7.01 (dd, *J* = 2.3, 8.9 Hz, 1H, Ar), 6.82 (d, *J* = 2.3 Hz, 1H, Ar), 5.24 (s, 2 H, CH₂), 4.05 (t, *J* = 6.3 Hz, 2 H, CH₂), 2.85 (t, *J* = 7.5 Hz, 2 H, CH₂), 2.18 (quintet, *J* = 7.1 Hz, 2 H, CH₂); ¹³C NMR (CDCl₃): δ 175.78 (C=O), 163.93 (C, Ar), 158.35 (C, Ar), 154.63 (CH), 151.44 (CH, triazole), 144.12 (CH, triazole), 140.92 (C, Ar), 128.53 (2 x CH, Ar), 128.48 (2 x CH, Ar), 127.16 (CH, Ar), 126.15 (CH, Ar), 118.63 (C, Ar), 117.55 (C, Ar), 115.43 (CH, Ar), 100.91 (CH, Ar), 67.63 (CH₂), 44.90 (CH₂), 31.96 (CH₂), 30.41 (CH₂); Anal. Calcd for C₂₁H₁₉N₃O₃ (361.4010): C 69.79%, H 5.30%, N 11.63%. Found: C 69.89%, H 5.30%, N 11.68%.

3-((1*H*-triazole-1-yl)methyl)-7-(3-(4-fluorophenyl)propoxy)-4*H*-chromen-4-one (13b)

Prepared from 3-(bromomethyl)-7-(3-(4-fluorophenyl)propoxy)-4*H*-chromen-4-one (**11b**) (0.12 g, 0.312 mmol). The pure compound was obtained as a white solid, yield: 0.11 g (91%); m.p. 145–147 °C; TLC (CH₂Cl₂-MeOH 95:5 v/v) *R_f* 0.36; ¹H NMR (DMSO-*d*₆): δ 8.57 (s, 1H, triazole), 8.52 (s, 1H, triazole), 7.93 (s, 1H, CH), 7.92 (d, *J*=8.9 Hz, 2H), 7.26 (m, 2H, Ar), 7.16 (d, *J*=2.4 Hz, 1H, Ar), 7.10 (m, 3H, Ar), 5.18 (s, 2H, CH₂), 4.10 (t, *J*=6.3 Hz, 2H, CH₂), 2.74 (t, *J*=7.7 Hz, 2H, CH₂), 2.04 (m, 2H, CH₂); ¹⁹F NMR (DMSO-*d*₆): δ -117.43; ¹³C NMR (DMSO-*d*₆): δ 175.38 (C=O), 163.75 (C, Ar), 161.11 (d, ¹*J*_{CF} = 240.9 Hz, Ar), 158.31 (C, Ar), 156.66 (CH), 151.74 (CH, triazole), 144.99 (CH, triazole), 137.73 (d, ⁴*J*_{CF} = 3.0 Hz, C, Ar), 130.53 (d, ³*J*_{CF} = 7.6 Hz, 2 x CH, Ar), 126.88 (CH, Ar), 118.62 (C, Ar), 117.47 (C, Ar), 115.74 (CH, Ar), 115.44 (d, ²*J*_{CF} = 20.9 Hz, 2 x CH, Ar), 101.71 (CH, Ar), 68.13 (CH₂), 44.71 (CH₂), 30.92 (CH₂), 30.51 (CH₂); HPLC: 100%, RT=4.60 min; HRMS (ESI, *m/z*): Theoretical mass: 379.1332 [M+H]⁺, observed mass: 379.1340 [M+H]⁺.

3-((1*H*-triazole-1-yl)methyl)-7-(3-(4-chlorophenyl)propoxy)-4*H*-chromen-4-one (13c)

Prepared from 3-(bromomethyl)-7-(3-(4-chlorophenyl)propoxy)-4*H*-chromen-4-one (**11c**) (0.15 g, 0.37 mmol). The pure compound was obtained as a white solid, yield: 0.13 g (88%); m.p. 156–158 °C; TLC (CH₂Cl₂-MeOH 95:5 v/v) *R_f* 0.37; ¹H NMR (DMSO-*d*₆): δ 8.58 (s, 1H, CH), 8.52 (s, 1H, triazole), 7.93 (d, *J*=8.7 Hz, 1H, Ar), 7.92 (s, 1H, triazole), 7.34 (d, *J*=8.4 Hz, 2H, Ar), 7.27 (d, *J*=8.4 Hz, 2H, Ar), 7.15 (d, *J*=2.3 Hz, 1H, Ar), 7.08 (dd, *J*=2.4, 8.9 Hz, 1H, Ar), 5.18 (s, 2H, Ar), 4.10 (s, 2H, CH₂), 2.75 (t, *J*=7.7 Hz, 2H, CH₂), 2.05 (m, 2H, CH₂); ¹³C NMR (DMSO-*d*₆): δ 175.34 (C=O), 163.73 (C, Ar), 158.32 (C, Ar), 156.69 (CH), 151.77 (CH, triazole), 145.00 (CH, triazole), 140.67 (C, Ar), 130.95 (C, Ar), 130.72 (2 x CH, Ar), 128.72 (2 x CH, Ar), 126.88 (CH, Ar), 118.65 (C, Ar), 117.51 (C, Ar), 115.74 (CH, Ar), 101.77 (CH, Ar), 68.10 (CH₂), 44.70 (CH₂), 31.09 (CH₂), 30.29 (CH₂); Anal. Calcd for C₂₁H₁₈ClN₃O₃ (395.8430): C 63.72%, H 4.58%, N 10.62%. Found: C 63.48%, H 4.77%, N 10.43%.

3-((1*H*-triazole-1-yl)methyl)-7-(3-(4-bromophenyl)propoxy)-4*H*-chromen-4-one (13d)

Prepared from 3-(bromomethyl)-7-(3-(4-bromophenyl)propoxy)-4*H*-chromen-4-one (**11d**) (0.12 g, 0.265 mmol). The pure compound was obtained as a white solid, yield: 0.11 g (96%); m.p. 160–162 °C; TLC (CH₂Cl₂-MeOH 95:5

v/v) *R_f* 0.40; ¹H NMR (DMSO-*d*₆): δ 8.57 (s, 1H, CH), 8.51 (s, 1H, triazole), 7.93 (d, *J*=8.9 Hz, 1H, Ar), 7.92 (s, 1H, triazole), 7.47 (d, *J*=8.3 Hz, 2H, Ar), 7.21 (d, *J*=8.3 Hz, 2H, Ar), 7.15 (d, *J*=2.3 Hz, 1H, Ar), 7.08 (dd, *J*=2.3, 8.9 Hz, 1H, Ar), 5.18 (s, 2H, CH), 4.10 (t, *J*=6.3 Hz, 2H, CH₂), 2.73 (t, *J*=7.6 Hz, 2H, CH₂), 2.04 (quintet, *J*=7.3 Hz, 2H, CH₂); ¹³C NMR (DMSO-*d*₆): δ 175.38 (C=O), 163.73 (C, Ar), 158.30 (C, Ar), 156.67 (CH), 151.74 (CH, triazole), 144.99 (CH, triazole), 141.09 (C, Ar), 131.63 (2 x CH, Ar), 131.12 (2 x CH, Ar), 126.89 (CH, Ar), 119.37 (C, Ar), 118.62 (C, Ar), 117.48 (C, Ar), 115.74 (CH, Ar), 101.74 (CH, Ar), 68.09 (CH₂), 44.71 (CH₂), 31.14 (CH₂), 30.21 (CH₂); Anal. Calcd for C₂₁H₁₈BrN₃O₃ (440.2970): C 57.29%, H 4.12%, N 9.54%. Found: C 57.58%, H 4.30%, N 9.55%.

3-((1*H*-triazole-1-yl)methyl)-7-(3-(4-methoxyphenyl)propoxy)-4*H*-chromen-4-one (13e)

Prepared from 3-(bromomethyl)-7-(3-(4-methoxyphenyl)propoxy)-4*H*-chromen-4-one (**11e**) (0.11 g, 0.27 mmol). The pure compound was obtained as a white solid, yield: 0.09 g (84%); m.p. 161–163 °C; TLC (CH₂Cl₂-MeOH 95:5 v/v) *R_f* 0.35; ¹H NMR (DMSO-*d*₆): δ 8.57 (s, 1H, CH), 8.51 (s, 1H, triazole), 7.93 (d, *J*=8.9 Hz, 2H, Ar), 7.92 (s, 1H, triazole), 7.14 (m, 3H, Ar), 7.08 (dd, *J*=2.3, 8.9 Hz, 1H, Ar), 6.84 (d, *J*=8.5 Hz, 2H, Ar), 5.18 (s, 2H, CH₂), 4.09 (t, *J*=6.3 Hz, 2H, CH₂), 3.71 (s, 3H, OCH₃), 2.69 (t, *J*=7.6 Hz, 2H, CH₂), 2.02 (t, *J*=7.4 Hz, 2H, CH₂); ¹³C NMR (DMSO-*d*₆): δ 175.35 (C=O), 163.81 (C, Ar), 158.32 (C, Ar), 157.95 (C, Ar), 156.68 (CH), 151.77 (CH, triazole), 144.99 (CH, triazole), 129.74 (2 x CH, Ar), 126.89 (CH, Ar), 118.65 (C, Ar), 117.48 (C, Ar), 115.75 (CH, Ar), 114.23 (2 x CH, Ar), 101.75 (CH, Ar), 68.18 (CH₂), 55.41 (OCH₃), 44.71 (CH₂), 30.85 (CH₂), 30.72 (CH₂); HPLC: 98%, RT=4.56 min; HRMS (ESI, *m/z*): Theoretical mass: 392.1605 [M+H]⁺, observed mass: 392.1598 [M+H]⁺.

Biological assays

Antimycobacterial activity was determined using either the Spot culture growth inhibition (SPOTi) assay [27] or the Resazurin Microtiter Assay (REMA) assay [29] with full details provided in the supporting information. The different *Mtb* strains used are detailed in Table S3.

Full details for the UV-Vis CYP121A1 spectral binding assay [26] for *K_D* determination, bacterial minimum bactericidal concentrations (MBC₉₅) determination and MTS cytotoxicity assays [30] are reported in the supporting information.

Computational studies

Molecular docking was performed using Molecular Operating Environment (MOE 2024.0601) software [32] to generate protein-ligand complexes, which were then subject to molecular dynamics simulations using the Desmond programme of Schrödinger (Maestro 2025-2) [34], the full methods are provided in the supporting information.

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1007/s00044-026-03631-5>.

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Author contributions JMA and LAA performed the chemistry and computational studies supervised by CS. AK and SYK performed the CYP121A1 UV-Vis binding studies supervised by DFE. SW performed the SPOTi assay. AKB performed the REMA and MBC assays while AYGA performed the MTS assay supervised by AKB. CS, LAA and JMA wrote the manuscript, and all authors revised and approved the final manuscript.

Data availability The data supporting this article is included in the main text or is included as part of the Supplementary Information.

Declarations

Competing interests The authors declare no competing interests.

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