AMPA Receptor Positive Allosteric Modulators: Potential for the Treatment of Neuropsychiatric and Neurological Disorders

Tristan Reuillon*, Simon E Ward and Paul Beswick

Sussex Drug Discovery Centre, School of Life Sciences, University of Sussex, Falmer, Brighton, BN1 9QJ, UK

Abstract: The neurotransmitter glutamate and its receptors have long been of interest to scientists involved in pharmaceutical research since dysfunction of the glutamatergic signalling pathway has been associated with the pathophysiology of several psychiatric and neurological disorders. The research on AMPAR positive allosteric modulators offers opportunities to modulate fast excitatory synaptic transmission and identify new potential therapeutic agents for a range of neurodiseases. The field of AMPAR modulators continues to be a dynamic area of drug discovery with a pronounced diversification of the chemotypes explored in recent years. This article reviews literature published in this area in the last 6 years, focusing on the new core templates, some derived from high-throughput screens, with an emphasis on structure-activity relationships, drug metabolism and pharmacokinetics proper-ties, and pharmacological profiles of these series.

GLUTAMATE AND ITS IMPLICATION IN NEURO-PSYCHIATRIC AND NEUROLOGICAL DISORDERS

In the mammalian central nervous system (CNS), the neurotransmitter glutamate, which is released from presynaptic terminals, mediates the greater part of excitatory signal-ling through binding to glutamatergic receptors widely distributed on neurons and glia [1]. Glutamate acts on two distinct classes of receptors: ionotropic (ion channels) and metabotropic (G-protein coupled receptors) [2]. There are three types of glutamate ionotropic receptors named according to the chemical entity originally discovered to selectively activate each channel: AMPA (α-amino-3-hydroxyl-5-methyl-4-isoxazolepropionic acid), NMDA (N-methyl-D-aspartate) and kainite [3]. The pathophysiology of several psychiatric and neurological disorders has been associated with glutamate neurotransmission dysfunction, such as schizophrenia, Alzheimer's disease, Parkinson's disease and mood disorders [4-6]. Consequently modulation of the glutamatergic signalling pathway attracted the interest of the pharmaceutical research community since it could potentially lead to the discovery of innovative treatments for a range of conditions. However the complex pharmacology of glutamatergic neuro-transmission and the absence of highly selective chemical tools have restricted major advancement in this neurological area for many years. Recent developments in the understanding of this physiological pathway [7] and the identification of novel, rationally designed, AMPA receptor modulators with targeted pharmacokinetic profiles represent great promise and could lead to new therapies for a range of neurological and psychiatric conditions in the near future.

Hypofunction of fast excitatory synaptic transmission has been linked to cognition deficit, a major symptom in Alzheimer's disease and schizophrenic patients.[4] In the case of schizophrenia, it has been proposed that the disease pre-dominantly originates from NMDA receptors' (NMDARs) glutamatergic synaptic underactivity, with non-selective NMDAR antagonists causing schizophrenia-like symptoms in healthy volunteers [8,9]. In addition, post-mortem analysis of schizophrenic hippocampus has revealed a subnormal density of AMPA receptors (AMPARs) and an increased concentration of the glutamate receptor antagonist N-acetylaspartyl glutamate, emphasizing the link

with hypo-glutamatergic neurotransmission [10]. Dynamic regulation of AMPARs in postsynaptic membranes and activation of the receptor are intimately associated with synaptic plasticity, a mechanism believed to underlie learning and memory [11,12]. Under normal physiological conditions, repetitive AMPAR stimulation induces postsynaptic neuronal mem-brane depolarization, provoking colocalized NMDARs activation, following release of the Mg2+ block. This enhances NMDAR Ca2+-gating [13], which concomitantly with AM-PAR-mediated cation influx eventually results in synapses strengthening (synaptic plasticity) [12,14]. In turn, the NMDAR-increased synaptic signalling influences receptor trafficking and additional AMPARs are in-serted into the synapses.[15]

STRUCTURE AND MODULATION OF AMPA RE-CEPTORS

AMPARs are tetrameric complexes, organized as dimers of dimers.[16] Four different subunits (GluA1 to GluA4), composed of about 900 amino acids and sharing 65 to 75% sequence homology, can combine to form homo- or heterodimers, which subsequently combine to form a tetramer.[17] Distribution of AMPAR subunits in the CNS has been demonstrated to be uneven between the various brain regions, with different cellular expression within each region.[18] Over the last decade, single electron microscopy and X-ray crystallography have enabled to develop a better understanding of the structure and assembly of the AMPAR subunits.[19] Recently, the combined use of X-ray crystallography and cryogenic electron microscopy has enabled to obtain structural information for GluA2-containing AMPAR heteromers and has revealed new insights into the dynamic arrangement of the extracellular region of the receptor.[20] Each subunit is composed of an extracellular domain, a transmembrane domain and an intracellular domain.[21] The extracellular domain comprises a large N-terminal region with binding sites for regulatory proteins, and a ligand binding domain (LBD) consisting of two discontinuous sub-domains, S1 and S2 arranged in a clamshell motif allowing glutamate binding. The transmembrane section is composed of three α -helices (TM1, TM3 and TM4) and a re-entrant hairpin loop (TM2), which constitutes part of the ion channel pore.[22] The intracellular section of each subunit displays the C-terminal domain, which possesses interaction sites for a range of accessory proteins, contributing to AMPARs trafficking and regulation of the receptor kinetics.[23–26] A diverse range of subunit assembly can lead to functional

AMPARs. The composition of the channel in vivo is extremely complicated and influences receptor kinetics and glutamate binding. An extra layer of structural diversity arises from the presence of sites of post-translational modifications and the existence of splice isoforms, known as flip (i) (Ser775; UniProt ascension number P42262-2) and flop (o) (Asn775; UniProt ascension number P42262-1).[27,28] The flip/flop variation, found in the S2 sub-domain of the LBD, controls the kinetics of channel closure and desensitization.[29,30] Incorporation of flip or flop isoforms in a tetrameric complex is influenced by transmembrane proteins [23].

In view of the connection of glutamatergic neurotransmission dysfunction with diverse neurological and psychiatric disorders, generation of glutamate receptor ligands has been of interest and a range of different approaches investigated. In particular, according to the mechanism of synaptic plasticity and the causes of cognition deficit in diseases, such as schizophrenia, enhancement of AMPAR-mediated synaptic transmission has been extensively explored.[31,32] Originally direct or partial AMPAR agonists were searched, but it quickly became clear that such compounds were associated with high risk of toxicity following over excitation of the receptor signalling.[33,34] This approach precluded the precise and refined spatial and temporal control, required for optimal cognitive functioning, and was overcome by the discovery of AMPAR positive allosteric modulators (PAMs), termed potentiators, which lack intrinsic activity in absence of glutamate. Nonetheless, when AMPARs are functionally activated via binding of the endogenous substrate, they enhance the agonistic effect by modulating the channel biophysical properties.[35] Potentiators enhance AMPAR response

through two distinct effects on synaptic currents: inhibiting desensitization and/or slowing deactivation.[36] Unlike orthosteric agonists, PAMs maintain the magnitude and/or duration of channel opening during synaptic activity. However AMPAR potentiators can still cause excitotoxicity, resulting in motor coordination disruption and/or convulsion,[37,38] and therefore carefully determining pre-clinical therapeutic indexes of these molecules is critical for onward development. The majority of modulators described to date have limited subunit selectivity but excellent selectivity against the other ionotropic and metabotropic glutamate receptors. It has been hypothesized that some AMPAR sub-types might govern the 'positive' pharmacology of AMPAR PAMs whereas other subtypes might cause the undesired adverse events. Hence selectively targeting the 'positive' subtypes would prevent side-effects and offer an optimal therapeutic index. Unfortunately it is currently impossible to biologically test this assumption.

After years of intense research, recent studies have established that AMPARs are macromolecular complexes with a large molecular diversity.[39] These result from the assembly of the GluA tetramer with auxiliary proteins including the transmembrane AMPAR regulatory proteins (TARPs), the cornichon homologs (CNIH) and the CKAMP44 protein.[40] Findings into the heteromultimeric nature of AMPARs indicate that the receptor functions beyond glutamate binding may be largely influence by non-GluA subunit constituents. In particular, members of the TARP family of proteins regulate AMPAR trafficking and pharmacology, and also control sensitivity to AMPAR potentiators. [41,42] TARPs have different regional expression across the CNS, hence providing the innovative and attractive opportunity to modulate AMPARs in specific brain regions to access potentiators with potential therapeutic advantage (Fig. 1). This would be achieved by identifying TARP-dependent AMPAR PAMs and a similar strategy has recently been successfully implemented by Lilly in their search for safer AMPAR antagonists for the treatment of epilepsy.[43] Positive emission tomography studies with a range of AMPAR modulators have indicated that different concentrations of potentiators are required to selectively activate the CNS regions associated with procognitive and undesired effects, suggesting a potential role of localized auxiliary proteins resulting in AMPAR complexes with different biophysical properties [44].

The LBD of the channel has an opened bilobate structure which closes upon binding of glutamate, initiating the gating cascade.[45,46] According to X-ray cocrystal structures of positive modulators bound to the LBD of AMPAR, all potentiators bind at the S1S2 interface of the LBD, resulting in increased stability of the closed glutamate-bound dimer.[47] Several studies have demonstrated that the isolated LBD possesses similar pharmacological properties to the native membrane-bound AMPAR.[48] This has enabled to significant advance in the understanding of the biophysical and structural properties of the channel, allowing structure-based drug design (SBDD) of agonists and antagonists, but also the identification of binding sites for PAMs.[49] Irrespective of their mechanistic properties and mode of action, AMPAR potentiators have been shown to enhance glutamatergic synaptic activity in several in vitro experiments both on recombinant and native tissue preparation,[14,50] and improve cognition in preclinical animal models and in small but not all clinical trials.[51–55] In addition, AMPAR PAMs have been therapeutically beneficial to treat a range of neuropsychiatric and neurological conditions in animal models, such as anxiety, attention-deficit hyperactivity disorder (ADHD), Parkinson's disease and depression.[56–60]

CLINICAL STUDIES WITH AMPAR POTENTIA-TORS

Successful medicinal chemistry programmes have delivered a number of clinical candidates that have entered clinical development. Encouragingly no major mechanism-related safety issues have been reported and some com-pounds have shown promising efficacy in early patient-based studies, however no compound has yet demonstrated sufficiently robust efficacy to merit large scale efficacy

studies. Table 1 summarizes compounds that have entered clinical evaluation but have been terminated.

Cortex pioneered early work in the area and reported positive clinical data on early compounds such as CX516, which demonstrated positive effects in studies with healthy volunteers[55] and as an adjunct therapy in patients with schizophrenia.[61,62] The early promise with CX516 did not continue as the compound failed to demonstrate efficacy in a number of later studies when tested in patients with mild cognitive impairment,[63] autism[64] or Alzheimer's Disease.[65] A subsequent compound CX717 (structure has not yet been revealed) also showed equivocal efficacy results, failing to demonstrate beneficial effects in healthy subjects[66] but demonstrating efficacy in ADHD patients.[67] Cortex failed to obtain funding to follow up on the positive results seen in patients with ADHD, however they are still developing CX717 for respiratory depression (Table 2). Cortex have also taken another compound CX691 into the clinic for psychiatric indications either alone or following licensing deals with other companies, but the com-pound failed to demonstrate beneficial effects.[68–70] Organon, Lilly and GlaxoSmithKline (GSK) have also reported negative data.[63,71–74] Understanding the factors which have contributed to the positive results seen with some com-pounds and the failures with others has been and will be key in the design of the next generation of AMPA modulators.

A number of AMPAR modulators remain under clinical investigation (Table 2). Cortex is again a leading player in the field, and have changed their therapeutic focus from psychiatric indications to respiratory conditions and consequently have changed the company name to RespireRx. They are currently progressing three compounds for sleep apnoea and drug induced respiratory depression.[75] The only company currently known to be active in the psychiatry area is Pfizer with a compound PF-4958242 11 in Phase I studies. A total of eleven Phase I studies have been con-ducted over an extended period,[76] many of these studies focus on pharmacokinetic evaluation of investigation of drug-drug interaction potential, and suggest that there are potentially a number of safety concerns with the compound. The results of one study with PF-04958242 have been published, where it showed beneficial effects in treating age related hearing loss.[77]

CHEMOTYPES OF AMPAR POSITIVE ALLOSTERIC MODULATORS

A number of excellent comprehensive reviews, describing the historical chemotypes originally explored as selective AMPAR positive modulators, have been published in recent years.[78–80] Briefly, three major classes have been explored: the benzamides, the thiadiazines and the phenethyl sulfonamides. Chronologically, the benzamide series, derived from the nootropic agent aniracetam 1, was first looked into and developed by Cortex into derivatives such as CX516 2, CX691 3 and ORG26576 4, which were all tested in the clinic. Then the thiadiazine series, originating from the diuretic agent cyclothiazide 5, was also extensively investigated, leading to Servier’s clinical candidate S-18986 6. Finally, a class of phenethyl sulfonamides, discovered by Lilly, generated significant interest with a number of subsequent patent applications from several pharmaceutical companies also covering sulfonamide derived structures. This class has yielded candidate compounds including LY451395 7 and LY450108 8 from Lilly, GSK729327 9 from GSK and PF-04958242 11 from Pfizer. Over time the modulators developed have become increasingly more potent for the AM-PAR, with the original benzamides being weaker than the phenethylsulfonamides. [78]

As highlighted in our 2015 review of the AMPAR patent literature,[81] a number of novel chemotypes have recently been discovered, some of which are suggested to have a different mode of action and potentially a different clinical profile. This article will concentrate on new developments reported in the scientific literature over the last six years and not fully covered by previous reviews. We will mainly

focus on the new core templates, some derived from high throughput screens (HTS), with an emphasis on structure activity relationships (SARs), drug metabolism and pharmacokinetics (DMPK) properties and pharmacological profiles of these series. Whilst only GSK and Pfizer have described in detail the discovery and profiles of development candidates (9, 10 and 11), [82, 83, 44] other organisations have published descriptions of a range of different chemotypes for which no development has been reported. It is also interesting to note that many recent scientific articles and patents in this area have been published by academic groups with a reduced number coming from industrial laboratories, this perhaps has been influenced by the decision of many companies to withdraw from the neuroscience area, and also in some cases the move of experts in this area from industry to the academic sector.

TWO NOVEL TEMPLATES FROM GSK

Between 2005 and 2010, GSK have filed a large number of patent applications for novel AMPAR modulators, indicating a strong interest of the company in this area of neuro-science [84–95]. In the period covered by this review, they published three scientific articles, each focusing on a different chemotype [82, 83, 96].

Table 1. Compounds which entered clinical evaluation but for which progression has been terminated

Molecule	Trial	Status
CX516 (2) Ampalex	Cognitive performance in healthy young and aged volunteers[55,61]	Improved cognitive performance
	CX516 added to Clozapine, Olanzapine or Risperidone in Patients with Schizophrenia[62]	Inconsistent cognitive benefits
	Efficacy and Safety of CX516 in Elderly Participants with Mild Cognitive Impairment[63]	Completed
	Effects of CX516 on Functioning in Fragile X Syndrome and Autism[64]	Completed
	Treatment of Alzheimer's Disease with CX516[65]	Completed
CX691 (3) ORG24448 SCH900460 Faramaptor	Cardiac Safety Study of Phase I and Phase II ORG24448 patients[69]	95 patients Completed
	Depression: 8 week treatment, includes PET analysis Excluded patients with propensity for seizure following EEG assessment[68]	180 patients Completed
	TUNRS NIMH-funded cognitive impairments in schizophrenia: 8 weeks adjunctive therapy to existing atypical antipsychotic medication[70]	Terminated
ORG26576 (4)	Phase II Major Depressive Disorder: 54 patients[72] Part 1: 100-600 mg bid up to 16 days Part 2: 30 subjects; high dose group and low dose group; 28 days dosing	Completed
	ADHD: 60 patients[71]; 100-300 mg BID; 8 weeks	Completed
LY451395 (7) (Mibampator)	Aggression and Agitation in Alzheimer's Disease: 132 patients; 3 mg bid 12 weeks[63]	Comparable to placebo on all endpoint except Frontal Systems Behavior Inventory where a significant improvement was demonstrated ($p=0.007$) (38)
	Alzheimer's Disease: 200 patients[74]	Negative
GSK729327 (9)	Phase I: Safety and Tolerability in healthy volunteers: 79 volunteers, 1-6 mg[73]	Completed

Table 2. Compounds currently under active clinical investigation

Molecule	Trial	Status
CX717	DARPA-funded small scale trial in healthy volunteers subjected to simulated night shift work[66]	No improvement in cognitive performance
	ADHD small (n = 23-28) study of 200 mg bid and 800 mg bid for 3 weeks in adults with moderate-severe symptoms	Significant improvements at highest dose on hyperactivity and inattention indices
	Drug-induced respiratory depression	Pending
CX1739	Sleep apnoea Drug-induced respiratory depression	No effect on mean apnoea/hypopnea index, however significantly reduced apnoea time
CX1942	Respiratory depression (intra-venous)	Pending
PF-04958242 (**11**)	Phase I[76]	On-going

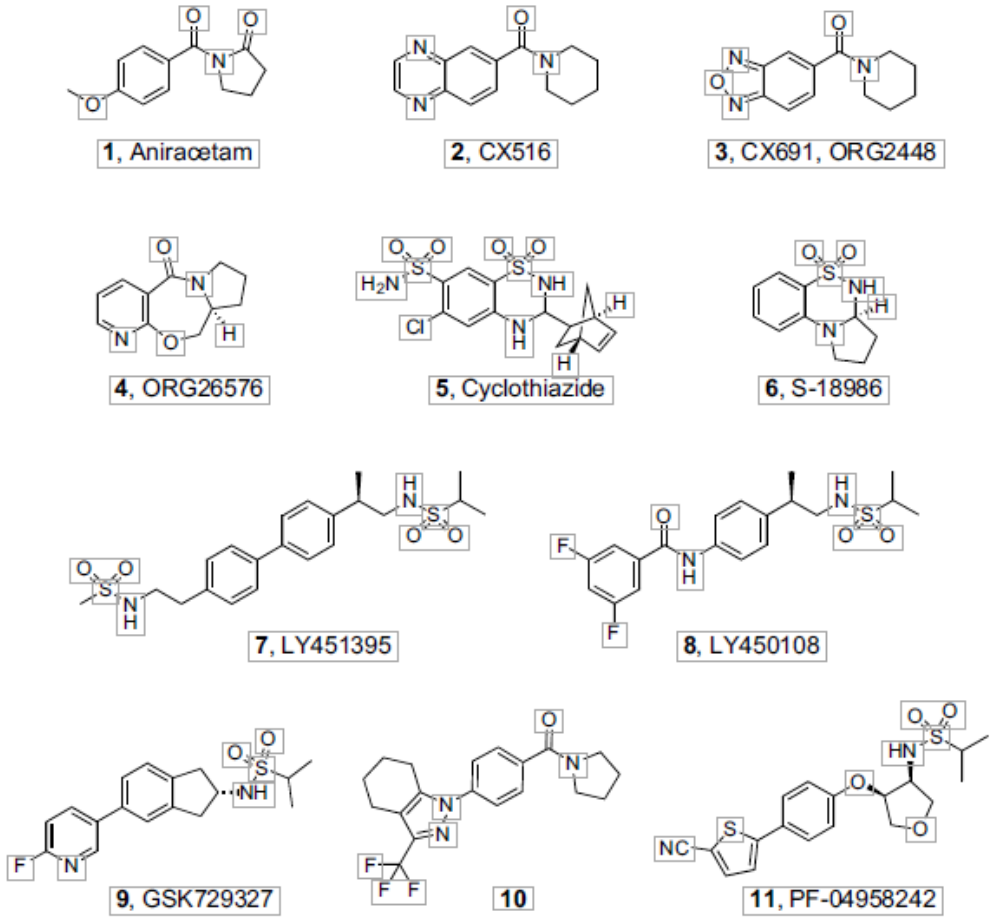


Fig. (1). Representative chemotypes of AMPAR positive allosteric modulators.

In a short communication,[96] they reported the discovery of a series of N-substituted pyrrolidines and tetrahydrofurans, related to the phenethyl sulfonamide modulators de-scribed by Lilly. We can assume that this exploratory work, based on existing literature, was conducted when the company first started to develop an interest in this area and can be considered as GSK's initial attempt to identify AMPAR positive modulators. The original Lilly phenethyl sulfonamide series generally suffered from poor physicochemical properties, such as low solubility, high polar surface area and high lipophilicity, which might have contributed to the failure of the compounds which progressed to the clinic (LY451395 7 and LY450108 8). By introducing heteroatoms on the phenethyl moiety, using 3,4-disubstituted pyrrolidines (Fig. 2) and 3,4-disubstituted tetrahydrofurans (Fig. 3) link-

ers, and exploring replacement for the N-phenyl methanesulfonamide motif, the GSK team was attempting to modulate these undesired properties. Interestingly the reported pyrrolidine linker derivatives have relative trans stereochemistry whereas the tetrahydrofuran (THF) linker derivatives have relative cis stereochemistry. This renders direct comparisons between the two sub-series challenging and it is assumed that direct matched-pairs have not been prepared, since according to the authors these stereochemistry were only selected because of their synthetic tractability. The compounds were screened for their ability to potentiate glutamate-induced calcium influx against recombinant hGluA2i receptors in a Fluorometric Imaging Plate Reader (FLIPR) based assay, with the maximal response of cyclothiazide defined as 100%.

Rigidification of the linker and introduction of a heteroatom in this part of the template was well tolerated since the first derivative prepared 12 (R = Me) induced strong potentiation of the hGluA2i recombinant receptor (EC50 = 0.3 µM, Asym Max = 124%). Different substituents on the pyrrolidine nitrogen were investigated (12 and 13, R = Me, Et,

iPr, Ph) and showed that N-methylated pyrrolidines afforded the best activities with bulkier groups leading to decreased potentiation. Reduction of the polar surface area was examined by replacing the meta methylsulfonamide moiety by a para nitrile in 13 or various meta substituents in 14, such as a methylsulfone, an acetamide, a methylketone or a trifluoromethyl group. All of these modifications resulted in at least a 10-fold loss in potency. Changing the phenyl ring for a pyridyl 15 (R = R' = H) or a substituted pyridyl 15 (R = H, R' = F or R = F, R' = H) produced compounds with higher EC50s than 12 (R = Me), in the micromolar range (2.5 to 5 µM). Thiophenes were also tried as potential phenyl replacement but afforded weaker compounds (16, R = 2-thiophene, EC50 = 20 µM; 16, R = 3-thiophene, EC50 = 3.2 µM).

A major downside of the majority of these pyrrolidine-linked isopropylsulfonamides was an undesired off-target inhibition of the hERG cardiac channel. Despite a reduction in lipophilicity, these molecules contain a basic centre, a common feature in compounds with affinity for hERG, and

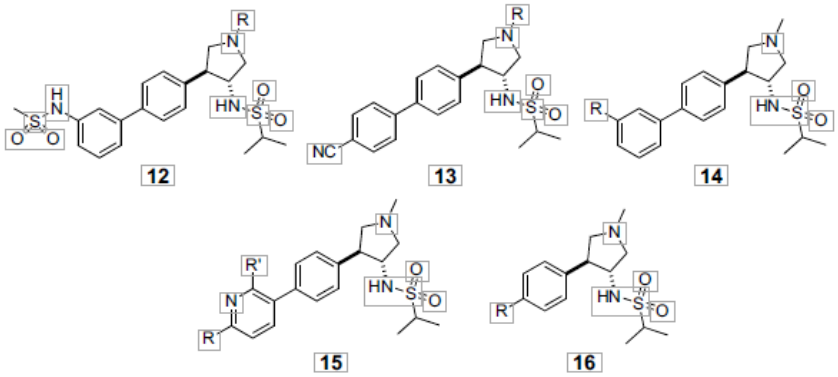


Fig. (2). Representative structures of the pyrrolidine-linked isopropylsulfonamide derivatives.

some derivatives were very potent hERG inhibitors, such as 12 (R = Ph, hERG IC50 = 1.3 µM) or 15 (R = F, R' = H, hERG IC50 = 1.0 µM). An increase in solubility was success-fully achieved, with compound 12 (R = Me) and 13 (R = Me) achieving levels of > 9 mg/mL and > 2.7 mg/mL, respectively. Intrinsic clearance was high in both rat and human liver microsomes (RLM and HLM) for N-methylated derivatives, with N-dealkylation being identified as a major route of metabolism. Compounds with larger N-substituents generally achieved good microsomal stability but had similar or higher affinity for hERG relative to AMPAR. It was hypothesized that substituting the pyrrolidine ring by a THF could

mitigate the hERG and metabolic liabilities of this series and afford selective compounds with a better pharmacokinetic profile.

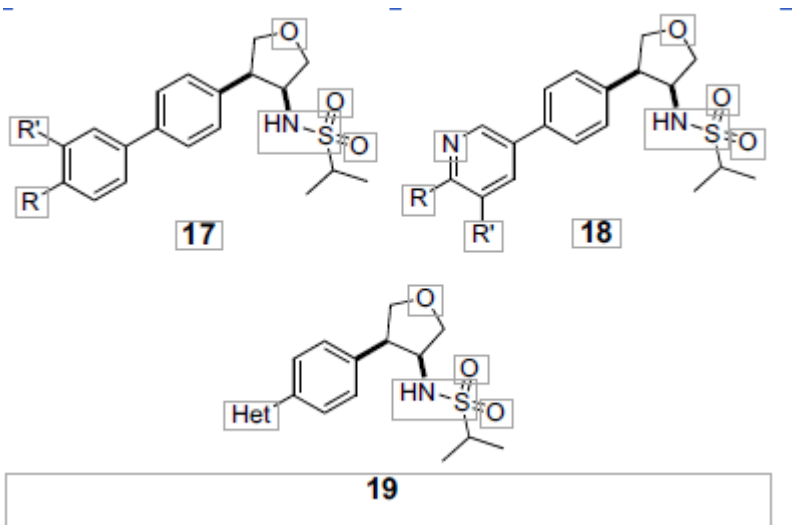


Fig. (3). Representative structures of the tetrahydrofuran-linked isopropylsulfonamide derivatives.

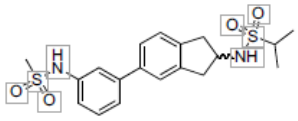
All the THF analogues reported (7 in total) showed excellent potentiation of the AMPAR (EC50 < 0.5 µM; Asym Max > 112%) and were significantly more active than the N-methyl pyrrolidine derivatives. This could be explained by the reduced steric bulk of the THF and also possibly by the different relative stereochemistry. The calculated lipophilicity and polar surface area of the THF derivatives was lower than that of the pyrrolidine analogues. With the exception of 17 (R = CN, R' = H) and 18 (R = F, R' = H), all the molecules in this subseries had more than 100-fold selectivity for hGluA2i over hERG. Unfortunately only a limited set of in vitro DMPK data was reported for these THF modulators and it is impossible to know if these molecules successfully achieved the targeted properties discussed earlier. Indeed, the intrinsic clearance of only two analogues 18 (R = H, R' = F) and 19 (Het = 2-(5-F)-pyridyl) was disclosed and both com-pounds had high turnover in RLM. In addition, no solubility or CNS permeability data are given for the THF derivatives.

The limited success in identifying AMPAR modulators with desirable physicochemical and pharmacokinetic proper-ties starting from a literature chemotype probably prompted GSK to identify their own chemical hits through screening of in-house compound libraries. Hence in a second publication from 2010, scientists at GSK reported the discovery and optimization of indane clinical candidate 9. [82] From this publication, it appears that the indane series was the first novel chemical core extensively explored by GSK in their search for AMPAR positive modulators. Interestingly in addition to a high potency, scientists at GSK were also targeting a specific pharmacokinetic profile, which would deliver high unbound compound concentrations. In this respect, the article not only presents the SAR around the indane core but also detailed in vitro and in vivo DMPK data, which led to the selection of the clinical candidate.

The exploratory work in this series started with the identification of lead amino indane 20, a rigid analogue of the phenethyl series, which comprises the two sulfonamide moieties of Lilly's clinical candidate LY451395 7. Com-pound 20 induced significant potentiation of the AMPAR-mediated response against recombinant hGluA2i receptors in a FLIPR-based fluorescent-indicator dye assay (pEC50 = 6.1, Asym Max = 123%). Owing to its encouraging calculated physicochemical properties (see Table 3), lead 20 was pro-filed in an array of in vitro assays, and found to have accept-able rat protein plasma binding (95.9%) and good selectivity for AMPAR over closely related ion channels. Additionally, it did not cause CYP450 inhibition at low concentrations but was identified as a moderate P-gp

substrate with a moderate efflux ratio (ER = 5.8) in the permeability MDCK-MDR1 assay. This was assumed to be caused by the high polar sur-face area (PSA) (92 Å²) and resulted in low CNS penetration in a rat oral pharmacokinetic study (Table 1, brain:blood ratio = 0.1) despite good systemic exposure.

Table 3. Physicochemical and DMPK properties of lead compound 20

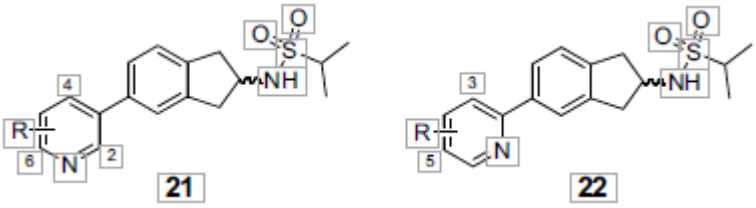


20

PSA (Å²)	MWt (g/mol)	clogP[a]	PPB[b]	AUC_{0-t} (ng·h/mL)	Brain:Blood AUC_{0-t} ratio	P_{app} (+inh) (nm/s)[c]	ER[d]
92	409	2.7	95.9%	1130	0.1	608	5.8

a) clogP Daylight Chemical Information Systems Inc., Alisa Viejo, CA, http://www.daylight.com; b) Rat plasma protein binding determined *via* a dialysis method at 1 µg/mL; c) Apical (A) to basolateral (B) transport rates of molecules (0.5 µM) across MDCK-MDR1 cells in presence of a potent P-gp inhibitor; d) Efflux ratio: the ratio of transport from B-A/A-B in the absence of a P-gp inhibitor

With the preliminary SAR indicating that modulation of the isopropylsulfonamide moiety was not tolerated, attention turned to modification of the phenyl ring. Deletion of the meta substituent and introduction of ortho or para groups led to a significant reduction in potency. A broad range of alternative meta substituents were investigated but none retained the activity of compound 20, with the exception of sulfonamide derivatives (secondary and tertiary), but which also maintained undesirable high PSA. In vivo exposure of some of these potent analogues was determined in rat pharmacokinetic studies but similarly to 20 all suffered from poor CNS penetration. As a result, a number of aromatic rings with significantly lower polar surface area was explored, with the objective to reduce affinity for P-gp while maintaining high free drug concentration and good potency. This led to the identification of 3-pyridyl and 2-pyridyl indane analogues (21, R = H and 22, R = H). Although, unsubstituted 3-pyridyl analogue 21 (R = H) had lower potency in the FLIPR assay (pEC50 = 4.8, Asym Max = 101%), inhibited CYP isoform 1A2 (IC50 = 0.6 µM) and was moderately stable in rat microsomes (Cli = 2.1 mL/min/g), it possessed significantly improved physicochemical properties, such as lower PSA (59 Å²), molecular weight (316 g/mol) and clogP (2.3). These pyridyl derivatives were deemed interesting starting points for the identification of compounds with improved CNS penetration and a series of close analogues was prepared. To avoid an increase of the polar surface area (PSA < 60 Å²), pyridyl substituents were limited to alkyl and halo groups.



21 22

Fig. (4). Chemical structure of the racemate pyridyl indane derivatives.

In the 3-pyridyl indane series 21, introduction of a single substituent at any position of the pyridyl ring prevented CYP450 inhibition, but only para-substitution provided stronger potentiators (21, R = 6-F, pEC50 = 5.3, Asym Max = 106%; 21, R = 6-Me, pEC50 = 5.5, Asym Max = 107%). Intrinsic clearance across this set of substituted 3-pyridyl analogues was variable, all compounds had noticeably higher human microsomal turnover than the unsubstituted pyridyl 21 (R = H) and the fluoro analogues were generally more stable than the methyl substituted pyridines. The same pattern in terms of potency (22, R = 5-F, pEC50 = 5.2, Asym Max = 114%; 22, R = 5-Me, pEC50 = 5.5, Asym Max = 90%), CYP450

inhibition and microsomal stability was observed in the 2-pyridyl indane series 22. To validate the initial hypothesis that a reduction in PSA would increase CNS exposure, a selection of compounds from these series (21 and 22) with lower PSA than 20 were assessed in rat in vivo pharmacokinetic studies and found to have significantly improved brain to blood ratios (21, R = 2-F, brain:blood ratio = 1.5; 22, R = 6-Me, brain:blood ratio = 1.4) (Fig. 4).

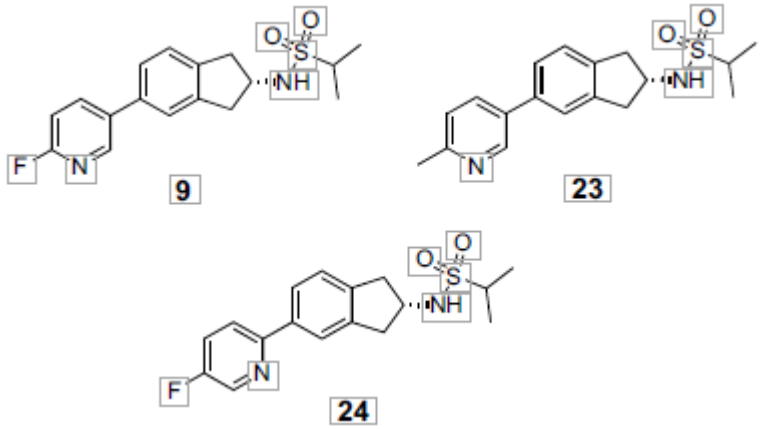


Fig. (5). Structure of the three enantiopure indanes extensively profiled.

The exploratory work described above was conducted using racemic mixtures of indanes, comprising R and S enantiomers. Testing of single enantiomers indicated that the S enantiomers were significantly more potent, hence further exploration and characterisation then focussed on derivatives with the S stereochemistry. Three enantiomerically pure modulators (9, 23 and 24) were profiled in detail. Indanes 9, 23 and 24 all produced good potentiation of AMPAR in the FLIPR assay, did not inhibit the CYP450 isoforms tested and retained the good microsomal clearance of the racemic mixtures, with methyl derivative 23 having higher intrinsic turn over than fluoro derivatives 9 and 24. In a whole-cell patch clamp electrophysiology assay, these compounds also showed clear potentiation of AMPAR-mediated currents over a range of concentrations in HEK293 cells expressing hGluA2i homomeric receptors. Furthermore, all three derivatives (9, 23 and 24) had excellent selectivity against a wide range of ion channels, enzymes and GPCRs. When tested in two in vivo behavioural cognition models, compounds 9 and 24 had a significant effect. Administration of both com-pounds at 0.3 mg/kg (PO) improved recognition memory of male rats, in the 24 h delay-induced deficit in a novel object recognition (NOR) experiment. [97] In the passive avoidance task assay,[98] modulator 9 attenuated the scopolamine-induced amnesic effect when administered at 3 and 10 mg/kg (PO), while compound 24 was active at 1 and 3 mg/kg (PO). Based on the efficacy and DMPK data, only derivatives 9 and 24 were progressed to safety and tolerability studies. From these studies (no direct comparison is given), 9 was selected as the development compound (Fig. 5).

The binding mode of the indane series was determined from a 1.8 Å resolution crystal structure of the functional hGluA2i S1S2 ligand binding domain in complex with modulator 9 (PDB code 2xhd; Fig. 6). The compound was found to bind in an analogous manner to the phenethyl sulfonamides described by Lilly, with the isopropylsulfonamide moiety pointing into one of the two deep hydrophobic pockets present at the dimer interface and the sulfonamide NH making a hydrogen bond with the backbone carbonyl of Pro515.

Clinical candidate 9 had low equilibrium solubility (≤ 0.1 mg/mL) across the range of pH (2 to 10) and physiological fluids (water, simulated gastric fluid, and simulated intestinal fluid) tested, but high permeability (Papp (+inh) = 688 nm/s; ER = 1.1), which delivered attractive pharmacokinetic profiles. Indeed, 9 had good oral bioavailability (51 to 61%) and low blood clearance (3 to 11% liver blood flow)

in the three preclinical species tested (rat, dog and monkey). In addition to these suitable developability properties, compound 9 had excellent solid and solution stability under a range of conditions, did not induce time-dependent inhibition of CYP450 and no formation of glutathione conjugate was detected, eliminating the potential liability associated with the presence of the α-fluoropyridine. AMPAR modulator 9 induced weak PXR activation in both rat and human, and as such only had a low propensity for drug-drug interactions through induction of CYP3A4. The in vitro plasma protein binding of the compound was measured in five species (mouse, rat, dog, monkey and human), found to be moderate (from 79.5 to 90.8%) with low interspecies variability, and was fitting with the targeted pharmacokinetic profile sought by the GSK group.

A metabolite identification study was conducted using in vitro rat, dog, monkey and human microsomes. Five metabolites were observed at low abundance relative to the parent drug, and all metabolites detected in HLM were also present in the other microsomal species. Three metabolites resulted from hydroxylation of the aliphatic part of the indane ring and one of these was a major metabolite across all species.

Oxidation of the isopropyl moiety was also detected but rep-resented a minor route. Aryl hydroxylation was the final metabolic route identified and was only significant in rat microsomes. Furthermore, development compound 9 was not genotoxic in the selected in vitro genetic toxicity screens and did not exhibit any cardiovascular risk in an in vitro hERG assay or following single oral dosing up to 2.5 mg/kg in cynomolgus monkeys. Finally, clinical candidate 9 had no observed adverse effect levels and acceptable safety margins in preclinical safety screening up to 6 weeks. All of these results prompted evaluation of 9 in the clinic.

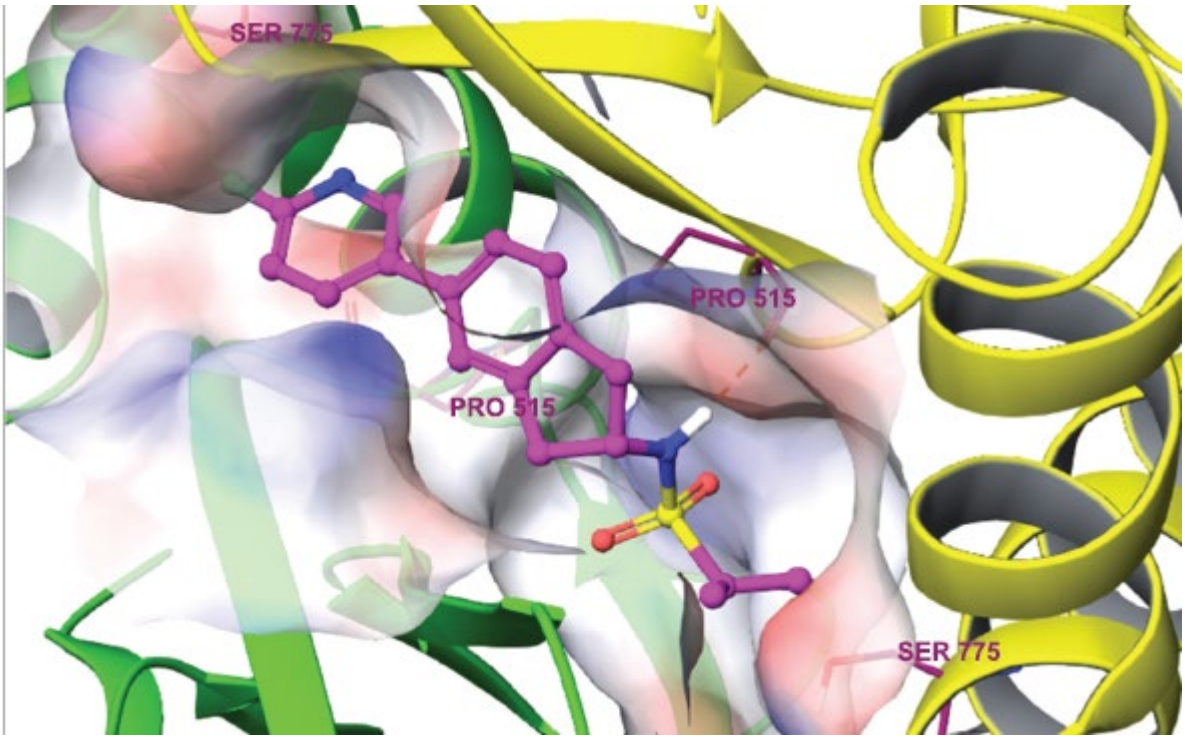


Fig. (6). X-ray crystal structure of clinical candidate 9 in complex with hGluA2i S1S2 LBD (PDB code 2xhd, 1.8 Å resolution)*

* All the X- Ray images presented in this review were generated using Maestro, version 10.5, Schrödinger, LLC, New York, NY, 2016

An initial phase I study was conducted in healthy volunteers to explore the safety and tolerability of the candidate, as well as its pharmacokinetics in human. Two dosing regimens were investigated: single doses (0.25 to 6 mg) and multipledose of 0.1 mg administered daily for 28 days. In both cases, indane 9 was rapidly absorbed (Cmax reached between 0.5 and 3 h post-administration) and had a very long half-life (107-168 h). This resulted from a very low apparent oral clearance and a large apparent volume of distribution, in excess of total body water. This pharmacokinetic profile was not predicted from preclinical species and prevented further clinical evaluation of the compound. Importantly, both dosing regimens were well tolerated with no safety issues raised or withdrawal caused by adverse events (Fig. 8).

In a third publication from 2011, GSK report the identification of a novel chemical series originating from a high-throughput screen and optimized using ligand-bound X-ray crystal structures.[83] The optimization work in that series led to the identification of GSK second clinical candidate, compound 10. The HTS was run using a FLIPR-based fluorescent indicator dye protocol in HEK cells expressing re-combinant hGluA2i receptors, indicating the ability of the screened molecules to potentiate glutamate-induced calcium influx. Despite a moderate AMPAR potentiation (pEC50 = 4.6, Asym Max = 94%) in the FLIPR assay relative to other hits, compound 25 was selected as an attractive starting point from the screen output because of its superior preliminary developability profile. Indeed, hit 25 had good physicochemical properties (MWt = 337 g/mol and logD = 3.0) and performed well in a battery of in vitro as-says. The molecule had moderate protein binding (plasma and brain), was devoid of significant CYP450 inhibitory activity, and had good selectivity against a range of ion channels, receptors and enzymes. Low kinetic solubility and high metabolic turnover in microsomes (rat Cli = 12.7 mL/min/g; human Cli = 3.5 mL/min/g) were identified as the major shortcomings of 25 and resulted in low systemic exposure (AUC0-t = 78 ng·h/mL) when the compound was tested in a rat pharmacokinetic assay. However in that assay hit 25 had good CNS exposure (brain:blood AUC0-t ratio = 1.3), suggesting high permeability.

In order to rapidly identify a development candidate, the screening cascade used in the optimization work not only relied on in vitro functional efficacy screens (FLIPR assay and electrophysiology), but also extensively on physicochemical properties calculation. Unusually for a membrane-bound ion channel target, X-ray crystallography was also routinely employed enabling SBDD and a focused exploration of chemical space to quickly access highly potent modulators and identify a clinical candidate. The crystal structure of tetrahydroindazole 25 in complex with the rat GluA2i S1S2 LBD and glutamate was determined at 1.55 Å (PDB code 2xx8; Fig. 7), and indicated that the phenyl ring sits at the S1-S2 interface on the 2-fold axis, similarly to other AMPAR PAMs. The trifluoromethyl group occupies one end of the inverted U shaped binding pocket and has displaced a cluster of four water molecules, while the carbonyl of the amide points down into the symmetrical end of the inverted U, making a hydrogen bond with one of the four water molecules. The aliphatic part of the tetrahydroindazole heterocycle makes contact with solvent, suggesting that modification of this ring might not be tolerated. Several serine residues, which can interact with small-molecule modulators via hydrogen bonding, are present in the binding pocket but were not exploited by hit 25. The dimethylamide group was identified as amenable for modification and as a handle to potentially reach Ser750, Ser775 or the water molecules as hydro-gen bonding partners.

The hit optimization campaign focussed on improvement of potency, solubility and also metabolic turnover. The high metabolic clearance of compound 25 was assumed to be caused by the dimethylamide moiety and other acyclic am-ides (26, R = CH2CH2Ph or R = Bu) where one of the methyl groups was replaced by a larger substituent were prepared. These derivatives retained similar

potency to 25 but had reduced solubility and similar or higher microsomal instability.

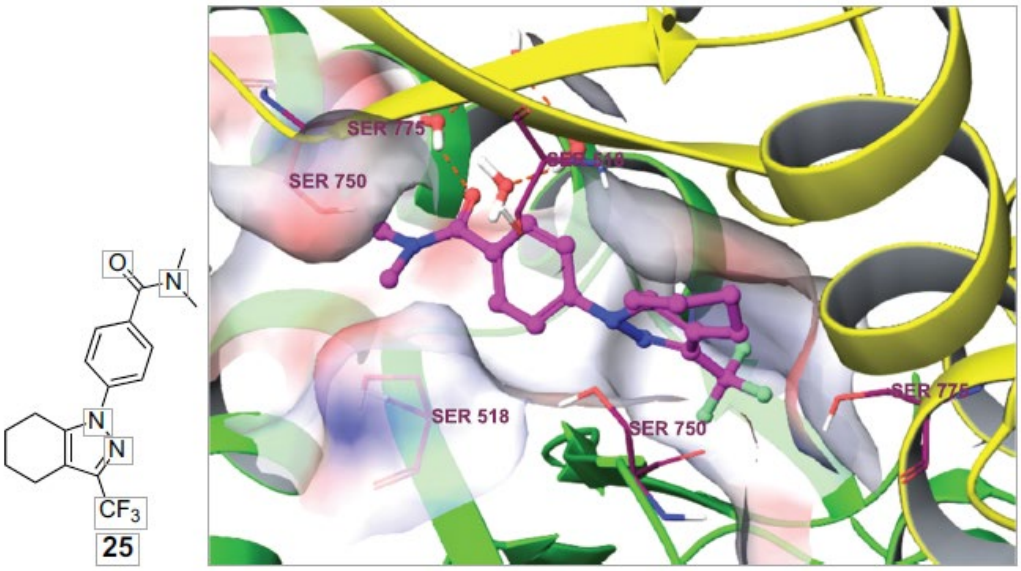


Fig. (7). Chemical structure of hit 25 and its X-Ray crystal structure with the hGLuA2i S1S2 LBD (PDB code 2xx8, 1.55 Å resolution).

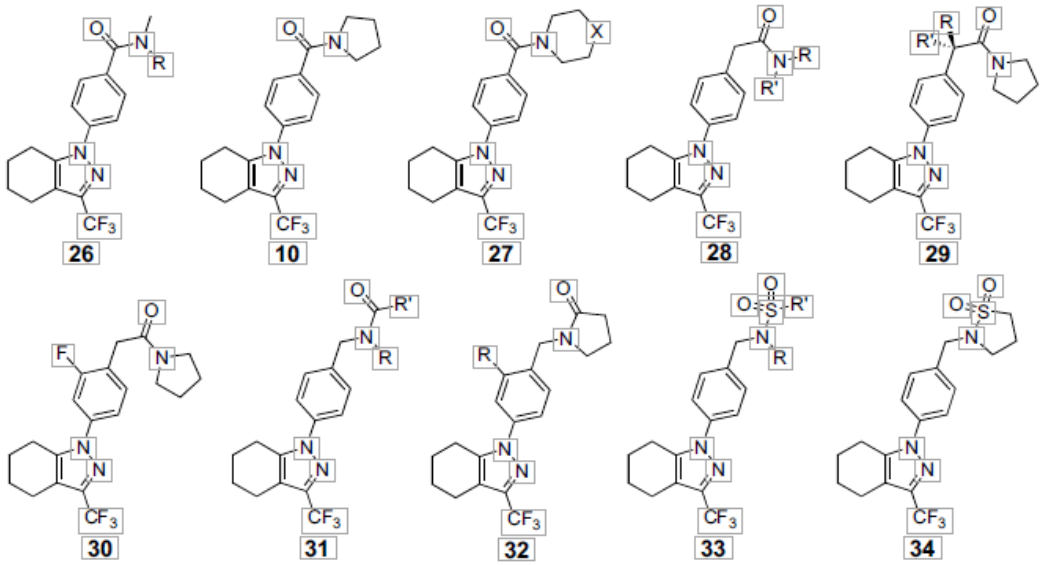


Fig. (8). Chemical structures of the tetrahydroindazole derivatives investigated during the lead optimization program

Attention then turned to cyclic amides (10 and 27 (X = CH2 or O)), which were predicted to fit optimally in the binding pocket according to the crystal structure of 25 (Fig. 7) and were expected to have reduced metabolic instability. Inter-estingly in the FLIPR assay pyrrolidine derivative 10 was more potent than 25 (pEC50 = 5.0, Asym Max = 123%), while six-membered ring analogues 27 were less active (X = CH2, pEC50 < 4, Asym Max = 100%; X = O, pEC50 < 4.7, Asym Max = 81%). Despite its moderate po-tency, morpholine derivative 27 (X = O) had an attractive profile since it retained similar solubility to 25, did not cause CYP450 inhibition and had significantly improved metabolic stability in both RLM and HLM (rat Cli < 0.5 mL/min/g; human Cli = 1.3 mL/min/g). Encouragingly, compound 10 was also more stable than 25 when tested in microsomes (rat Cli = 2.4 mL/min/g; human Cli < 0.5 mL/min/g). Derivatives 10 and 27 (X = O) had good physicochemical properties (MWt < 380 g/mol and

logD < 3.1), high permeability in an artificial membrane permeability assay (10, 415 nm/s; 27 (X = O), 455 nm/s) and moderate to high rat plasma and brain protein binding. Owing to their favourable in vitro pro-file, compound 10 and 27 (X = O) were progressed to a rat in vivo pharmacokinetic study and found to have good systemic exposure (10, AUC0-t = 472 ng·h/mL; 27 (X = O), AUC0-t = 1984 ng·h/mL) with reduced blood clearance rela-tive to the HTS hit. Both compounds also retained high CNS penetration (brain:blood AUC0-t ratio > 1) comparable to 25. Interestingly a cocrystal structure of amide 10 (PDB code 2xx7; Fig. 9) showed a similar binding mode to compound 25, with an unpredicted reorientation of the amide carbonyl and differences in the network of water molecules. The carbonyl group of 10 is making a hydrogen bond with a water molecule, sitting at the top of the benzene ring, and which is itself making a second hydrogen bond with Ser518. In the meantime, the pyrrolidine ring has displaced one of the four water molecules found at the end of the inverted U shaped binding pocket.

Replacement of the amide moiety with a sulfonamide, a ketone or a sulfone was also investigated but afforded com pounds with lower or at best similar potency to hit 25. Kinetic solubility of these derivatives was also significantly low. Owing to the difficulties in balancing high potency and DMPK properties, introduction of a linker atom between the phenyl and the amide group was explored and this modification was suggested to be tolerated by docking studies. Such molecules were expected to have an increased flexibility that would lead to a superior solubility. The homologated analogue of 25, compound 28 (R = R’ = Me), induced a substantial increase in calcium influx in the FLIPR assay (pEC50 = 5.7, Asym Max = 107%) but retained high microsomal turnover. The SAR in this series appeared to be quite narrow since a close acyclic homologated amide 28 (R = cPen, R’ = H) had reduced efficacy and very high microsomal clearance (rat and human Cli > 45 mL/min/g). The successful improvement in metabolic stability with cyclic amides 10 and 27 (X = O), prompted the GSK team to look at similar structural transformation in the homologated series (29 and 30). Derivative 29 (R = R’ = H) maintained the improved potency of 28 (R = R’ = Me) (pEC50 = 5.5, Asym Max = 82%) but also the undesired high microsomal instability (rat Cli = 14.6 mL/min/g; human Cli = 24.9 mL/ min/g). When both compounds were tested in a rat in vivo pharmacokinetic experiment, moderate systemic exposure and high blood clearance were observed, emphasizing the need to identify compounds with much improved metabolic stability. The initial attempt to enhance metabolic stability focussed on blocking the benzylic position of 29 (R = R’ = H), a likely site of metabolism, by introduction of small alkyl and fluorine groups. Monomethyl, cyclopropyl, mono and bisfluorinated analogues 29 were prepared, but all gave reduced potency relative to methylene benzylic modulator 29 (R = R’ = H) and retained high metabolic turnover. The methylene spacer not only created a potential site of metabolism but also increased the electron density of the phenyl ring, making it more prone to oxidation. Lowering the aromatic electron density by introduction of substituents was considered but had to be limited to fluorine atoms since the crystal structure of 29 (R = R’ = H) (PDB code 2xxh; Fig. 10) indicated that the benzene ring was in a narrow channel and that large substituents would not be tolerated. Fluorophenyl analogue 30 was synthesized and had superior potency to 29 (R = R’ = H) (pEC50 = 5.9, Asym Max = 79%), slightly better human microsomal stability but significantly reduced solubility (Fig.8).

The lack of success in identifying homologated amides with a favourable in vitro DMPK profile prompted scientists at GSK to consider reversed amides with an embedded benzylamine core motif (31 and 32). Tertiary 31 (R = R’ = Me) and secondary 32 (R = H, R’ = Et and R = H, R’ = Me) acyclic amides were prepared and found to be highly potent (pEC50 > 5.6), but retained high to moderate intrinsic clearance in both RLM and HLM (3.3 < Cli < 22.4 mL/min/g). With the previous success of cyclic amides 10 and 27 (X = O) to yield compounds with reduced turnover, pyrrolidinone 32 (R = H) was synthesised and induced excellent potentiation of the AMPA ion channel (pEC50 = 6.0, Asym Max = 132%). Despite

a similar moderate rat microsomal clearance to 10 (Cli = 2.4 mL/min/g), modulator 32 (R = H) had lower systemic exposure (AUC0-t = 231 ng·h/mL) and notably larger estimated blood clearance when tested in rats in vivo. Blocking a potential site of metabolism by introduction of a fluorine on the phenyl ring in 32 (R = F) success-fully afforded a potent compound (pEC50 = 6.0, Asym Max = 112%) with slightly higher intrinsic stability in microsomes (rat Cli = 2.0 mL/min/g; human Cli = 2.9 mL/min/g). However this did not translate to a remarkably superior in vivo pharmacokinetic profile and compound 32 (R = F) did not achieve the blood and brain levels observed with 10. The majority of the reversed amides retained some undesired CYP450 inhibition and solubility in this series was variable with the cyclic derivatives being the most soluble. Replacement of the amide moiety by sulfonamides, such as in 33 (R = H or Me, R' = Me or cPr or iPr) or in 34, produced active compounds, although moderately less potent than the amide analogues, but lacking any CYP450 inhibitory activity. These compounds also successfully achieved moderate to high microsomal stability in both rat and human (Cli < 2.5 mL/min/g) but were unfortunately for the vast majority too poorly soluble to be tested in vivo.

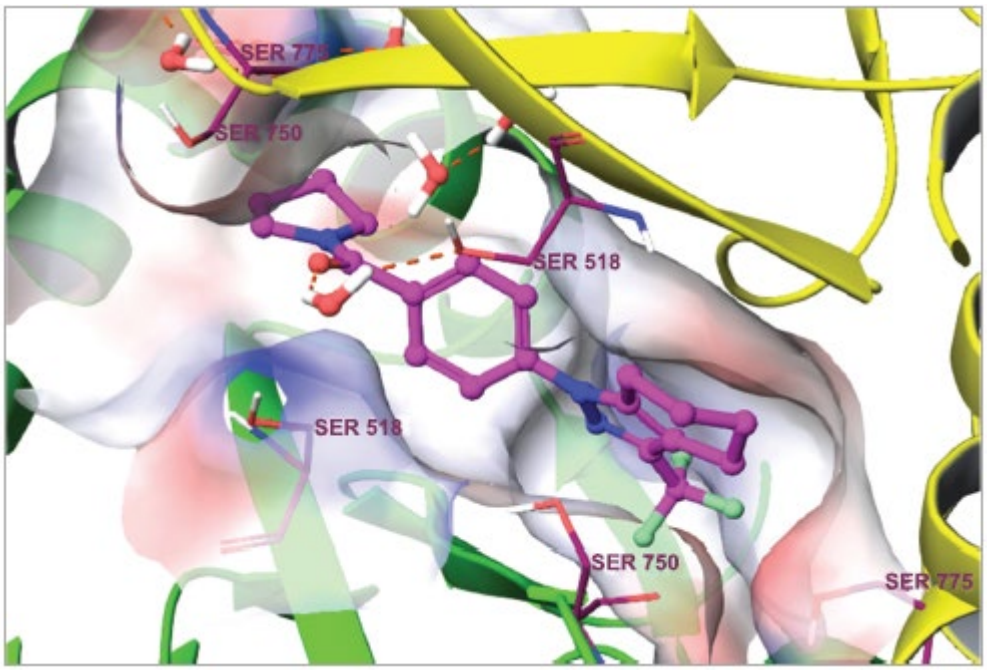


Fig. (9). X-Ray crystal structure of pyrrolidine amide 10 in complex with the hGLuA2i S1S2 LBD (PDB code 2xx7, 2.2 Å resolution).

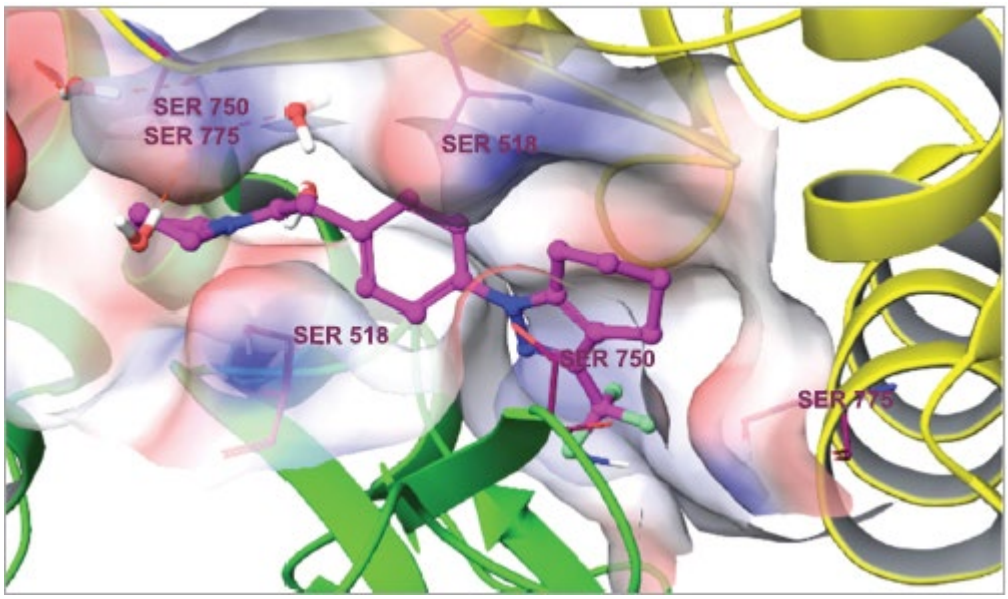


Fig. (10). X-Ray crystal structure of homologated amide 29 with the hGLuA2i S1S2 LBD (PDB code 2xxh, 1.5 Å resolution).

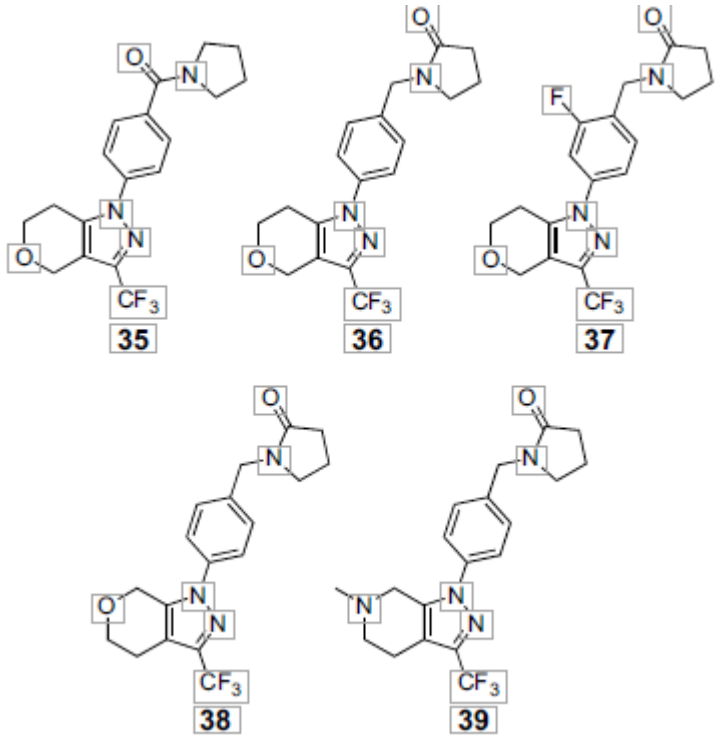


Fig. (11). Indazole derivatives containing a heteroatom in the saturated ring.

Having failed to identify compounds with low in vitro and in vivo turnover using a range of strategies, a selection of compounds was progressed to an in vitro metabolite identification study to develop a better understanding of the metabolic liabilities of the series. Interestingly the saturated part of the tetrahydroindazole was detected as a major site of metabolism and stabilization of this heterocycle was subsequently investigated. Introduction of heteroatoms (oxygen or nitrogen) in the aliphatic part of the tetrahydroindazole moiety was explored (Fig. 11) and successfully delivered active compounds with much improved in vitro properties. Overall, modulators 35 to 39 had lower intrinsic clearance in rat and human microsomes, higher solubility, lower logD, higher free fraction in both plasma and

brain, and did not inhibit CYP450 enzymes. This resulted in high oral exposure, with low blood clearance and good CNS penetration, when the compounds were tested in rat pharmacokinetic experiments. These molecules alongside the ones which previously gave the best exposures when profiled in vivo (AUC0-t > 400 ng·h/mL) were tested in electrophysiology, selectivity, developability and early toxicology screens. From the results of these assays (data not disclosed), a set of five molecules (10, 27 (X = O), 33 (R = H, R' = Me), 35 and 37), with excellent wider selectivity and clear potentiation of AMPAR-mediated currents in patch clamp assays, were identified as potential development candidates.

As mentioned earlier, AMPAR positive modulators have a tendency to induce convulsion, a recognized sideeffect of this class of drugs.[37] The maximum electroshock seizure threshold (MEST) assay enables to identify compounds which reduce seizure threshold and possess an undesirable proconvulsant activity.[99] The five selected compounds were profiled in MEST experiments and found to be devoid of any proconvulsant adverse effect. With the exception of compound 33 (R = H, R' = Me), all the modulators were clean in preliminary genotoxic screens and were subsequently tested in in vivo cognition models. In the passive avoidance task model,[98] only modulators 10, 35 and 37 attenuated the scopolamine induced amnesic deficit while modulator 27 (X = O) showed no effect. The three active molecules were then assessed in the NOR experiment,[97] a second in vivo behavioural model to evaluate cognition. In this model, only compound 10 showed a significant pro-cognitive effect when administered at doses ranging from 0.1 to 1 mg/kg (PO). Hence tetrahydroindazole derivative 10, which was identified early in the program, was selected as the preclinical candidate and evaluated further in a battery of in vitro and in vivo assays.

Similarly to hit 25, development candidate 10 exhibited low solubility in water, simulated gastric fluid and fasted simulated intestinal fluid. Nonetheless the compound had excellent solution stability in all media tested and also high solid stability over time. In addition, compound 10 did not induce time-dependent inhibition of CYP450 isoforms 2D6 and 3A4, and did not inhibit hERG currents at 10 μM. Intrinsic turnover of the molecule was studied in both microsomes and hepatocytes across five different species: rat, dog, minipig, monkey and human. Microsomal and hepatocyte clearances were correlating well and highlighted interspecies differences, with low to moderate instability in rat, dog and human, and high turnover in minipig and monkey. In vivo pharmacokinetic profiling of modulator 10 in four species (rat, dog minipig and monkey) indicated a good tissue distribution, with a volume of distribution at steady state in excess of total body water, a rapid oral absorption (0.5 h to 1 h) and variable blood clearance across species (moderate in rat and high in the others). This resulted in short half-lives (0.5 to 1.6 h) and moderate to low oral bioavailability (2 to 54%). The general good agreement between in vitro and in vivo clearances suggested a likely high systemic exposure in humans. In single and repeat dose oral toxicity studies of up to four weeks in rats and dogs, the clinical candidate exhibited a safe profile, giving a large therapeutic window. Surprisingly, AMPAR PAM 10 never entered clinical evaluation and GSK did not report or patent further exploratory work in the trifluoromethylpyrazole series.

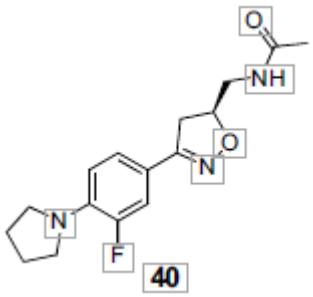
HTS AND HYBRIDIZATION STRATEGIES BY PFIZER

Pfizer is another pharmaceutical group which has ex-pressed a strong interest in AMPAR modulation and has filed 4 patent applications between 2008 and 2010.[100–103] The company has published the most recent scientific literature in the area, with three journal articles since 2013,[44,104,105] and is probably the last pharmaceutical group to have joined the race to discover and commercialize an AMPAR PAM. In these recent publications, Pfizer describes the discovery and optimization of two chemical series, one of which has led to the discovery of clinical candidate PF-04958242,[44] believed to be the only AMPAR potentiator still actively evaluated in clinical trials for neuro-logical disorders.

The majority of AMPAR modulators described in the literature were identified and characterized in vitro using recombinant systems, which do not reproduce the structural complexity of AMPARs found in vivo (see introduction) and possess a risk that data generated using homotetrameric receptors will not translate reliably in native tissues. To mitigate this risk early in projects, Pfizer developed an assay employing mouse embryonic stem (mES) cell-derived neurons, which are expected to represent more accurately the considerable heterogeneity of composition of AMPARs found in biological organisms and afford composite pharmacological values.[106] This assay, which was optimized for a HTS campaign and ran on a FLIPR platform, provided two quantifiable parameters: an EC50 obtained from a 9-point concentration-response curve and a maximal efficacy percentage (Emax), normalized to the average response to 100 µM of AMPA in the presence of 32 µM of cyclothiazide, which was defined as 100%. Screening of a Pfizer com-pound library using this newly developed assay led to the identification of dihydroisoxazole (DHI) hit 40.[104] Despite a moderate potency in the FLIPR mES assay (EC50 = 4.4 µM), modulator 40 had favourable calculated physicochemical properties and was as a result profiled in an array of in vitro DMPK assays (see Table 4). Interestingly compound 40 had good permeability with no P-gp liability and moderate clearance in HLM, confirming that it was an attractive starting point for an optimization program that would primarily focus on improving the potency while maintaining optimal CNS physicochemical and ADME (absorption, distribution, metabolism and excretion) properties.

A co-crystal structure of DHI 40 with the LBD of hGluA2o was determined (PDB code 4lz7; Fig. 12) and revealed that the phenyl ring sits on the 2-fold axis and that the acetamide projects into one of the deep hydrophobic pocket, similarly to the sulfonamide group in LY451395 7. The pyrrolidine ring approaches Asn775 but does not participate in any distinct interaction. Intriguingly, unlike GSK researchers, scientists at Pfizer did not comment on the network of water molecules present at the dimer interface of the two LBD subunits and their potential displacement or interaction with modulator 40.

Table 4. Physicochemical and DMPK properties of DHI 40



40

TPSA (Å^2)	MWt (g/mol)	clogP	$Cl_{int,app}$ (µL/min/mg)	P_{app} (cm/s)[a]	ER[b]
54	305	2.0	26	35×10^{-6}	0.98

a) Absorptive apparent permeability of a potentiator (2 µM) across a population of MDCK cells with reduced transporter expression; b) Efflux ratio: the ratio of secretory (B to A) to absorptive (A to B) apparent permeabilities of a potentiator (2 µM) in MDR1 transfected MDCK cells.

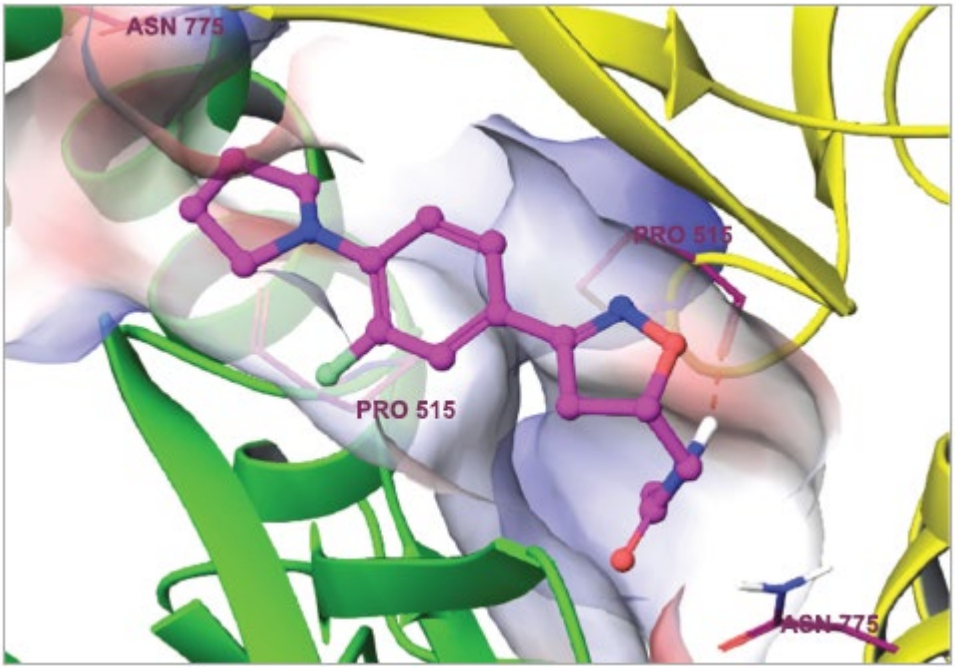


Fig. (12). X-Ray crystal structure of DHI hit 40 with the hGLuA2o S1S2 LBD (PDB code 4lz7, 2.1 Å resolution).

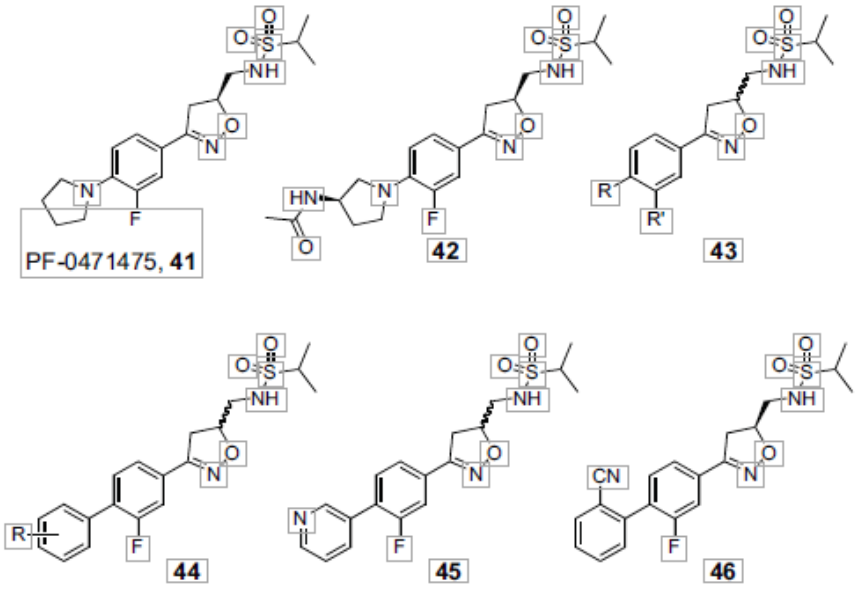


Fig. (13). Representative structures of DHI derivatives prepared during the lead optimization campaign.

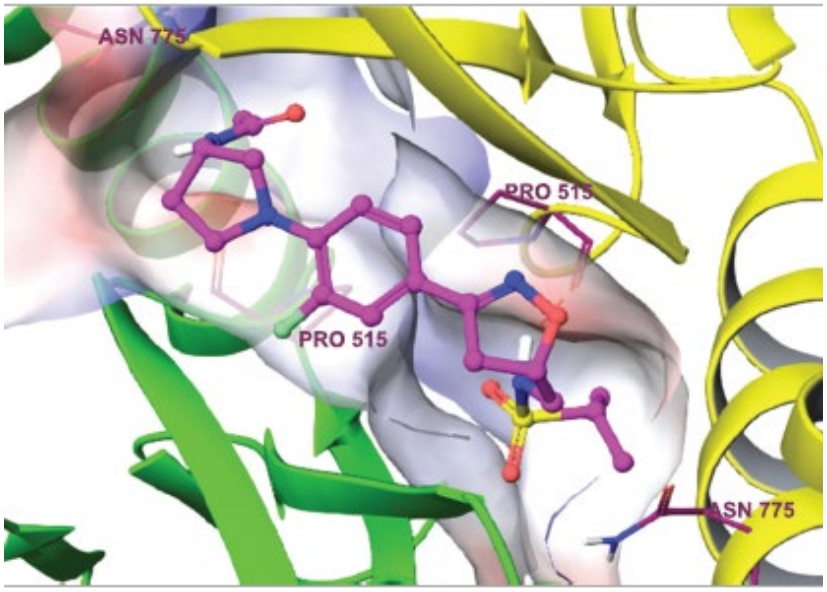


Fig. (14). X-Ray crystal structure of compound 42 with the hGLuA2o S1S2 LBD (PDB code 4lz8, 1.85 Å resolution).

From this crystal structure, it was initially suggested that replacement of the acetamide by a sulfonamide could afford molecules with superior binding affinity since docking of a sulfonamide group was providing a better fit for the hydro-phobic pocket. The isopropylsulfonamide of the phenethyl series was introduced on the dihydroisoxazole core, giving 41, which was 35-fold more active than 40 (EC50 = 123 nM, Emax = 147%), confirming the design hypothesis. Compound 41 retained desirable CNS drug-like properties (MWt = 369 g/mol, clogP = 3.0, TPSA = 79 Å2), had moderate predicted human blood clearance (Cl = 13.5 mL/min/kg), and was permeable with no P-gp efflux in the MDR1-MDCK in vitro permeability assay (Papp = 18.6 × 10- cm/s, ER = 1.45). The enantiomeric pair of 41 was significantly less active, confirming that molecules with the S stereochemistry provided the best fit in the binding pocket. A variety of small amides and sulfonamides were prepared to probe the SAR of this part of the scaffold, but all induced a loss of potency. Replacing the amide with carbamates or ureas was also unsuccessfully investigated. Removal of the pyrrolidine produced an inactive compound (43 (R = H, R' = F), EC50 > 32 µM), while desfluoro racemate 43 (R = pyrrolidine, R' = H) was only slightly less active (EC50 = 253 nM, Emax = 169%) but was attractively slightly less lipophilic (clogP = 2.75).

Docking of 41 in the crystal structure of 40 was per-formed to identify additional means to increase the functional potency of this series, and indicated that the second symmetrical hydrophobic pocket could be accessed through substitution of the pyrrolidine. Hence an acetamide was introduced at the 3-position of the pyrrolidine affording 42, an exceptionally active potentiator (EC50 < 10 nM, Emax = 143%), whose postulated binding interactions were confirmed by X-ray cocrystal structure (Fig. 14, PDB code 4lz8). A tritiated form of this potent modulator was prepared to develop an in-house AMPAR competition binding assay.

The development of a low nanomolar potentiator which occupies both hydrophobic pockets of the LBD was done at the expense of the physicochemical properties of 42 (MWt = 427 g/mol, TPSA = 109 Å2) and resulted in poor permeability and affinity for the P-gp transporter protein (Papp = 3.9 × 10-6 cm/s, ER = 12.1). Consequently, compound 42 had low CNS exposure in a mice pharmacokinetic study, precluding its use in vivo. Therefore the SAR around the pyrrolidine ring was revisited with the objective to obtain compounds with lower TPSA and also to remove the aniline moiety which

represented well-documented concerns.[107,108] From the crystal structure of 40 and 42, it appeared that replacing the pyrrolidine with a second aromatic ring would be tolerated, and a series of biaryl derivatives was prepared. While phenyl analogue 44 (R = H) had low affinity for AMPARs (EC50 = 3.2 μM, Emax = 138%), pyridyl derivative 45 retained acceptable potency (EC50 = 294 nM, Emax = 157%). Molecular modelling of the biaryl template suggested that a nitrile group at the ortho position of the distal aromatic could access the hydrophobic pocket, providing superior binding affinity. This design hypothesis was confirmed since racemate 44 (R = 2-CN) had similar potency to 41 (EC50 = 87 nM, Emax = 152%) and the S-enantiomer 46 was again the most potent (EC50 < 10 nM). Modulator 46 retained good calculated physicochemical properties (MWt = 401 g/mol, clogP = 3.8, TPSA = 100 Å2), had moderate predicted human hepatic clearance (Cl = 9.67 mL/min/kg) and was highly permeable (Papp = 29.1 × 10- cm/s; ER = 1.18). Disappointingly, no additional ADME, s electivity or in vivo pharmacokinetic data were given for this very promising compound (Fig. 13). In addition, it should be noted that no solubility or CYP450 inhibitory data are communicated on any of the compounds described above. [104]

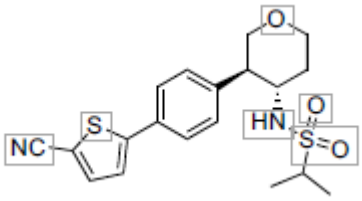
In this publication, detailed pharmacokinetics and pharmacodynamics were only reported for compound 41, despite modulator 46 being in appearance the most attractive compound. In their in vivo assessment, the Pfizer group demonstrated a marked interest to the unbound brain compound concentration (Cb,u), which determines AMPAR LBD inter-actions, and developed a Cb,u-normalized AMPAR-mediated exposure-response continuum to define an acute dose rodent-based therapeutic index (TI). Such studies were carried out since AMPAR overstimulation has been associated with ex-citotoxicity [33, 37, 109] and for this reason defining the toxicity threshold in rodents was viewed as crucial by Pfizer scientists before advancing any drug of this pharmacological class to large animal safety and efficacy models.

Potentiator 41 had high rat protein plasma binding (97.3%) and rat brain tissue binding in brain homogenates (98.6%). In a rat pharmacokinetic study, following single subcutaneous (SC) dosing (0.2 mg/kg), compound 41 had a short terminal half-life (t1/2 = 0.4 h), a total brain to total plasma concentration ratio of 1.04 and an unbound brain to unbound plasma concentration ratio of 0.54, confirming that DHI 41 was CNS-penetrant. The convulsion liability, a recognized side-effect of AMPAR PAMs,[109] was first studied in CD-1 mice and detected 5 to 11 min post-dose in one third of the animals following SC administration of 0.32 mg/kg of 41 and in all animals at a higher dose (0.56 mg/kg, SC). Bioanalysis of brain samples from these mice indicated that a 2.1 nM mean Cb,u of potentiator induced convulsion. The same study was repeated in Sprague-Dawley rats where the exposure convulsion threshold was similar with a toxic Cb,u of 2.3 nM.

The efficacy of potentiator 41 was first assessed in a modified version of the cerebellum cyclic guanosine monophosphate (cGMP) assay, published by Lilly[110] to evaluate the effect of their AMPAR modulators on the accumulation of cGMP, a downstream messenger involved in a signal-ling cascade regulating excitatory neurotransmission and neuroplasticity. It was determined that cGMP levels in-creased with statistical significance in CD-1 mouse cerebellum 30 min after SC dosing of 0.32 mg/kg of 41, corresponding to a mean Cb,u of 0.4 nM. Surprisingly, it is not mentioned if the effect of 41 on cGMP elevation occurred in a dose-dependent manner and as mentioned earlier, this same dosing regimen (0.32 mg/kg, SC) also induced convulsion in one third of the mice, supporting the idea that lower doses must have been investigated but for which no result is re-ported. Urethane-anesthetized rats, treated with the NMDAR antagonist MK-801 which induces a schizophrenic state, were used as an in vivo model to measure the nootropic electrophysiological effects of 41 administered intravenously. A minimal effective dose of 0.003 mg/kg (IV) of 41 was required to significantly reverse the MK-801-induced schizophrenic effect. From a pilot rat IV pharmacokinetic

study (0.01 mg/kg), it was extrapolated that the reversal effect which occurred after 5 min corresponded to a Cb,u of 0.004 nM, giving a 500-fold TI for convulsion relative to procognitive electrophysiological enhancement in rats. In the majority of the pharmacokinetic and pharmacodynamic experiments described above, DHI 41 was administered subcutaneously and no justification was given in this first publication for choosing this preferred mode of administration, which is not desired for clinical evaluation. However in a subsequent article, [105] it was mentioned that subcutaneous administration was predominantly used to control the variability in (and rise to) Cmax and prevent the generation of any potential first-pass active metabolites. Pfizer did not mention if further biological studies were being undertaken with this compound, such as in vivo cognition behavioural experiments, or assessment of memory related effects in non-rodent species or if focus had shifted to potentiator 46. No additional data has been reported on either compound since 2013. From this study, it should be noted that the use of SBDD positively informed GluA2-based SAR and allowed to quickly prepare potent modulators.

In a second publication from 2013,[105] Pfizer presented extensive efficacy and safety profiling on a single compound, PF-04778574 47, which was originally disclosed in a patent[100] and for whom no medicinal chemistry rational design has yet been revealed. As previously mentioned, AMPAR overstimulation can be excitotoxic and AMPAR potentiators have been associated with marked adverse events in preclinical studies.[37] Conscious of this risk, Pfizer designed a sequence of in vitro and in vivo assays in a selection of preclinical species to assist in the identification of safe compounds early in lead optimization campaigns and understand potentiator exposures causing desired versus harmful effects. In this paper, the Pfizer scientists reported how they applied their safety and efficacy assessment cascade to carefully define a PF-04778574 mechanism-based therapeutic index in mice and study its translatability from rodents to higher order species, by developing an interspecies exposure response model.



PF-04778574, 47

Fig. (15). Chemical structure of PF-04778574.

In the in vitro mES FLIPR assay, which was used for the screening campaign in the work described above, PF0477-8574 concentration-dependently enhanced S-AMPA calcium influx through the heterogeneous mixture of AMPARs ex-pressed (pEC50 = 6.05 ± 0.19 and Emax = 162 ± 24%). In the in-house competitive binding assay, modulator PF-04778574 had a Ki of 85 nM and was also found to be devoid of any agonistic effect in three different assays, corroborating the assumed mode of action. Results from these two experiments confirmed that PF-04778574 was a subtype-independent AMPAR PAM. As expected, the compound also potentiated

the effect of S-AMPA on HEK293 cells expressing either recombinant hGluA2i or hGluA2o in a FLIPR assay, with similar efficacy on both isoforms (flip: pEC50 = 7.35 ± 0.11 and Emax = 111 ± 6%; flop: pEC50 = 7.05 ± 0.07 and Emax = 112 ± 5%), demonstrating an absence of selectivity between these two receptor subtypes. Whole-cell patch clamp electrophysiology on primary cultures of rat cortical neurons, expressing native populations of AMPARs, was used to study the functional potency of PF-

04778574 on S-AMPA-evoked currents. In that assay, PF-04778574 clearly increased the electric current induced by 30 μM of S-AMPA from a concentration of 30 nM and had an EC50 of 282 nM.

The protein plasma binding of PF-04778574was measured across a range of species (CD-1 mice, Sprague-Dawley rats, beagle dogs, cynomolgus monkeys) and found to be moderate (91.2% to 95.2%). Interestingly the brain tissue binding was markedly higher (98.0%) and considered species independent.[111] These two parameters were used to calculate the unbound drug concentration in the plasma (Cp,u) and the brain (Cb,u) from measured total plasma and total brain concentrations. To reduce the number of animals sacrificed in the efficacy and safety pharmacodynamic studies, Cb,u:Cp,u ratios in four animal models (mouse, rat, dog and Cb,u:Cp,u ratios in four animal models (mouse, rat, dog and monkey) were experimentally determined to subsequently allow calculation of a Cb,u from a measured Cp,u, when brain tissues were not collected. This conversion of a Cp,u into a Cb,u relies on the assumption that Cb,u:Cp,u ratios are constant over time, which is not always the case. The free drug ratios determined in neuropharmacokinetic experiments ranged from 0.42 (mouse) to 0.73 (monkey), indicating good CNS permeability and low interspecies variability from rodents to large animals (< 1.7 fold differences). A number of acute pharmacokinetic experiments in different animal models, using alternative mode of administration and various dose of PF-04778574 were then conducted to obtain a selection of dose-Cb, u-time relationships.

From these experiments, it should be noted that following intravenous administration of PF-04778574 (0.2 mg/kg) in rat, the blood clearance was more than twice in excess of total liver blood flow (Cl = 250 mL/min/kg), the volume of distribution was high (Vd = 4.59 L/kg) and the half-life very short (t1/2 = 0.24 h), indicating poor systemic stability of PF-04778574 in rats. The compound was noticeably more stable in dogs following oral dosage (0.1 mg/kg) with a moderate blood clearance (Cl = 8.01 mL/min/kg), a high volume of distribution (Vd = 2.1 L/kg) and a moderate half-life (t1/2 = 3.1 h). PF-04778574 was rapidly absorbed in dogs (Tmax = 0.75 h) and had acceptable systemic exposure (Cmax = 56.0 ng/mL and AUC0-∞ = 210 ng·h/mL). In mon-keys, PF-04778574 was administered subcutaneously at two doses (0.1 and 0.32 mg/kg) affording a moderate mean half-life (t1/2 = 3.4 h) and a Tmax around 2 h. Positively, maximal plasma drug concentration (Cmax) and AUC0-∞ values suggested linear pharmacokinetics across this dosing range. Un-fortunately no in vitro clearance assessments in microsomes or hepatocytes were given and it is impossible to say if the overall high in vivo metabolic turnover of PF-04778574 could have been predicted from the in vitro data.

Once all the pharmacokinetic studies had been carried out, modulator PF-04778574 was characterized in a selection of in vivo pharmacodynamic models to correlate the unbound brain drug concentration (Cb,u) with the observed pharmacological effects. To rule out any non-AMPAR-mediated in vivo pharmacological effect, PF-04778574 was screened at 10 μM against a panel of 118 enzymes and receptors, and found to express a high degree of selectivity for the AMPAR with only minimal inhibitory activity on the dopamine trans-porter (IC50 = 0.95 μM).

The in vivo efficacy of PF-04778574 was first assessed in the cGMP assay similarly to compound 41. cGMP elevation in the cerebellum of CD-1 mice occurred dose-dependently and was statistically significant from a SC dose of 0.32 mg/kg of drug, the other doses evaluated being 0.1 and 1 mg/kg SC. Using the pharmacokinetic models developed in CD-1 mice and assuming linear pharmacokinetics across the doses tested, it was determined that Cb,u superior to 1.7 nM would induce significant increase in cerebellar cGMP levels. In this paper, Pfizer also suggested that changes in cerebellar cGMP levels correlate with the potential of compounds to display adverse events. However it should be noted that this assay was originally developed to study the efficacy of the AMPAR modulators but not their safety.[110] In urethane-anesthetized rats, a dose of 0.1 mg/kg (IV) of PF-04778574 significantly overcame the electrophysiological hypoglutamatergic conditions induced by the NMDAR antagonist

MK-801. The restorative effect occurred between 5 and 25 minutes post-dose and was projected to correspond to Cb,u ranging between 0.98 and 0.35 nM, respectively. Additionally, a 0.03 mg/kg dose (IV) of compound, translating to a maximal projected Cb,u of 0.30 nM, was ineffective in this assay, indicative of a minimal effective Cb,u close to 0.30 nM. Since modulator 47 improved impaired synaptic transmission over a large concentration range, it was then tested in a behavioural cognition model, the monkey ketamine-disrupted spatial delayed response (SDR) task.[53] In this behavioural model, PF-04778574 was tested over a 100-fold dose range (0.001, 0.01 and 0.1 mg/kg, SC), but only significantly reversed the ketamine-induced impaired performance at 0.01 mg/kg (SC), suggesting that the compound had a hormetic exposure-response,[112] typical of nootropic drugs. From the pharmacokinetic model experiments in primate, this dose projected to a mean Cb,u of 0.38 nM. Interestingly the readouts of the two in vivo efficacy models were consistent and suggested that 47 induced procognitive effects from an unbound brain drug concentration of around 0.35 nM (Fig. 15).

The amplitude of adverse events caused by AMPAR potentiators is generally progressive, first manifesting as disruption of motor coordination and ultimately leading to convulsion. The tremorgenic potential of AMPAR modulator 47 was evaluated in mice undergoing an accelerating rotarod assay 30 min post-dose. In that assay, low doses (0.178 and 0.32 mg/kg, SC) were not associated with a decrease animal fall latency and only 0.56 mg/kg (SC) of PF-04778574, projecting to a Cb,u of 4.8 nM, induced statistically meaningful motor deficits. In addition to the motor coordination disruption observed in the rotarod test, a number of tremorgenic events were unexpectedly recorded during the initial pharmacokinetic studies. Hence general tremor was observed in dogs at Cb,u ranging from 6.0 to 15.6 nM and movement-related tremor/ataxia were detected in monkeys at Cb,u ranging from 8.0 to 24.1 nM. In these two species, the Cb,u inducing tremorgenic side-effects were similar but were two to three-times higher than in mice. Convulsion, a more severe side-effect, which has been associated with a significant number of AMPAR PAMs, was assessed in dose-response studies across three species (mice, rats and dogs) to evaluate a potential interspecies consistency in Cb,u causing convulsions. In CD-1 mice, among the three doses tested (1, 1.78 and 3 mg/kg, SC), only the highest two induced general convulsions and this extrapolated to a minimal convulsion causing Cb,u of 9.6 nM. The delay to observe convulsions following dosing was greater for the 1.78 mg/kg (SC) group (23 minutes) relative to the 3.2 mg/kg (SC) group (7 minutes), which was consistent with the “threshold” nature of this adverse event. In rats, the two highest doses evaluated (3.2 and 5.6 mg/kg, SC) induced convulsions and the same dose-dependent adverse event delay as in mice was observed. The convulsive exposure threshold in this species translated to a projected Cb,u of 10.7 nM. In dogs, tremorgenic events were observed at the low doses investigated (0.2 mg/kg and 0.25 mg/kg, PO), but generalized convulsions only occurred following a dose of 0.5 mg/kg (PO) of 47, corresponding to a Cb,u of 17.4 nM. The PF-04778574 Cb,u causing convulsion was consistent in rodents (9.6 and 10.7 nM), slightly higher in dogs (17.4 nM) and could be expected to be even higher in primates, since tremorgenic events happened at doses of up to 24.1 nM.

The data generated showed that motor coordination disruption was the dose-capping adverse effect and that PF-04778574 showed adequate separation between Cb,u linked to efficacy and harmful effects with TI of 7-13, 16-29 and 22-42 in mice, dogs and monkeys, respectively. Overall, the exposure-response physiological effects were mainly consistent across species, suggesting a translatable relation-ship from rodent to higher-order species. Escalating unbound brain concentrations progressively led from efficacy to cerebellar cGMP elevation to motor coordination disruptions to convulsion. From these studies and a number of subsequent assumptions, the scientists at Pfizer concluded that PF-04778574, with a projected 8 to 16-fold TI in humans for self-limiting tremor, might be safely evaluated clinically as a nootropic drug in schizophrenic patients. Despite this

conclusion, the compound never entered clinical evaluation and no further results with this AMPAR potentiator have been reported since 2013.

In a final publication from 2015, scientists at Pfizer re-ported the discovery, SAR and optimization of clinical candidate PF-04958242 11.[44] In addition to the identification of new templates from HTS such as the DHI series described previously, Pfizer also explored modification of Lilly's biaryl isopropylsulfonamide LY451646 48, with the objective to discover a series with improved efficacy, selectivity and metabolic stability relative to 48. Synthetic tractability strongly influenced chemistry route design to enable library synthesis and quick expansion of the SAR around the biaryl scaffold. From their initial studies with AMPAR modulators, the scientists at Pfizer understood the importance of identifying compounds with low human pharmacokinetic variability to accurately predict the Cmax and prevent occurrence of threshold-mediated adverse events.[113] With this in mind, physicochemical properties of virtual targets imparting ADME properties were carefully examined to only prepare compounds likely to develop into good oral CNS drugs with the targeted pharmacokinetic profile.

LY451646 48 was tested in the FLIPR mES assay devel-ped by Pfizer to set a benchmark and only produced moderate activity (EC50 = 3.4 μM, Emax = 132%). A number of initial design hypotheses, including investigation of different vectors around the central aryl ring, replacement of the middle benzene with an heteroaliphatic ring and sulfonamide variation/replacement were unsuccessfully explored and afforded inactive compounds. Modulation of the linker was more successful, in particular introduction of an oxygen be-tween the phenyl and the two-carbon linker resulted in active modulators, such as 49. In this ether subset, para-substitution was preferred, mimicking the SAR of the all-carbon linker series. Interestingly while the unsubstituted distal aromatic analogue 49 (R = H) had similar potency to 48 but was significantly less efficacious (EC50 = 2.44 μM, Emax = 32%), introduction of an orthocyano 49 (R = CN) had an opposite effect, giving a less potent compound with greater efficacy (EC50 = 7.35 μM, Emax = 218%), suggesting a potential functionally favourable contact of the cyano with the AMPAR LBD interface.

The X-ray crystal structure of LY451646 48 in complex with the LBD of hGluA2o (PDB code 4lz5) indicated that the isopropylsulfonamide NH was forming a hydrogen bond with the carbonyl oxygen of Pro515 and was occupying one of the deep lipophilic pockets, similarly to modulators of the sulfonamide class. The biaryl core was sitting along the two-fold axis, projecting to the symmetrical binding pocket. Docking of 49 (R = CN) into this crystal structure indicated that the orthocyano was ideally positioned to access the symmetrical hydrophobic space occupied by the isopropylsulfonamide at the other end of the inverted U-shaped binding pocket. This binding model prompted the team at Pfizer to conformationally restrict the amido-alkoxyethane linker to preorganize the ligand for binding and obtain potentiators with superior pharmacological activity. Hence a number of cis and trans 1,2-dihalo 5- and 6-membered ring were de-signed and prepared. Examination of the physicochemical properties of these molecules and consideration of prior finding from GSK on an almost identical series, incited Pfizer to focus on cistetrahydrofuran ether analogues (Fig. 16).

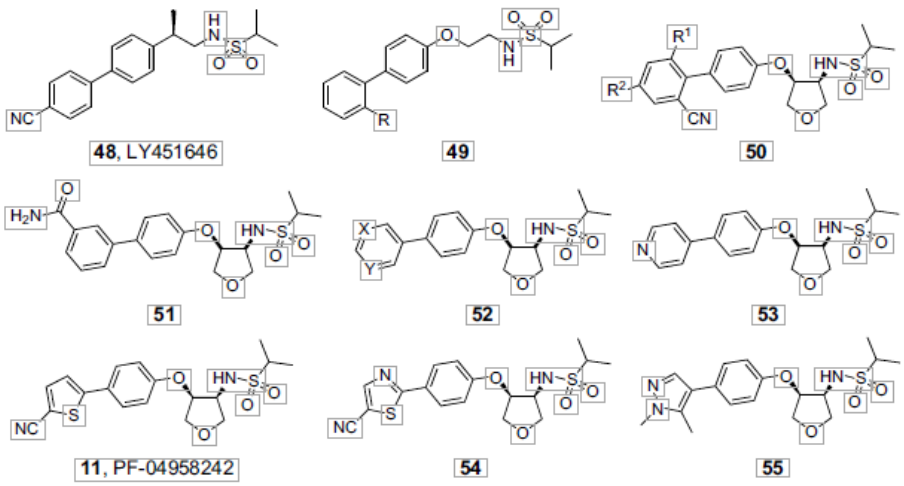


Fig. (16). Representative structures of the derivatives investigated during the lead optimization which led to the identification of PF-04958242.

A significant improvement in functional efficacy was observed following introduction of the THF-ether linker and chiral separation indicated that the (3S,4S)-isomers were notably more potent than their (3R,4R)-counterparts. Despite high functional efficacy (EC50 = 217 nM, Emax = 143%) and good permeability with no P-gp efflux in the in vitro MDR1-MDCK assay (Papp = 32.3 × 10-6 cm/s; ER = 1.39), com-pound 50 (R1 = H, R2 = H) suffered from undesirable high turnover in HLM (Cli = 158 mL/min/kg). To improve the metabolic stability, fluorine atoms were introduced on the distal phenyl, which was a likely source of CYP450-mediated hydroxylation, and afforded functionally active compounds with improved but still suboptimal human microsomal turnover, such as 50 (R1 = F, R2 = H, EC50 = 14.1 nM, Emax = 126%, Cli = 89 mL/min/kg) and 50 (R1 = H, R2 = F, EC50 < 10 nM, Emax = 190%, Cli = 39 mL/min/kg). Reduction of the phenyl ring lipophilicity by adding polar substituents also successfully lowered the clearance but concomitantly induced a reduction of potency (51, EC50 = 1.11 µM, Emax = 102%) and negatively impacted properties governing CNS permeability (51, Papp = 17.4 × 10-6 cm/s; ER = 7.84). Replacement of the orthocyanophenyl with

6-membered heteroaromatics in 52 (X = N, Y = C or X = Y = N) and 53 caused a reduction of clogP and delivered compounds with low HLM clearance, high permeability, no P-gp liability and acceptable functional potency (0.62 <

EC50 < 1.16 µM, 110 < Emax < 161%). A selection of substituted 5-membered heteroaromatic derivatives (11, 54, 55) was also synthesized and afforded compounds with superior functional activity compared to the 6-membered heteroaromatics. Of these compounds, potentiator 11 which had good activity in the FLIPR mES assay (EC50 = 310 nM, Emax = 110%), low HLM clearance (Cli < 8 mL/min/kg) and high predicted CNS exposure from the MDR1-MDCK assay (Papp = 19.3 × 10-6 cm/s; ER = 1.05) was selected for further profiling.

An X-ray cocrystal structure of 11 bound to the LBD interface of hGluA2o (PDB code 4x48, Fig. 17) indicated that the isopropylsulfonamide was making similar contacts with the protein as in 48, and that the cyano group was in the vicinity of Asn775 possibly impacting on the flip/flop selectivity of modulator 11.

The in vitro potency and efficacy of PF-04958242 11 was assessed in a selection of assays to evaluate its species, subunit and splice variant selectivity profile. Although no data is given, the authors mentioned that 11 was equipotent in mouse and human ES cell-derived neurons, indicating interspecies uniformity. In cells expressing recombinant hGluA2 receptors, compound 11 increased S-

AMPA-mediated Ca2+ influx with some selectivity for the flip (hGluA2i (Ser775), EC50 = 24 nM, Emax = 124%) over the flop isoform (hGluA2o (Asn775), EC50 = 880 nM, Emax = 124 %). In a whole-cell patch clamp electrophysiology experiment on primary cultures of rat cortical neurons, modulator 11 significantly enhanced S-AMPA-evoked cur-rent (EC50 = 43 ± 10 nM). To ascertain that the pharmacological effects observed when testing PF-04958242 in vivo were solely AMPAR-mediated, the selectivity of 11 was determined against a panel of 118 human-based ion channels, receptors and enzymes. At the concentration tested (10 µM), compound 11 had good selectivity for AMPAR with detectable affinity for only three other receptors and enzymes, the most potent interaction being with the serotonin transporter (Ki = 530 nM). The plasma protein binding of 11 was moderate in the three species tested (rat: 95.2%; dog: 93.0%; monkey: 89%) while its rat brain protein binding was relatively high (98.1%), consistent with the moderate calculated lipophilicity of 11 (clogP = 3.54). Inhibition of seven major CYP450 isoforms by modulator 11 was low, with only detectable inhibition of CYP2D6 (IC50 = 18 µM). Unfortunately only this limited set of in vitro ADME properties was given, and important parameters, such as compound solubility, hERG inhibition, CYP induction, reactivity with glutathione, or genotoxicity, were not discussed.

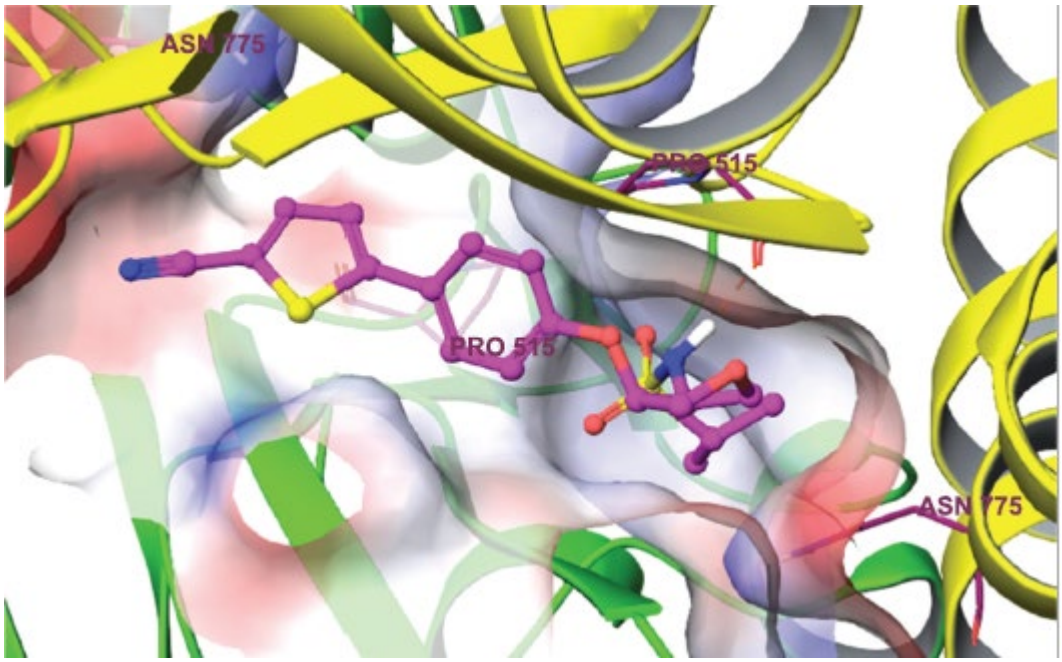


Fig. (17). X-ray crystal structure of 11 bound to hGluA2o S1S2 LBD (PDB code 4x48, 1.89 Å resolution).

A similar approach to the one used to study the effects of PF-04778574 in vivo was employed and Cb,u (unbound brain concentrations) were calculated for each animal/dose/time/administration mode to establish a Cb,u-normalized AMPAR-mediated exposure response continuum for clinical candidate 11, enabling to assess AMPAR-dependent activities related to nootropic effects and safety. Single-dose neuropharmacokinetic experiments were con-ducted in CD-1 mice and Sprague-Dawley rats to determine Cb,u:Cp,u ratios, treated as constant over time, and subsequently allow calculation of a Cb,u from a measured Cp,u at multiple time points. The Cb:Cp and Cb,u:Cp,u ratios experimentally measured ranged from 0.59 to 1.5, confirming the good CNS permeability of 11 predicted by the MDR1-MDCK assay. The neuropharmacokinetic experiments in rat (1 mg/kg, SC; 0.01 mg/kg, IV) indicated that 11 had similar terminal half-life, ranging from 0.53 h to 0.76 h, and Tmax (0.75 h) in plasma, cerebrospinal fluid and brain. Following intravenous administration in rat (0.2 mg/kg), PF-04958242 had very high blood clearance (Cl = 169 mL/min/kg), very short half-life (t1/2 = 0.31 h), high volume of distribution (Vd = 3.6 L/kg) and low systemic exposure (AUC0-∞ = 20.1 ng·h/mL), indicating

poor plasma stability in rats. When administered orally to rats (3 mg/kg), 11 was rapidly absorbed (Tmax = 0.25 h), had a longer half-life (t1/2 = 0.84 h) and acceptable systemic exposure (Cmax = 134.0 ng/mL and AUC0-∞ = 175 ng·h/mL), giving a rat oral bioavailability of 58%. In higher order species, the half-life was greater (t1/2 = 6.21 h in dogs (0.1 mg/kg, PO), t1/2 = 12.3 h in monkey (0.032 mg/kg, SC)), the Cmax was lower and the systemic exposure consistent (in dogs, Cmax = 23.9 ng/mL and AUC0-∞ = 158 ng·h/mL; in monkeys, Cmax = 6.87 ng/mL and AUC0-∞ = 149 ng·h/mL).

Elevation of cerebellar cGMP in CD-1 mice occurred dose-dependently 30 min after administration of compound 11 and was statistically significant from a dose of 1 mg/kg (SC). From the neuropharmacokinetic models previously established in CD-1 mice following SC dosing of 11, it was determined that a projected Cb,u of 6.7 nM induced cGMP elevation in CD-1 mice cerebellum. In urethane anesthetized rats, a dose of 0.01 mg/kg (IV) of 11 significantly overcame the electrophysiological hypoglutamatergic conditions induced by the NMDAR antagonist MK-801, projecting to Cb,u ranging between 0.23 and 0.18 nM. The compound was then tested in a second behavioural cognition model, the keta-mine-disrupted spatial working memory in the radial arm maze (RAM), in which it attenuated the ketamine-evoked memory loss at doses ranging from 0.0032 to 0.032 mg/kg (SC), protecting to Cb,u between 0.029 and 0.29 nM according to the subcutaneous rat pharmacokinetic study. Higher doses (0.1 and 0.32 mg/kg, SC) were ineffective, indicating a hormetic exposure response for this nootropic agent.

The tremorgenic potential of potentiator 11 was assessed in C57BL/6J mice undergoing an accelerating rotarod assay 30 min post-dose. In that test, low doses (0.1 and 0.178 mg/kg) were safe and only 0.32 mg/kg (SC) of PF-04958242, projecting to a Cb,u of 10.8 nM, induced statistically meaningful motor deficits. The convulsion liability of potentiator 11 was not described, although it can be assumed from the previous studies that Cb,u superior to 10.8 nM will be required to cause convulsion. From these studies, PF-04958242 had a 37-fold therapeutic index in rodents.

Intrinsic turnover of 11 was studied in rat and dog hepatocytes (rat Cli ≤ 63.6 mL/min/kg and dog Cli ≤ 7.4 mL/min/kg) and found to be consistent with the respective in vivo clearance. The good agreement between in vitro and in vivo clearances, and the moderate human hepatocyte clearance of the molecule (Cli ≤ 7.1 mL/min/kg) suggested a likely moderate to high systemic exposure in humans. Metabolite identification studies, indicated that compound 11 underwent similar turnover in dog and human systems, with THF hydroxylation and O-dealkylation identified as major route of metabolism. Studies using major recombinant human cytochrome P450 isoforms indicated that hepatic turn-over via CYP2C19 and CYP3A4 would be the predominant human clearance pathway.

These positive results prompted Pfizer to evaluate PF-04958242 in 90 day regulatory toxicology studies in rats and dogs, in which clinical candidate 11 achieved adequate TIs, suggesting that the compound could be safely evaluated in the clinic. Simcyp software was used to project clinical pharmacokinetic parameters from experimentally determined preclinical data and predicted an efficacious dose of 0.3 mg with a 12 h dosing interval. At the time of publication, AM-PAR potentiator 11 had been tested in 11 Phase I clinical trials and found to be safe and well tolerated in healthy volunteers, in patients with age related sensorineural hearing loss and schizophrenic subjects on stable doses of antipsychotics and other psychotropic medications. The authors then claimed that further clinical studies would focus on evaluating the nootropic effects of 11 in select neurodisease populations.

EVOLUTION AND OPTIMIZATION OF THE COR-TEX CX614 SERIES

Cortex has worked in the area of AMPAR positive modulators for many years and have identified many compounds which have entered clinical evaluation (Table 1 and 2). The majority of the chemical information was until recently contained within patents.[114–118] Only the structures of the early development compounds (CX516, CX546, CX614 and CX691, Fig. 18) are in the public domain and little information is available regarding SAR and DMPK properties of these compounds. Four recent publications describe the discovery of further compounds using CX614 as a starting point.[119–122] In the first of the papers,[121] the authors describe how CX614 57 was designed using the knowledge of how aniracetam 1 binds to the AMPA protein and applying a conformational restriction strategy to stabilise the com pound chemically and enhance activity to achieve micromolar potency. The activity of the modulators developed by Cortex was assessed using patch clamp electrophysiology on cultured rat embryonic hippocampal neurons. The activities were reported as EC2x, which is the concentration of potentiator required to double the current flow through the per-fused neuron.

Initial investigations focussed on exploration of the importance of the fused dioxane ring, analogues demonstrated that the oxygen at position 1 was important for retention of activity; analogues in which this oxygen was absent were much less active, with the exception of the compound with a carbonyl group at position 1 which retained activity. In c-trast, the oxygen at position 4 was not required for activity. Introducing a carbonyl group at position 4, together with a fused pyrrolidine, encouragingly produced a more potent derivative (58, EC2x = 0.06 μM) than CX614. The discovery of 58 prompted further investigations in this area, initially focussing on simple N-alkyl analogues 59. Results indicated that a small alkyl group was optimal for potency, with the methyl analogue being the most potent (EC2x = 0.1 μM). Larger groups, which included cycloalkyls and branched alkyls, were generally less active, and the unsubstituted analogue (59, R = H, EC2x = 5 μM) had similar potency to CX614. Compounds were also tested in a ligand binding assay and a good correlation with the electrophysiology as-say was observed. Some of the active compounds in electrophysiology also showed weak activity when assayed in a rat hippocampal slice preparation.

In a second publication,[122] Cortex reported further SAR investigations around the N-substituent. Initial studies focused on alkyl chains (59, R = alkyl) bearing substituents. Starting from the N-ethyl derivative (59, R = Et) work investigated the effect of introducing functionality on the terminal carbon atom. A wide variety of groups were tolerated, producing compounds with similar potency to the N-ethyl derivative (59, R = Et, EC2x = 0.2 μM). The two modifications which showed marginally increased potency were the introduction of chemically reactive groups azide (59, R = N3(CH2)2-, EC2x = 0.08 μM) and isothiocyanate (59, R = NCS(CH2)2-, EC2x = 0.06 μM). Increasing the chain length by one atom and appending a nitrile group gave a butanenitrile derivative (59, R = NC(CH2)3-, EC2x = 0.05 μM). The indication from this work is that a wide range of functionality is tolerated at this position.

Further studies investigated the effect of aromatic groups appended to the alkyl chain (60, R = Ar(CH2)n). Activity was shown to be dependent on the chain length in the initial studies (Ar = phenyl) with two carbon atoms being optimal (EC2x = 0.25 μM), longer (n = 3) and shorter (n = 1) chains gave compounds with activity similar to CX614 (Table 5). Working in the two carbon linker series, the introduction of small halogen substituents (fluorine, chlorine) at the 3-position of the phenyl moiety gave a 10-fold increase in potency, larger or more polar groups resulted in reduced activity or at best retention. Substituents in the 2-or 4-position led to reduced potency. Interestingly the 3,5-difluorophenyl derivative was highly potent in both the electrophysiology as-say (EC2x = 0.005 μM) and binding assay (Ki = 0.3 μM). Following the investigations focussed on phenyl substituents, the final area of investigation looked at heterocycles, all linked to the core through a two carbon chain. All three isomeric pyridines were equipotent with the corresponding phenyl analogues (Table 5). With a few exceptions, the general trend amongst unsubstituted 5-membered heterocycles was an increase

in potency, typically 10-fold compared to the pyridyl groups. A limited range of substituents are described for certain heterocycles, thus it is difficult to draw conclusions regarding the SAR. There is a suggestion that in the case of the pyrazoles described, a small lipophilic group, such as chlorine, gave a further increase in potency.

No physicochemical property information or DMPK data was given for this series of compounds in the papers describing the SAR. However in a subsequent publication[119] the Cortex group referred to phenethyl compound 61, stating that despite its potency in a rat in vitro electrophysiology assay (EC2x = 0.011 µM), it is only weakly active when tested in an in vivo electrophysiology experiment, similarly to related compounds. High metabolism is suggested to explain the discrepancy between in vitro potency and in vivo efficacy. Metabolic route studies in RLM showed the major sites of metabolism to be the ethyl chain and the saturated carbon atoms of the oxazinone and pyrrolidine rings. The initial strategy adopted to improve the metabolic stability was to substitute the alkyl chain linking the phenyl group to tetracyclic core such as in 62 and 63. Substitution adjacent to the phenyl group (62) resulted in a large reduction of the in vitro potency in all cases. Substitution of the methylene adjacent to the tetracyclic core (63) (X = H, R1 = CO2Me, R2 = H; X = F, R1 = ethynyl, R2 = H) did result in compounds with comparable potency to the parent compound (61), but these derivatives failed to demonstrate improved metabolic stability.

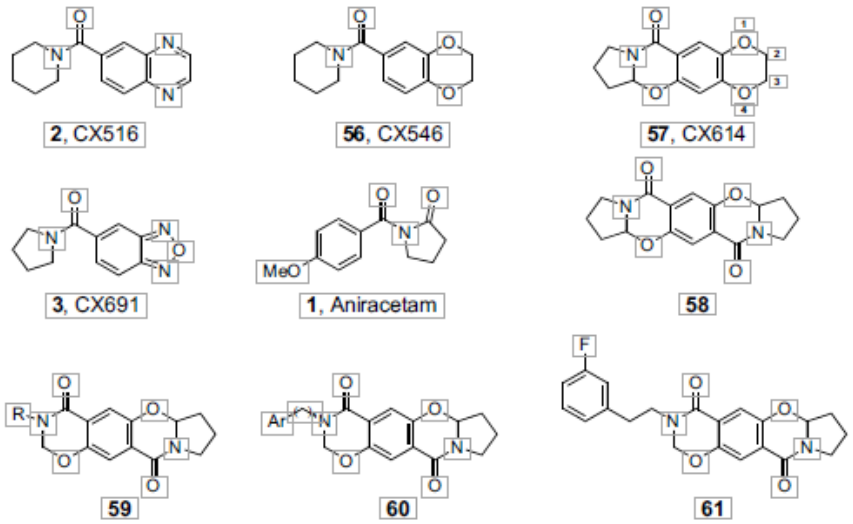
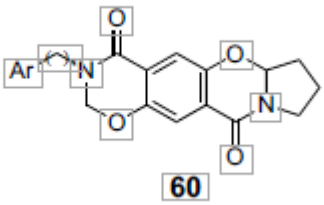


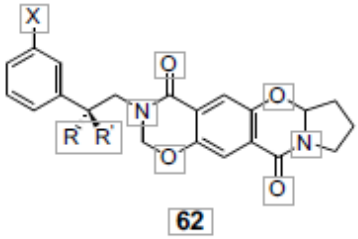
Fig. (18). Amide based AMPAR modulators describes by Cortex.

Table 5. Influence of chain length and appended aromatic groups on AMPAR modulation



Ar	n	EC2x (μM)
Ph	0	Inactive
Ph	1	6.0
Ph	2	0.25
Ph	3	3.6
3-F-Ph	2	0.011
3-Cl-Ph	2	0.02
3-Br-Ph	2	1.6
3-MeO-Ph	2	14
2-F-Ph	2	5.4
4-F-Ph	2	1.2
3,5-F_2-Ph	2	0.005
2-Pyridyl	2	0.6
3-Pyridyl	2	0.2
4-Pyridyl	2	0.24

The second strategy selected was to investigate replacement of the oxazinone ring with a pyrimidinone (64, X = CH) or triazinone ring (64, X = N). This proved successful with both the pyrimidinone and triazinone showing similar effects in an in vivo electrophysiology assay at 5 mg/kg (IP), when compared to 61 which showed very little response. The SAR of the pyrimidinones and triazinones regarding chain substitution mirrored that described for 62 and 63, with small lipophilic groups on the carbon atom adjacent to the hetero-cycle producing increased potency but substituents adjacent to the phenyl ring not being tolerated. Replacing the pendant fluorophenyl group with a 2-tetrazolyl group resulted in in-creased potency in the in vivo electrophysiology assay. This was particularly evident in the triazinone series and was also reported to correlate with increased exposure. Having identified compounds with improved metabolic stability, Cortex focussed on removing the chiral centre present in the pyrrolidinooxazinone motif, this was effectively achieved by appending a cyclopropyl group to the nitrogen of the oxazi-none. Combining the achiral ring system with the modifications, which gave optimal metabolic stability and potency, resulted in 65, which was active in a rat native tissue electro-physiology assay (EC2x = 0.46 μM), produced an increase in the amplitude of fEPSP in vivo (39% at 5 mg/kg, IP) and induced LTP in the rat (1 mg/kg, IP) (Fig. 19).



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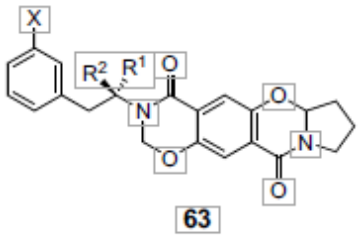

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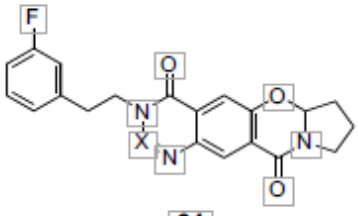

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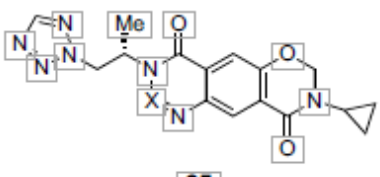

65

Fig. (19). CX614 analogues with enhanced potency.

The main issue with compound 65 was its short plasma half-life (0.27 h) and as a result in a fourth publication[120] Cortex described how the pharmacokinetic profile of this series was optimized. In addition to the short half-life, it was also suggested that 65 had low brain penetration, although no data is given. Initially further investigation of the core ring system was performed and replacement of the oxazinone with either pyrimidinone or triazinone was investigated, leading to the identification of compound 66, which had an encouraging profile. AMPAR modulator 66 was 100% bioavailable in the rat, had good half-life in all species tested (rat: t1/2 = 1.7 h; dog: t1/2 = 3 h; monkey t1/2 = 9.5 h), and retained the electrophysiological profile of 65 (EC2x = 0.28 µM; 37% increase in fEPSP amplitude at 5 mg/kg, IP). All four possible combinations of pyrimidinone and triazinone were prepared with 66 having the best profile. Extensive SAR investigation was subsequently performed looking at modifications of the tetrazole, pendant methyl and cyclopropyl groups. Few modifications gave a profile superior to 66 with the exception of replacing the cyclopropyl group by a methyl, which resulted in a 2-fold increase in the in vivo electrophysiology effect. Wider SAR investigation showed that dimeric tetrazole 67 was more potent than 66 (EC2x = 0.016 µM; 59% increase in fEPSP amplitude at 1 mg/kg, IP). No pharmacokinetic data was given on the later compound. Since the publication of this series of letters in 2011, Cortex has not reported further progress in the CX614 series or on other templates the company has explored (Fig. 20).

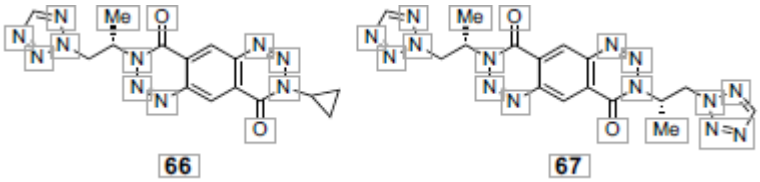

Fig. (20). Compounds with improved DMPK profiles.

OPTIMIZATION OF AN HTS-DERIVED HIT BY OR-GANON USING SBDD

Organon (now Merck) registered four patent applications between 2008 and 2010,[123–126] and relatively soon after published a series of three papers[127–129] which describe the discovery of advanced lead compounds and their evolution from a HTS-derived hit, compound 68.

In the first publication, [129] they describe the profile of 68 and strategies used to improve the in vitro developability profile with retention of potency. AMPAR modulator 68 had very encouraging in vitro functional potency (pEC50 = 6.7; calcium assay in HEK cells overexpressing hGluA1) for a screening hit but was poorly soluble (< 1 mg/L) and highly unstable in both rat and human liver microsomes (Cli > 270 µL/min/mg). To direct the medicinal chemistry, the group made good use of X-ray structures of compounds in complex with the LBD of the hGluA2i subunit. Analysis of the binding mode of 68 (PDB code 3O28, Fig. 21) suggested that the key interactions responsible for the activity were hydrophobic interactions made by (a) the trifluoromethyl on the tetrahydroindazole and (b) the cyclohexyl ring of the tetrahydrobenzothiophene.

Initial efforts concentrated on opening of the saturated ring of the tetrahydrobenzothiophene and preparing dimethyl or methyl and ethyl thiophene analogues (69) or introduction of heteroatoms into the saturated ring (70). Truncated analogues all exhibited reduced potency (≈ 30-fold in the best case) and a modest increase in kinetic solubility (10 mg/L) for some of them. Introduction of an oxygen atom into the saturated ring produced compound 70 which retained potency, but this modification did not improve solubility or microsomal stability. Attempts to modify the saturated ring of the tetrahydroindazole gave similar results and truncation was generally poorly tolerated. Active

compounds were obtained when the system was fully aromatised and contained nitrogen atoms into the 6-membered ring, but this failed to significantly improve the developability characteristics.

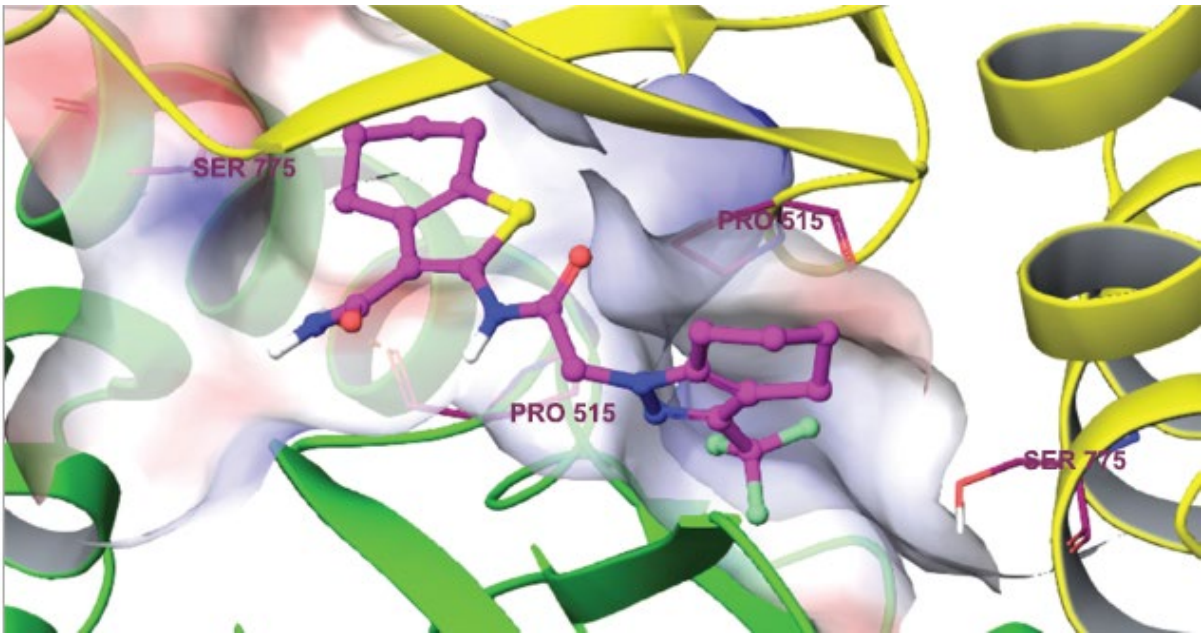

Fig. (21). X-ray structure of hit compound 68 in complex with the S1S2 LBD of hGluA2i (PDB code 3O28, 2.0 Å resolution).

Further examination of the structural information revealed a hydrophilic pocket close to the space occupied by the primary amide function. A series of analogues were pre-pared in which various hydrophilic groups were appended to the amide of 71. One of the early analogues (71, R = NMe2, n = 2) showed modest potency (pEC50 = 5.2) but was successfully crystallised with the hGluA2 ligand binding do-main (PDB code 3O29, Fig. 23). Analysis of the structure revealed a weak interaction with an aspartic acid residue (Asp781), which had the potential for further optimization. Interestingly both the tetrahydroindazole and tetrahydroben-zothiophene were binding similarly to what was observed with hit 68.

Further modification led to a more potent analogue (71, R = NH2, n = 3; pEC50 = 6.4) and an improved developability profile (solubility: 38 mg/L; Cli = 17 µL/min/mg (HLM), Cli = 77 µL/min/mg (RLM)). The ADME properties of this compound were still suboptimal since the permeability determined in a Caco-2 assay was low, but the compound was not a P-gp substrate (A-B = 34 nm/s, B-A = 55 nm/s). Unfortunately when 71 (R = NH2, n = 3) was tested in an in vivo rat pharmacokinetic study, the poor permeability caused low oral bioavailability (7.7%) and poor CNS penetration (brain to plasma ratio = 0.05), despite the compound demonstrating moderate blood clearance (21 mL/min/kg). Further analysis of X-ray data suggested that conformationally constrained analogues, which might enhance permeability, would bind well to the protein. Compound 72 was prepared and showed good potency (pEC50 = 6.6), which was slightly superior to the corresponding enantiomer (pEC50 = 5.8). Derivative 72 possessed promising solubility (20 mg/L) and microsomal stability (Cli = 41 µL/min/mg (HLM), Cli = 50 µL/min/mg (RLM)). However the Caco-2 permeability was still low resulting again in poor bioavailability (3.8%) despite low in vivo clearance (2.3 mL/min/kg, rat). Interestingly the brain to plasma ratio increased to 1.03:1.

Following extensive exploration of the tetrahydroben-zothiophene motif, which failed to deliver molecules with the desired in vivo profile, attention focussed on the tetrahy-droindazole group.[128] Structural data suggested that introduction of polar groups in this region was possible and could

potentially interact with two key residues, namely Pro515 and Ser750. Compounds with an opened saturated ring and basic functionality appended to the pyrazole ring were modelled as having the greatest potential. A series of analogues 73 was prepared; a wide variety of small alkyl groups were tolerated and good activity (pEC50 ≥ 6) was observed with both secondary and tertiary amines. Two secondary amines were chosen for further study: 73, R1 = Me, R2 = H; pEC50 = 6.5, Cli < 12 µL/min/mg (RLM), Cli = 33 µL/min/mg (HLM) and kinetic solubility = 65 mg/L and 73, R1 = Et, R2 = H; pEC50 = 6.1, Cli < 19 µL/min/mg (RLM), Cli < 12 µL/min/mg (HLM) and kinetic solubility = 107 mg/L. Both compound pharmacokinetics were studied in vivo and showed improved oral bioavailability in the rat compared to previous analogues (R1 = Me, F = 15%; R1 = Et, F = 43%). The ethyl compound was progressed further and found to have low CNS penetration (brain to plasma ratio = 0.05). However despite the poor CNS permeability, it did show effects in a rat in vivo electrophysiology assay, increasing the amplitude of evoked activity at a dose of 0.3 mg/kg (IV). The crystal structure of 73 (R1 = Et, R2 = H) bound to the S1S2 LBD of hGluA2i was obtained and con-firmed the initial designed hypothesis (PDB code 3O6H, Fig. 24). The authors conclude by saying that at the time of publication the advanced compound was under detailed investigation and further results will be published at a later date (Fig. 22).

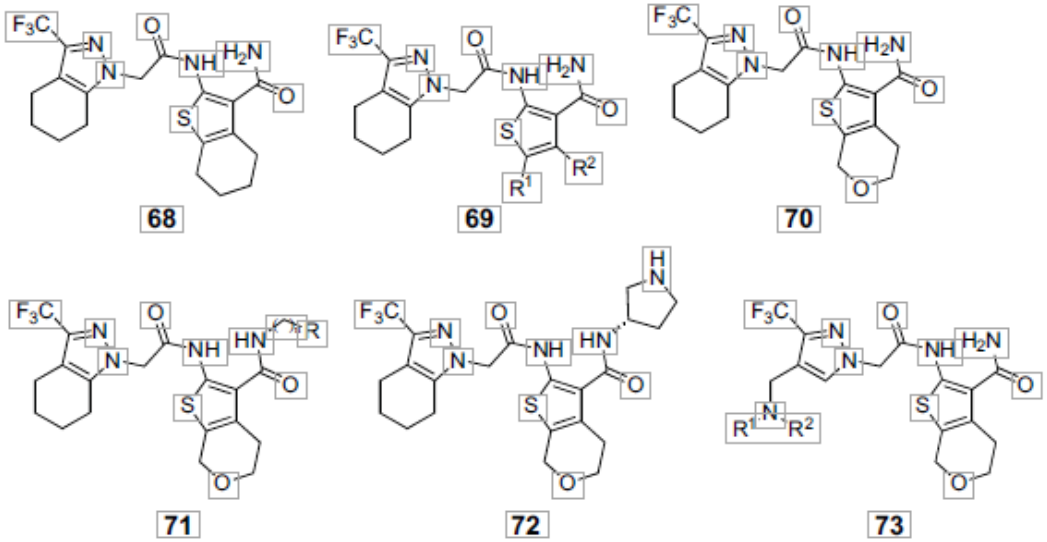

Fig. (22). Key compounds in the optimization of screening hit 68 published by Organon.

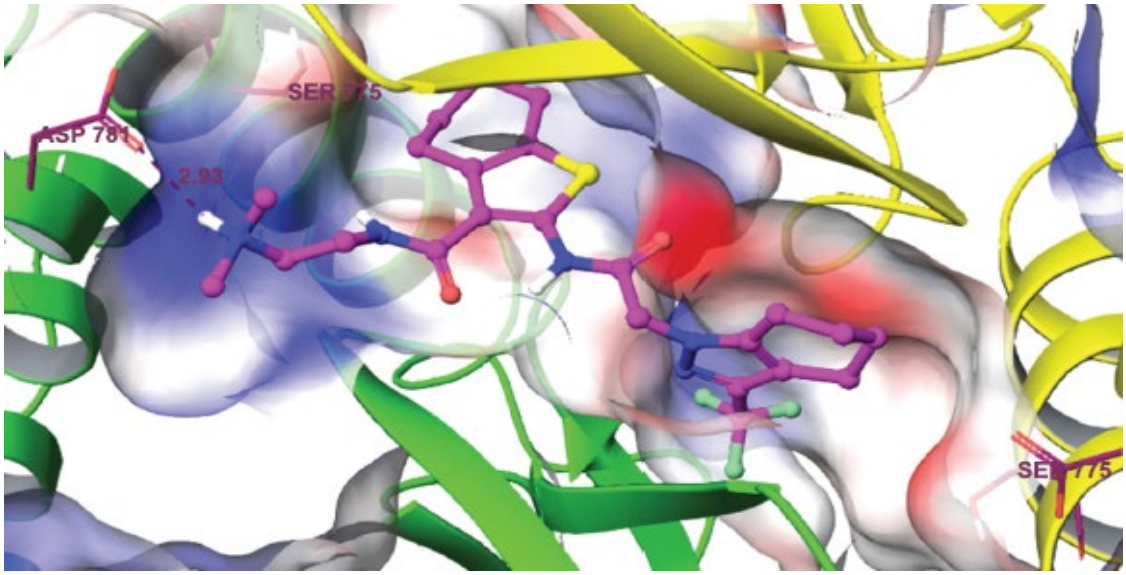

Fig. (23). Biostructure of lead 71 (R = NMe2, n = 2) bound to the S1S2 LBD of hGluA2i (PDB code 3O29, 2.02 Å resolution).

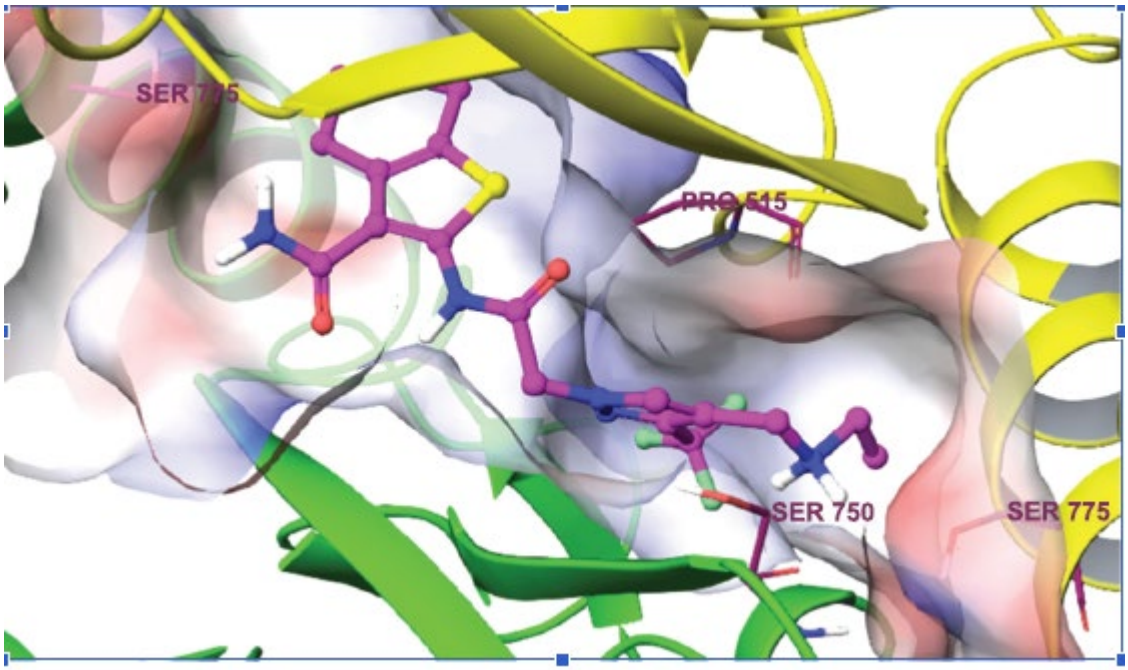

Fig. (24). X-ray structure of 73 (R1 = Et, R2 = H) in complex with the S1S2 LBD of hGluA2i (PDB code 3O6H, 2.1 Å resolution).

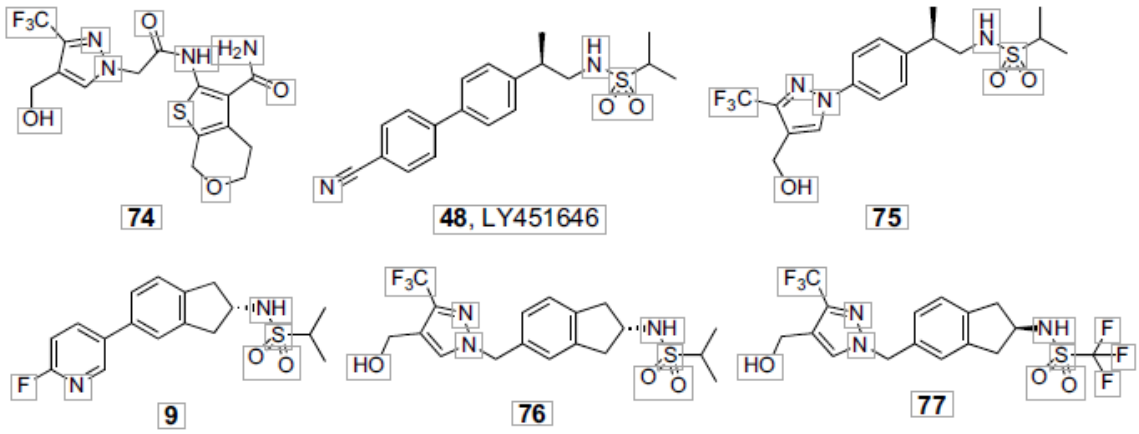

Fig. (25). Advanced compounds from Organon and comparison with similar templates from other groups.

In the final publication,[127] further evolution of the template to structurally diverse scaffolds was explored using a hybridisation strategy (Fig. 25). Structural components of other reported AMPA modulators were combined with key elements of the newly discovered scaffold, in order to identify a back-up series. The work resulted in the identification of compound 77. Examination of available X-ray structures suggested that 74 (hGluA1 pEC50 = 6.4, solubility = 20 mg/L) had the potential to be hybridised with the previously reported Lilly compound (LY451646, 48) by replacing the cyanophenyl group with the pyrazole moiety of 74 to give 75. The results were initially encouraging with 75 having the desired in vitro profile (pEC50 = 6.3, solubility = 73 mg/L and Cli < 12 µL/min/mg (rat and human)), combined with reasonable permeability (Caco-2) and no evidence of transporter interaction. However the in vivo rat profile was less than desired with high plasma clearance (49.7 mL/min/kg). It was

hypothesized that the introducing conformation constraint might have reduced CYP-mediated metabolism leading to a decreased clearance.

A second hybridisation strategy was employed to achieve the conformation restraint, incorporating features of a template described by GlaxoSmithKline 9[82] producing 76 (PDB code 3PMW, Fig. 26). A linker was introduced be-tween the indane and the pyrazole as molecular modelling suggested the directly linked pyrazole would not fit the binding site optimally. Derivative 76 had a very encouraging profile (pEC50 = 6.3, Cli = 31 μL/min/mg (RLM), 23 μL/min/mg (HLM)), improved in vivo clearance (21.6 mL/min/kg, rat) and excellent oral bioavailability (F% = 95). Having identified 76 as a promising new scaffold, the Organon group explored further the SAR around that template. Changing the stereochemistry of these compounds had little effect on the overall profile of the compounds. The pyrazole ring could be replaced by other 5-membered heterocycles including furan and pyrrole. A range of substituents in place of the alcohol are tolerated including hydrophobic groups such as an additional trifluoromethyl and polar groups such as secondary amines. Exploration of the sulfonamide demonstrated that a wide range of hydrophobic groups are tolerated in this position including aromatic groups such as methoxyphenyl and trifluoromethyl pyrazole. Compound 77 resulted from this exploration and it was noted that replacement of the isopropyl sulfonamide by the corresponding trifluoro methyl analogue gave a compound which retained much of the potency of 76 but with further improved metabolic stability. The profile of 77 is as follows: pEC50 = 5.6, solubility = 83 mg/L, Cli < 12 μL/min/mg (RLM and HLM), in vivo (rat) Cl = 1.9 mL/min/kg, t1/2 = 7.4 h, F% = 100, and evidence of good CNS penetration. No report describing the in vivo pharmacological effects of the AMPAR potentiators described in these three papers has been published by Organon and it is unclear if these series are still being developed (Fig. 27).

A recent publication from one of the Organon authors currently working at the University of Strathclyde described another hybridisation strategy,[130] in which the hydroxymethyl pyrazole used in the design of 77 is combined with a template related to those previously described by Cortex to produce hybrid 79. Only in vitro activity data is de-scribed in an electrophysiology assay with compounds being tested on both flip and flop isoforms of GluA2. Modulator 79 is significantly more potent than 74 and shows activity at both receptor subtypes. Also described in the paper are the sulfonamide analogue 80 and the compound in which the pyrazole is directly attached to the tricyclic core 81, both compounds are less potent than 79 (Fig. 28).

WORK FROM OTHER PHARMACEUTICAL AND ACADEMIC GROUPS

A number of groups at the universities of Liege, Copenhagen and Modena have published work centred around the thiadiazine template 5.[131–138] Servier also worked for many years on this template but have not described any new chemotypes related to this scaffold in the period covered by this article. This work has been comprehensively reviewed elsewhere. [139]

Takeda have demonstrated an interest in this area of neuroscience through a significant number of patent applications between 2009 and 2012. [140–145] However until now no scientific publication reporting on their strategy and discovery of advanced AMPAR PAMS has been published. Lilly which had a strong interest in the area before 2010 seems to have stopped working on AMPAR PAMS, since no patent or scientific articles originating from them has been reported in the last 6 years.

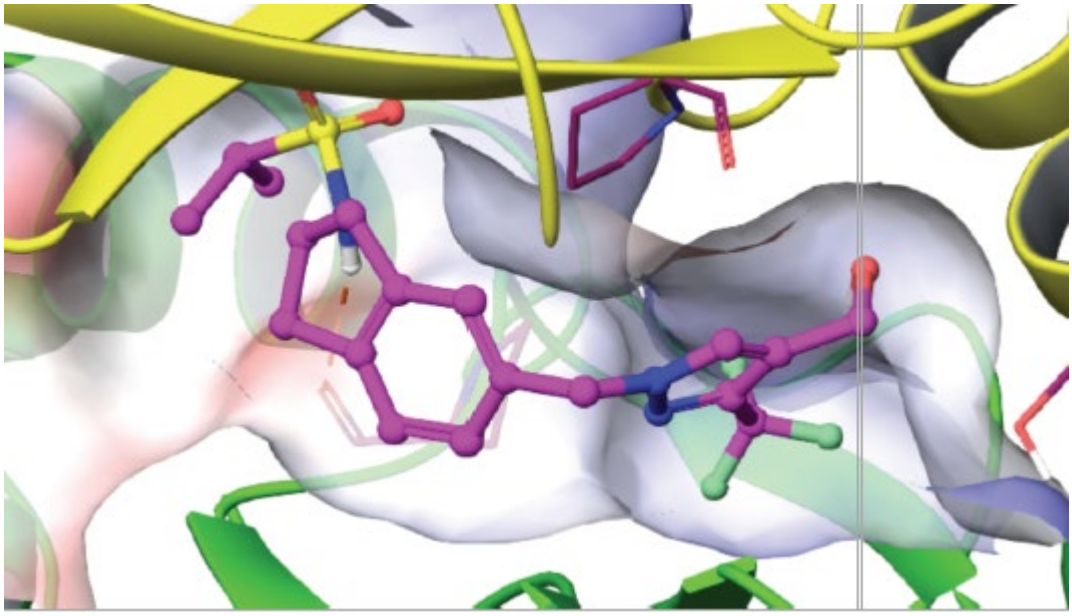

Fig. (26). X-ray structure of 76 bound to the S1S2 LBD of hGluA2i (PDB code 3PMW, 2.2 Å resolution).

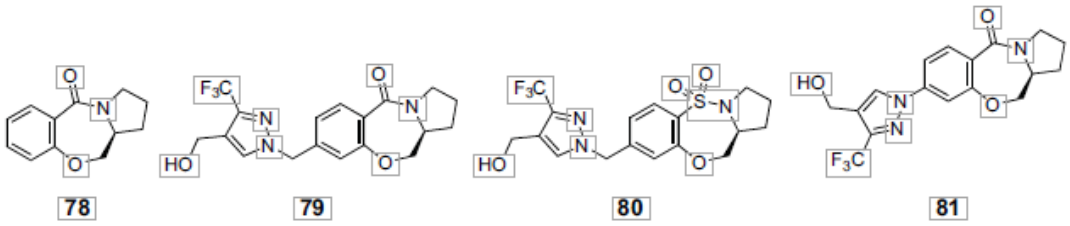

Fig. (27). Conformationally restrained molecules published by Organon.

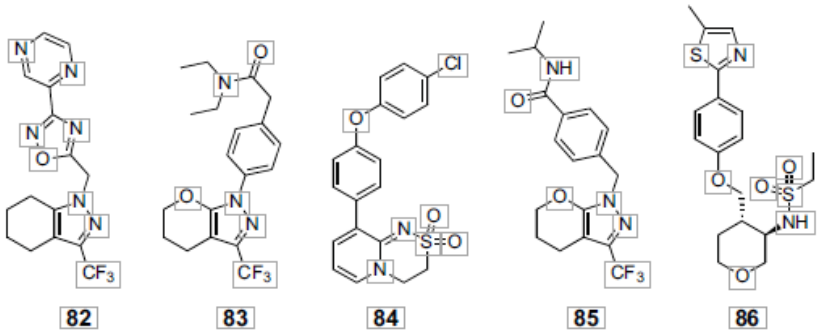

Fig. (28). Representative structures of the AMPAR modulators claimed by Takeda.

CONCLUSION AND FUTURE PERSPECTIVE

The large number of publications on AMPAR potentiators within the period covers by this article proves that it remains a fertile area of drug discovery and that interest will remain for the foreseeable future. It appears that the field has learnt from the failure of the early AMPAR PAMs which entered clinical evaluation and that more precise and defined properties are now sought. The use of SBDD has clearly assisted a number of projects and allowed the identification of very potent potentiators, active at very low doses in cognition behavioural models in vivo. Some pharmaceutical groups such as Pfizer and GSK have also aimed for a specific pharmacokinetic profile with the objective to achieve large TI, low intersubject pharmacokinetics variability and prevent the occurrence of the recognized adverse events associated with AMPAR modulators. We can expect a number of compounds derived from

series described in this review to enter clinical evaluation in the next few years, thus expectation of positive readouts of clinical trial data continues.

CONFLICT OF INTEREST

S. Ward holds a grant from the Wellcome Trust entitled 'Transforming the treatment of schizophrenia: Design and development of AMPA receptor modulators with a much improved safety profile as novel drugs for treating the cognitive dysfunction associated with schizophrenia and other CNS disorders.'

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

ACKNOWLEDGEMENTS

The authors thank Dr Ben Wahab for crystal structure processing and computational support.

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