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A novel series of positive modulators of the AMPA receptor: Structure-based lead optimization

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ABSTRACT

Starting from lead compound **1**, we demonstrate how X-ray structural data can be used to understand SAR and expediently optimize bioavailability in a novel series of AMPA receptor modulators, furnishing **5** with improved bioavailability and robust in vivo activity.

In the preceding Letter,[1] we illustrated how an HTS derived hit could be rapidly optimized through application of biostructural data to a lead compound (**1**), an AMPA receptor modulator worthy of further optimization. Key data associated with compound **1** is listed in Figure 1.

During the hit-to-lead phase, the strategy adopted to improve oral bioavailability was to introduce a conformational constraint while maintaining acceptable levels of solubility. A hydrophilic pocket on the receptor structure[2] was targeted in order to meet these two goals. However, as the summary data outlined in Figure 1 indicates, oral bioavailability remained sub-optimal despite the compound having an acceptable half-life and CNS penetration. Therefore, we sought an alternative approach to improving oral bioavailability in the series. Additionally, we sought to further establish SAR around the series, taking direction from our biostructural data.

As the mass of our lead compound (495 Da) was approaching the upper limits of what is generally regarded as being useful for an oral drug,[3] we turned our attention to how we could produce analogues of reduced complexity and molecular weight. A key objective was to retain a high degree of solubility in these simplified analogues since we anticipated that this property would be of benefit to bioavailability.[4] From the available crystal structures, we reasoned that a suitably sized solubilizing group could be appended either from the pyrazole region of the molecule or through modification of the external amide. The strategy adopted is outlined in Figure 2.

We initially targeted the amide region as a means of rationalizing our template and sought to introduce an alcohol moiety to furnish **2**. Compared to the progenitor compound, **2** was equipotent (pEC_{50} = 6.6) but lacked sufficient solubility (1 mg/L) and had unacceptable microsomal stability (rat/human intrinsic clearance, Cl_i >270 μL/min/mg protein). Additionally, we attempted to introduce amine functionality in this region, however, our attempts to do so were hamstrung by the innate chemical instability of such species.

We next turned our attention to introduction of amine functionality off the tetrahydroindazole species. Modeling based on our crystal structures suggested that changes to the fused ring system could be tolerated, and that main-chain atoms of Pro 494 and Ser 729 provided potential interaction points. This was in contrast to our previous experience[1] with the tetrahydrobenzothiophene moiety where preservation of the fused ring was essential to maintain modulatory activity at GluA1. As Table 1 illustrates, pendant amines were generally well tolerated with acceptable measured kinetic solubilities in the range 20–100 mg/L.

Further profiling of selected analogues revealed that secondary amines such as **4** and **5** displayed a good overall balance of potency, solubility and microsomal stability (e.g., **4** rat Cl_i <12 μL/min/mg protein, human Cl_i = 33 μL/min/mg protein, solubility = 65 mg/L; **5** rat Cl_i <19 μL/min/mg protein, human Cl_i <12 μL/

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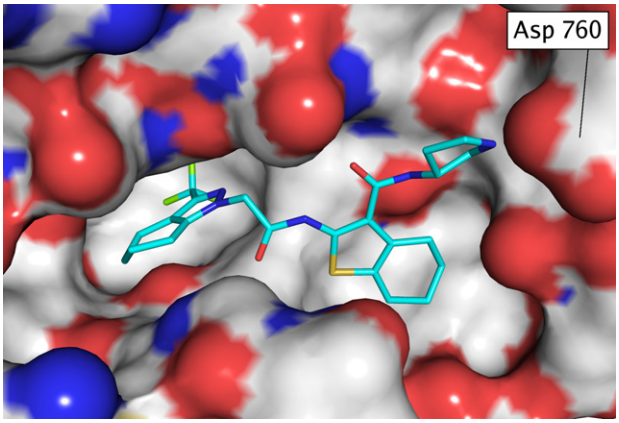
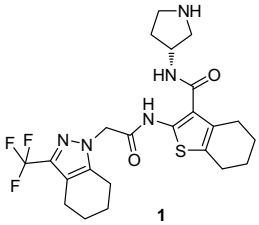




GluR1 pEC_{50} = 6.6
Solubility = 20 mg/L
$T_{1/2}$ = 3.8 h
F% = 3.8
B : P ratio = 1.03

1

Figure 1. Lead compound **1**, summary property data & structure in complex with S1S2 LBD. As the binding site spans an intramolecular two-axis, two orientations of **1** are observed in the crystal structure, but only one (pyrazole moiety on LHS, as shown in preceding Letter) is shown here for clarity.

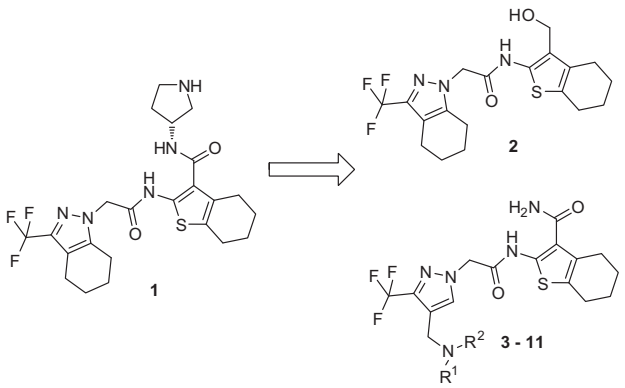
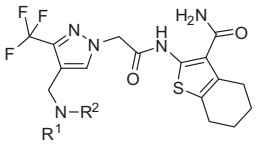


Figure 2. Simplification of lead compound **1**.

Table 1
Pyrazole substituted amines



Compds	R^1	R^2	pEC_{50}[a]
3	H	H	6.1
4	H	Me	6.9
5	H	Et	6.1
6	Me	Me	6.3
7	H	*i*Pr	5.5
8	H	CH_2CH_2OMe	5.8
9	Et	Et	6.2
10	H	*c*Pr	6.2
11	$-CH_2CH_2-CH_2-$		6.0

[a] Values are means of two experiments performed in duplicate.

min/mg protein, solubility = 107 mg/L). Within the series, tertiary amine derivatives generally exhibited poor microsomal stability (e.g. **9** rat/human Cl_i >270 μL/min/mg protein).

Having confirmed that introduction of polar functionality was tolerated in the pyrazole region, we then sought to establish additional SAR in this area. From inspection of the crystal structure of **5** bound to the S1S2 LBD of GluA2 (Fig. 3), we concluded that the hydrophobic interaction made by the trifluoromethyl group could be approximated by other moieties such as *tert*-butyl. To this end, we prepared a series of 3-*t*-butyl functionalized pyrazolyl amines shown in Table 2.

Again further profiling of selected analogues indicated that compounds such as **12** had a reasonable combined profile of GluA1 activity, solubility (79 mg/L) and microsomal stability (rat Cl_i = 15 μL/min/mg protein; human Cl_i = 15 μL/min/mg protein). The X-ray structure of **12** in complex with the S1S2 ligand binding domain confirmed the binding mode was as anticipated with the *tert*-butyl system creating a hydrophobic interaction with the receptor (Fig. 4).

Previously, we had not examined the role of the central methylene spacer, and in particular, determined the effect of extending this linker system on potency at GluA1. Although relatively close hydrophobic contacts were made by the distal ends of our modulators with the receptor, we hypothesized that this could be further optimized through increasing the length of the central linker system. A

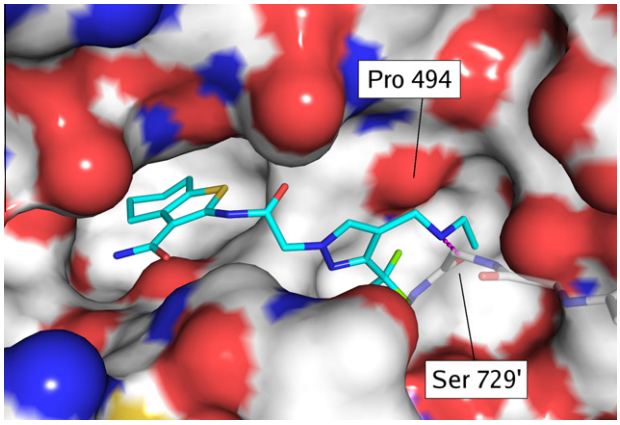
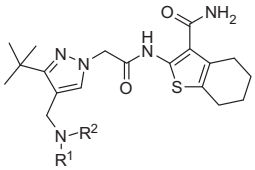


Figure 3. X-ray structure of compound **5** in complex with the S1S2 J LBD of GluA2 (Alternate orientation to that in Figure 1, with pyrazole moiety on RHS). The pendant amine function sits between Pro 494 from one monomer and Ser 729 from another, and forms a hydrogen bond with the backbone carbonyl of Ser 729. Residues 727–730 were omitted from the surface depiction for clarity. Here the main-chain atoms of those residues are shown as sticks to illustrate the hydrogen bond between **5** and Ser 729.

Table 2
tert-Butylpyrazole substituted amines



Compds	R^1	R^2	pEC_{50}[a]
12	H	Me	6.5
13	Me	Me	6.5
14	H	*c*Pr	5.8
15	H	Et	6.1
16	H	*i*Pr	5.8

[a] Values are means of two experiments performed in duplicate.

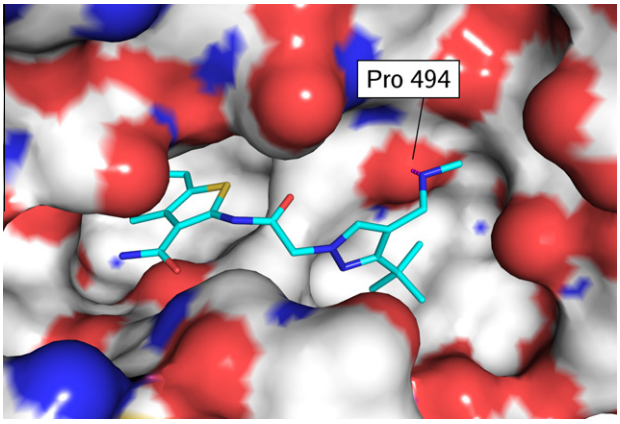


Figure 4. X-ray structure of compound **12** in complex with the S1S2 J LBD of GluA2. The orientation of **12** is as shown in Figure 3. In this case the pendant amine forms a hydrogen bond with Pro 494.

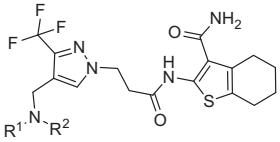
selected number of analogues were prepared and evaluated against GluA1 for their modulatory activity as summarized in Table 3.

The resulting activity data suggested the homologated species were up to ten fold less potent than their progenitor compounds. In addition, microsomal stability was eroded, presumably due to the higher lipophilicity associated with the compounds (e.g. **17** rat Cl_i 49 μL/min/mg protein, human Cl_i 89 μL/min/mg protein).

From the amine derivatives prepared from **3** to **16**, compounds **4**, **5**, and **12** were advanced to in vivo DMPK studies to assess oral bioavailability. Table 4 shows a summary of the key properties for each compound.

This subset of compounds showed encouraging oral bioavailability compared to our initial lead **1**, with **5** in particular being most promising. The bioavailability data prompted us to prepare a limited number of further analogues of **4** and **5** in order to determine if absorption could be further enhanced. The crystal structure of compound **5** prompted us to consider conformational constraint (**20**, Fig. 5) as we believed a heteroatom could successfully be incorporated into the tetrahydroindazole system. Another option explored was to reduce the H-bond donor count to determine if

Table 3
Homologated central linker analogues



Compds	R^1	R^2	pEC_{50}[a]
17	H	Me	5.8
18	H	Et	5.6
19	Et	Et	5.7

[a] Values are means of two experiments performed in duplicate.

Table 4
In vivo pharmacokinetic properties of selected compounds

Compds	$T_{1/2}$[a] (h)	V_{ss}[a] (L/kg)	Cl_p[a] (mL/min/kg)	F%[b]
1	3.8	0.5	2.3	4
4	1.5	2.4	23	15
5	1.1	0.6	15	43
12	1.9	1.1	9	16

[a] 2 mg/kg iv dose Wistar BRL rats.
[b] 10 mg/kg po dose Wistar BRL rats.

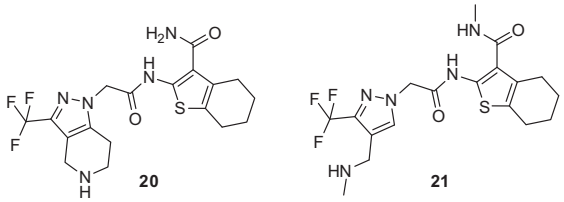


Figure 5.

this parameter could enhance bioavailability, which led to the design of **21**. Although **20** had acceptable potency (pEC_{50} = 6.0) it did not show sufficient microsomal stability (rat Cl_i 33 μL/min/mg protein, human Cl_i 57 μL/min/mg protein) to merit further progression. While compound **21** had similar potency to both **5** and **20** (pEC_{50} = 5.8) and good microsomal stability (rat Cl_i 19 μL/min/mg protein, human Cl_i 16 μL/min/mg protein), it displayed much higher clearance in vivo (Cl_p = 43 mL/min/kg), therefore, it was not further progressed.

Returning to compound **5**, further in vitro profiling indicated the compound was a selective AMPA positive modulator, with no activity at kainate, NMDA or GABA. Wider selectivity determination[5] indicated no off-target activity against a panel of receptors and channels. Determination of brain to plasma ratio in Wistar BRL rats gave a value of 0.05 which indicated modest exposure in the central compartment. However, testing the compound in vivo showed that **5** gave robust potentiation of AMPA evoked single unit activity as measured by electrophysiology in the hippocampus of anesthetized rats,[6] with an MED of 0.3 mg/kg when dosed intravenously. This mechanistic data gave us confidence that **5** was capable of modulating AMPA activity in vivo despite its apparently modest brain penetration.

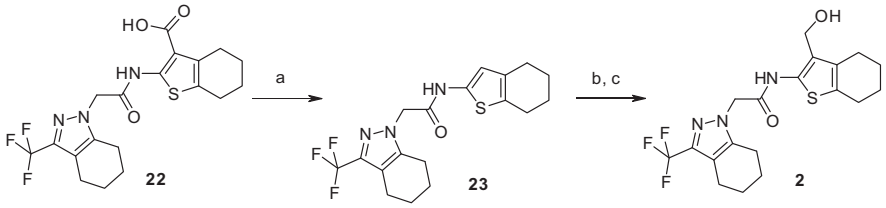
Syntheses of the compounds described above are outlined in Schemes 1–3. Compound **2** was prepared in a 3 step manner from the carboxylic acid **22** (prepared as described in the preceding publication[1]). Copper mediated decarboxylation gave the amide **23** followed by Villsmier formylation and subsequent borohydride reduction furnished **2**.

Compounds **3**–**16** and compound were accessed as illustrated in Scheme 2. Gewald cyclisation[7] provided the aminothiophene derivative **24** which could then be acetylated with using bromoacetyl bromide in the presence of base. Alkylation of the appropriate pyrazole system with **25** followed by reductive amination gave **3**–**11** and **12**–**16**, respectively. Compound **21** was accessed using chemistry analogous to that depicted in Scheme 2 replacing cyanoacetamide with *N*-methylcyanoacetamide in the Gewald step.

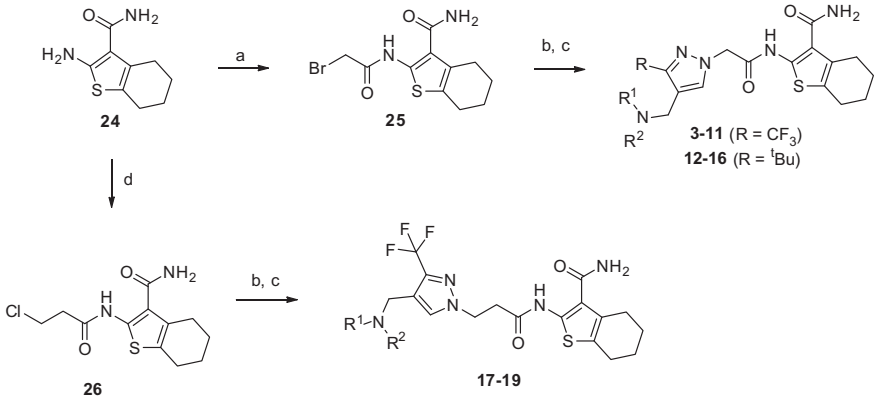
The homologated analogues **17**–**19** were prepared using similar methodology shown also in Scheme 2.

Compound **20** was realized utilizing the synthetic sequence shown in Scheme 3. The requisite pyrrolopiperidine system **29** was prepared according to literature methods.[8] Starting from Boc-piperidinone, enamine **28** was prepared. Subsequent reaction with trifluoracetic anhydride and trapping of the resulting β-diketone with hydrazine gave **29**. Alkylation of **29** with intermediate **25** (Scheme 3) followed by acidolysis of the Boc group provided compound **20**.

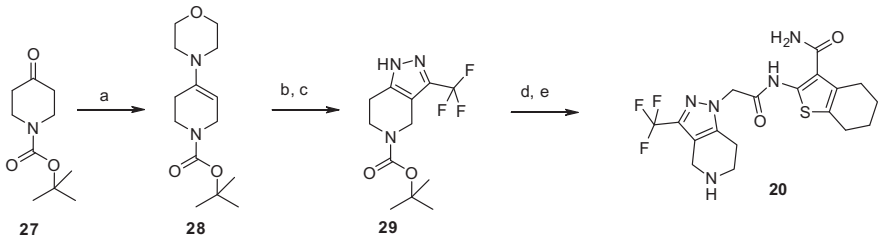
In summary, this work has demonstrated how Structure-Based Drug Design (SBDD) can be exploited within target families such as ion channels where previously its application had been hindered. Throughout our optimization trajectory, a central component has been the application of SBDD in directing our template modifications as well as offering key insights into SAR within the series. Starting from a lead compound **1** which exhibited poor oral bioavailability, we have demonstrated how this can be optimized to yield a rationalized entity (**5**) that has significantly enhanced



Scheme 1. Reagents and conditions: (a) Cu, quinoline 200 °C, μW, 95%; (b) $POCl_3$, DMF, DCE, reflux, 86%; (c) $NaBH_4$, THF, rt, 97%.



Scheme 2. Reagents and conditions: (a) bromoacetyl bromide, Et_3N, CH_2Cl_2, rt, 71%; (b) 3-trifluoromethyl pyrazole or 3-*tert*-butylpyrazole derivative, NaH, DMF, 0 °C to rt, 27–96%; (c) $NaBH(OAc)_3$, AcOH, CH_2Cl_2, rt, 12–50%; (d) 3-chloropropionyl chloride, Et_3N, CH_2Cl_2, rt, 79%.



Scheme 3. Reagents and conditions: (a) morpholine, cat. PTSA, PhMe, reflux, quant.; (b) trifluoroacetic anhydride, Et_3N, CH_2Cl_2, 5 °C to rt, quant.; (c) hydrazine hydrate, EtOH, 5 °C to rt, 43%; (d) **25**, K_2CO_3, DMF, 50 °C, 36%; (e) TFA/CH_2Cl_2, rt, 96%.

bioavailability with retention of receptor potency as well as promising in vivo activity.

We believe that **5** provides us with a valuable asset in order to delineate the role of AMPA receptors in neurological disorders. Additional pharmacological profiling of **5** is on-going and will be the subject of a separate publication.

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