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In vitro and in vivo SAR of pyrido[3,4-*d*]pyramid-4-ylamine based mGluR1 antagonists

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ABSTRACT

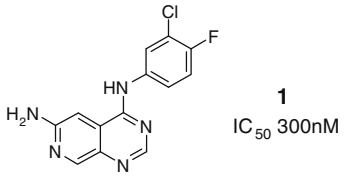
The SAR of a series of novel pyrido[3,4-*d*]pyramid-4-ylamine mGluR1 antagonists is described. The multiple of the unbound K_i in cerebrospinal fluid necessary to give morphine like analgesic effects in an electromyograph pinch model in rodents is determined and the effect of structure on CNS penetration examined.

Glutamate acts at two distinct classes of receptor; fast neurotransmission is mediated by ion channel coupled 'ionotropic' receptors that are widely distributed throughout the central nervous system (CNS). Glutamate can also activate G-protein coupled 'metabotropic' receptors (mGluRs), mGluR1 receptor antagonists have been found to be analgesic and in particular there is evidence that the mGluR1 receptor mediates the hyperalgesia seen in nociceptive and capsaicin-induced pain states.[1] The mGluRs have different distribution patterns raising the possibility that compounds acting at different receptors will have differing physiological effects. High throughput screening of the Pfizer compound file revealed that compound **1** had mGluR1 activity.

This compound contains a potentially toxic aniline moiety and similar compounds were potent kinase inhibitors[2] so the initial objective of the program was to determine whether the aniline could be removed and activity retained. As the literature suggested that an mGluR1 antagonist needs good CNS penetration to show analgesic effects small alkyl groups were also put on the nitrogen since H bond donors are known to impair CNS penetration.[3]

The primary amine in **1**[4] was replaced with dimethylamino and the aniline portion with cyclohexyl to give **2** which had slightly improved potency. Acyclic alkyl groups such as **3** lost a significant amount of activity. When the cyclohexyl ring in **2** is exchanged for indane to give **5** equivalents activity was observed. Addition of a methyl to the NH indane functionality in **5** gave **6** which lost activity slightly. The removal of a methyl group from the dimethylamino portion of compound **5** to give **8** produced a large jump in potency. Removal of methylamine from **8** and introduction of methoxy to give **9** gave a sevenfold drop in potency. This data showed that there was a rich seam of mGluR activity running through the compounds derived from the pyrido[3,4-*d*]pyramid-4-ylamine core (Fig. 1). Following profiling of this first wave of compounds, our key objective was to determine which parts of the core were necessary for activity (Table 1).

Replacement of the ring nitrogen of the quinazoline **10** with carbon to give **11** resulted in a large drop in potency as did converting the exocyclic nitrogen in **10** to oxygen to give **12** (Table 2). This data combined with the comparison of **7** with **8** indicated that it was likely that the hydrogen bond made by the exocyclic nitrogen contributed to potency.



1
IC_{50} 300nM

Figure 1. Lead compound derived from HTS.

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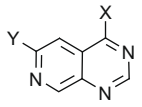
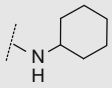
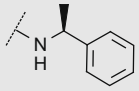
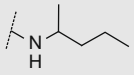
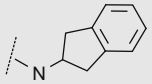
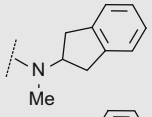
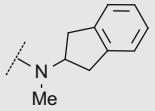
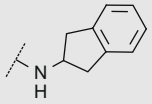
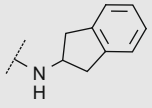
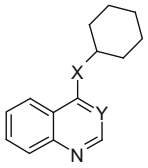
Table 1
SAR of aniline replacements



Compound	Y	X	mGluR1 K_i (nM)[a]
2	–NMe_2		199
3	–NMe_2		1400
4	–NMe_2		858
5	–NMe_2		185
6	–NMe_2		300
7	–NHMe		54
8	–NHMe		14
9	–OMe		115

[a] Assay details are given in Ref. 1.

Table 2
Quinazoline SAR



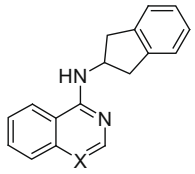
Compound	X	Y	mGluR1 K_i (nM)
10	NH	N	27
11	NH	C	6160
12	O	N	694

Likewise, replacement of the quinazoline nitrogen in **13** with carbon to give **14** led to a major drop in potency (Table 3).

It is clear from this SAR that the two pyramido nitrogens are important for activity of the pyrido[3,4-*d*]pyramid-4-ylamine core. In addition to the core modifications the effect of switching the large lipophilic substituent at **Y** to **X** of **8** was examined.

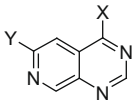
Comparison of **8** with **15** showed that some potency is retained if the positions of the indane and methylamine substituents are exchanged. Replacement of the indane substituent in **16** with

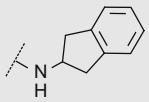
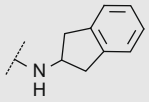
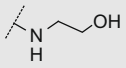
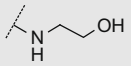
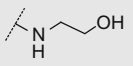
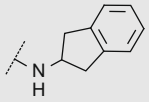
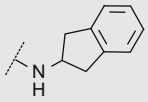
Table 3
Quinazoline SAR



Compound	X	mGluR1 K_i (nM)
13	N	34
14	C	1650

Table 4
Substituent disposition SAR



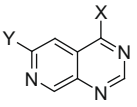
Compound	**Y**	**X**	mGluR1 K_i (nM)
15			160
16			125
17			6150
18			31

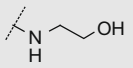
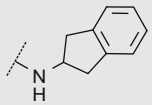
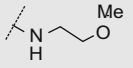
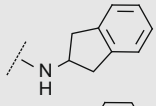
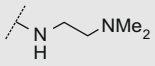
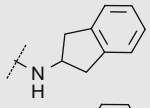
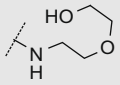
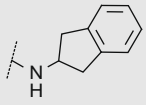
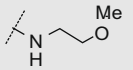
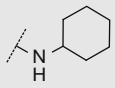
ethanolamine to give **17** gave a large drop off in activity (Table 4). However, increasing the size and lipophilicity of the **Y** and **X** substituents gave a sizable increase in potency compared to **15** and **16** as illustrated by **18**. Compounds such as **15** and **16**, where the positions of the large substituents are switched from **Y** to **X**, are of interest in that they may indicate an alternative binding mode. However, since they were significantly less potent than the original series they were not pursued any further. Having determined which parts of the diaminopyrido[3,4-*d*]pyramid-4-ylamines were necessary for activity our objective became improving the potency of the leads. Our synthetic resource was directed to optimising the series with the larger, more lipophilic substituent at **X**.

There was a broad trend towards substituents with H bond donors and acceptors at the **Y** position giving good potency. Neutral compounds as well as strong and weak bases were tolerated as shown by **19**, **20** and **22**. Across a range of examples cyclohexyl **Y** substituents have broadly similar activity to indane as illustrated by compound **23** (Table 5).

Having determined the scope of the pyrido[3,4-*d*]pyramid-4-ylamine mGluR activity the next objective was to obtain some compounds that could be used to determine whether the series would have in vivo activity in our EMG-pinch model of nociceptive pain. In order to give meaningful data in this model a compound must meet two key requirements, it must be possible to obtain enough systemic exposure for the activity to be expressed and sufficient selectivity over other mechanisms that could influence the results from a nociceptive pain model.

Table 5
SAR of nitrogen substituted analogues



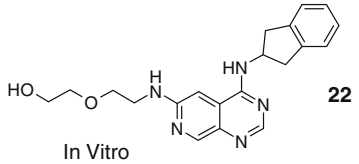
Compound	Y	X	mGluR1 K_i (nM)
19			21
20			5
21			102
22			3
23			11

The suitability of compound **22** (Fig. 2) for dosing into the Electromyograph (EMG) Pinch model of antinociception was determined (Fig. 3).

It is a commonly accepted assumption that unbound or free drug is the species available for interaction with drug targets within the body, and this is referred to as the free drug hypothesis. Since the site of action of mGluR1 is in the brain it was necessary to estimate the unbound exposure of compound in the brain. In this case, concentrations of compound were measured in the cerebrospinal fluid (CSF) as a surrogate measure of free brain concentration.[5] CSF concentrations at steady state are related back to unbound plasma concentration (unbound plasma concentration is subsequently referred to as C_u) to reflect blood brain barrier penetration. After IV infusion to steady state the CSF: C_u was found to be only 0.02. The compound has no off target pharmacology judged by its mGluR5 activity, mGluR5 is the closest mGluR receptor,[6] a panel of kinases and a selection of enzymes and receptors.

The compounds in vivo inhibition of nociception was assessed by measuring its ability to inhibit an EMG reflex produced in response to a noxious stimulus in anaesthetised rats.[7]

Compound **22** and morphine were dosed initially as an IV bolus and then by IV infusion to maintain a steady plasma level. Electrophysiological recordings of spinal pain signaling neurones demonstrated that the prototype mGluR1 compound **22** had a selective inhibitory effect on responses evoked by stimuli in the noxious range. Recordings were made from wide dynamic range neurones in the dorsal horn of the spinal cord and the responses to a range of sensory inputs assessed before and after administration of **22**. At the highest dose tested responses to noxious pinch were selectively inhibited whilst responses to low threshold tactile stimuli remained at control levels. Inhibition of noxious responses comparable to that produced by a maximal inhibition produced by morphine was only achieved when the concentration of **22** measured in CSF exceeded the K_i by a factor of 10. Since **22** has poor CNS penetration (CSF:C_u = 0.02), it appeared that relatively large free plasma concentrations (>1 μm) in the periphery had little or no effect on sensory input to the spinal cord. As well as poor CNS penetration, compound **22** also had poor solubility. Our strategy to address both these problems was to remove H bond donors from the molecule and examine the effect of introducing a basic centre. One particular area of focus for improving solubility was removing the rigid, planar indane. Replacement of the indane also had the benefit extracting two benzylic centres from the molecule. This would be expected to help to make the compounds more metabolically robust.[8] A series of analogues of compound **9** where one of the NH functionalities on the pyrido[3,4-*d*]pyramid-4-ylamine has been replaced with oxygen were profiled.



22

In Vitro

mgluR1 3nM
mgluR5 >3μM
Human Liver Microsome Cl_{int} <7 (μl/min/mg)
Rat Liver Microsome Cl_{int} 30 (μl/min/mg)

In Vivo Rat PK (n=2, 1mg/kg)

$T_{1/2}$ 0.3hr
Cl 26 (ml/min/kg)
Vd (L/kg) 0.6
Oral Bioavailability 33%
Rat PPB 81%

Figure 2. Pharmacological and pharmacokinetic profile of **22**.

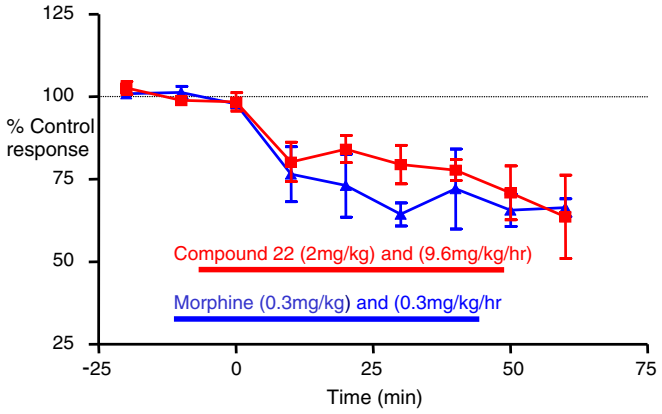


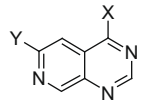
Figure 3. EMG-pinch data for **22**.

Addition of an ethane 1,2-diol substituent onto the azaquinazoline core gave compound **24** which had reasonable activity. The alcohol in **24** was methylated to give **25** which had slightly increased potency. However, incorporation of an extra methylene into the sidechain of **24** to give **26** increased potency fivefold (Table 6). The methoxy functionality in **25** was exchanged for pyrrolidinone to give compound **27** which again increased potency fivefold. This SAR tells us that substituents of a relatively large size and variety are tolerated. The CNS penetration of **27** was examined, see Table 8. A series of derivatives containing a basic centre were then synthesized.

The ethylene diamine adduct **28** had only weak activity. Extension of the chain length to give the propylene derivative **29** or increase of the size of the substituents on the amine increased activity. When the exocyclic nitrogen in **30** was exchanged for oxygen to give **31** this caused a drop off in activity. However, replacement of the cyclohexyl in **31** with cycloheptyl gave a compound with substantially increased potency, **32** (Table 7). The CNS penetration of compounds **30** and **32** were examined, see Table 8.

The CSF concentrations of **22**, **8** and **30** are low compared to **32**. This is probably due to **32** having a combination of high logD and a low number of H bond donors compared to the other compounds. The ability of compound **32** to inhibit in vivo nociception was as-

Table 6
SAR of oxygen substituted analogues



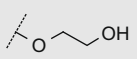
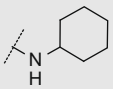
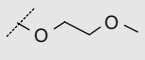
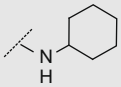
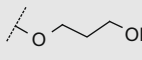
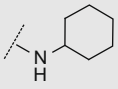
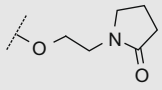
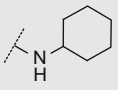
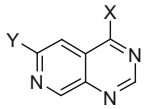
Compound	Y	X	mGluR1 K_i (nM)
24			177
25			101
26			33
27			20

Table 7
Basic substituent SAR



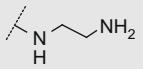
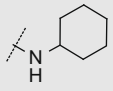
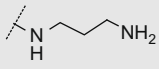
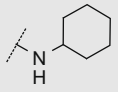
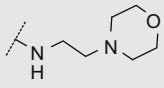
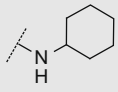
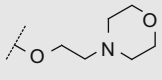
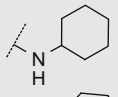
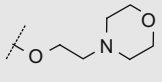
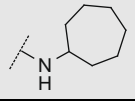
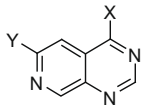
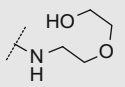
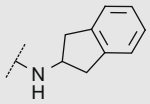
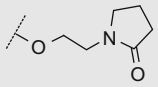
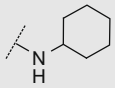
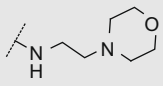
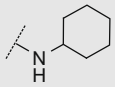
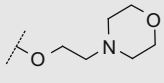
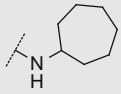
Compound	Y	X	mGluR1 K_i(nM)
28			2200
29			200
30			10
31			42
32			6

Table 8
CSF penetration of pyrido[3,4-*d*]pyramid-4-ylamine antagonists



Compound	Y	X	CSF:C_u	HBD[a]	logD
22			0.02	3	3.0
8			0.06	1	2.9
30			0.07	2	2.8
32			0.5	1	3.4

[a] HBD are hydrogen bond donors

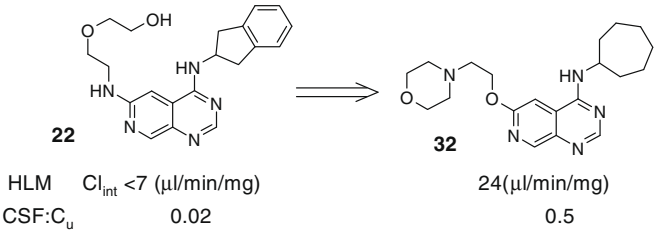


Figure 4. Metabolic stability and CNS penetration of **22** and **32**.

sessed by measuring its ability to inhibit an EMG reflex. This was produced in response to a noxious stimulus in anaesthetised rats[7] in an analogous manner to that used to determine the anti-nociceptive effects of **22**. The maximum level of inhibition was obtained at 8–16× free K_i in the CSF.

This level of inhibition is similar to the 10× K_i shown by compound **22**. This is despite the fact that **32** is able to penetrate the CNS much more effectively (Fig. 4). Therefore, it is probable that there is only a minimal peripheral component to the anti-nociceptive activity of mGluR1 antagonists. This finding is in accordance with that of other workers in the field.[9] While **32** clearly has excellent CNS penetration it also has fairly poor in vitro metabolic stability (24 μg/ml/min) in HLM. This is in contrast with **22** which had excellent in vitro stability but poor CNS penetration. The next step in moving this series forward was to combine metabolic stability and good CNS penetration into one molecule and examine the side effect profile and efficacy of our molecules in other models of nociceptive pain. The results of these investigations will be disclosed in a subsequent publication.

In conclusion, a novel class of mGluR1 antagonists has been disclosed. The compounds are able to inhibit nociceptive pain with the same efficacy as morphine when they are present in the CSF at approximately 10× the free K_i. The CNS penetration SAR of the series has been examined and a highly penetrant, potent compound **32** has been identified.

References and notes

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4. Procedures for the synthesis of or methods of preparation for all the compounds within this manuscript are contained within Harbottle, G. W.; Feeder, N.; Gibson, K. R.; Glossop, M.; Maw, G. N.; Million, W. A.; Morel, F. F.; Osborne, S.; Poinsard, C. *Tetrahedron Lett.* **2007**, *48*, 4293 or Edwards, P. J.; Gibson, K. R.; Mantell, S. J.; Maw, G. N.; Poinsard, C. US 2005038047.
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7. The EMG was recorded by a concentric electrode inserted into a flexor leg muscle and the reflex evoked by a 5 s application of an arterial clip. The clip exerts a constant force that was reported to be mildly noxious when the experimenters applied it to themselves. In this model iv administration of morphine at doses that have been reported to cause analgesia in awake rats inhibits the EMG reflex by approximately 40%. Drugs were administered iv; a loading bolus was given over 30 s and a maintenance infusion for the remainder of the experiment (1 h). The pinch stimulus was applied at 10 min intervals, when three consecutive pinch responses varied by less than 10% the drug or vehicle was applied. The data for each study was normalised with respect the mean of the three consistent baseline responses. At the end of the study plasma and CSF samples were taken to assess the drug level achieved peripherally and centrally for each study.
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