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An orally bioavailable positive allosteric modulator of the $mGlu_4$ receptor with efficacy in an animal model of motor dysfunction

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ABSTRACT

A high-throughput screening campaign identified 4-((*E*)-styryl)-pyrimidin-2-ylamine (**11**) as a positive allosteric modulator of the metabotropic glutamate (mGlu) receptor subtype 4. An evaluation of the structure–activity relationships (SAR) of **11** is described and the efficacy of this compound in a haloperidol-induced catalepsy rat model following oral administration is presented.

The metabotropic glutamate (mGlu) receptors are members of the Group C class of G-protein coupled receptors that bind the excitatory neurotransmitter glutamate. To date, there are eight known mGlu receptors (1–8) and they are sub-divided into three groups according to their amino acid sequence homology, signal transduction pathway and pharmacology (Group I: mGlu 1&5; Group II: mGlu 2&3; Group III: mGlu 4,6,7,8).[1–4] These receptors are distributed throughout the CNS and they are responsible for the regulation of a variety of physiological processes. Two different approaches to modulate the biological response of the mGlu receptors have been postulated. One approach is to identify ligands that compete with glutamate for binding to the orthosteric site and this can lead to direct activation or inhibition of the receptor. The alternative approach is to identify ligands that do not compete with glutamate but instead bind to an allosteric site. Allosteric ligands can potentially bind in the presence of glutamate and positively or negatively modulate the response of the receptor.

The debilitating side effects associated with the current treatments for patients suffering from Parkinson's disease (PD) coupled with an ageing population indicate that PD is an area of neurological research with a growing unmet medical need.[5] Stimulation of $mGlu_4$ is currently regarded as an attractive non-dopaminergic target for the palliative treatment of PD which might provide new drugs without the motor complications (dyskinesias) associated with the existing frontline therapy L-DOPA.[6,7] Recently orthosteric $mGlu_4$ agonists such as L-AP-4 or LSP1-2111[8] and $mGlu_4$ positive allosteric modulators (PAMs) such as (−)-PHCCC[9,10] (**1**) have demonstrated improvement of motor function in pre-clinical rodent models of PD. One of the key advantages of positive allosteric modulation of $mGlu_4$[11] is that the mGlu allosteric sites are likely to be less well conserved across the different mGlu subtypes and hence the specificity for $mGlu_4$ over other mGlu receptors should be achievable. Additionally positive enhancers do not activate the receptor on their own and thereby minimize interference with the highly regulated neurotransmission. From the medicinal chemistry perspective another advantage of targeting the allosteric site instead of the orthosteric site is that the allosteric ligands will be different to glutamate and so they will have markedly different properties with the potential for improved central nervous system

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(CNS) exposure. Several literature reports of $mGlu_4$ PAMs that detail structure–activity relationships (SAR) conducted on different chemical series have emerged recently and selected examples of compounds **2**,[12,13] **3**,[14] **4**,[15] **5**,[14] **6**,[16] **7**,[17,18] from these publications are illustrated in Figure 1.

Following a high-throughput screening campaign on $mGlu_4$ we identified compound **8** (Fig. 2) which showed an EC_{50} ~5 μM (82% glutamate max) in our $mGlu_4$ positive modulator assay.[19] Although **8** was a singleton it attracted our attention because of its structural similarity to SIB 1893 **9** and MPEP **10** which have been published previously as $mGlu_5$ negative allosteric modulators (NAMs) with weak $mGlu_4$ PAM activity.[20,21] During the resynthesis of **8** we profiled the parent aminopyrimidine **11** (the product of a two step synthesis from (*E*)-4-phenyl-but-3-en-2-one[22]) and found that this compound, and not the oxalate ester **8**, was the source of the $mGlu_4$ PAM activity with an EC_{50} ~1 μM (106% Glu max). With this result in hand we performed an SAR analysis around **11** in an effort to improve the potency and to identify compounds suitable for evaluation in vivo.

For our SAR exploration we divided compound **11** into four parts, the amino group, the pyrimidine, the olefin and the phenyl ring and made conservative modifications in each of these regions of the molecule.

Changes to the amino moiety on the pyrimidine ring are reported in Table 1. Methylation or dimethylation of **11** to provide **12** and **13** was detrimental to activity. Sulfonylation to give **14** also gave a compound that showed no activity at the highest concentration tested. Replacement of the amino group with either a methyl to give the SIB 1893 analogue **15** or a thiol to give **17** provided compounds that were ~7–8 μM but introducing a phenyl ring in place of the amino moiety (compound **16**) resulted in loss of activity. The results for **13, 14** and **16** suggest that there might be stringent steric requirements at this region of the binding site. Additionally it could be postulated that an H-bond donor is responsible for improved potency but it is perhaps inconclusive whether the amino group is forming a key hydrogen bond with the receptor. None of the modifications that were made to the amino group represented an advantage over compound **11** in terms of their positive modulatory effect on $mGlu_4$.

Minor changes to the pyrimidine moiety of **11** were generally well tolerated as shown for compounds **18**–**28** in Table 2. Addition of a methyl group in the 6-position of compound **11** led to the equipotent compound **18** whereas addition in the 5-position as in compound **19** led to an improvement in potency. Interestingly, when trying to combine this SAR by inserting methyl groups in the 5- and 6-position as in compound **20** no synergistic effect could be seen. Complete loss of activity was induced when a typical hydrogen donor group such as the amino or a hydroxy group (compounds **21** and **22**) were attached in position 4 whereas capping the hydroxyl in **22** to give the methoxy derivative **23** regained activity. Introduction of small electron-withdrawing groups (**24**, **25** and **26**) in **11** had minimal effect on the potency in the $mGlu_4$ positive modulation assay. When a nitrogen was removed from **11** to give aminopyridine **27** or when the pyrimidine was replaced with a pyrazine as shown with compound **28** it had a detrimental effect on the activity suggesting that the nitrogen in the 1-position of **11** might be important for binding to $mGlu_4$.

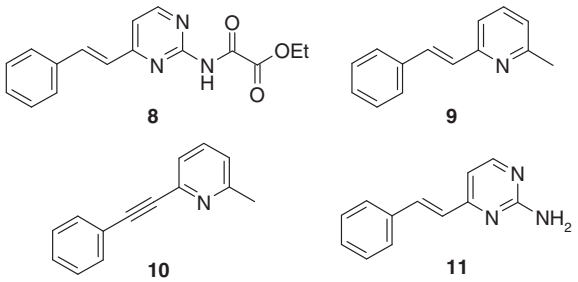
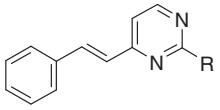


Figure 2. mGlu ligands.

Table 1
Modifications to the amino group



Compound	R	$mGlu_4$ (h) EC_{50} (μM)	% Glu max[a]
11	NH_2	1.0	106
12	NHMe	>30	–
13	NMe_2	>30	–
14	$NHSO_2Me$	>30	–
15	Me	6.9	67
16	Ph	>30	–
17	SH	7.8	115

[a] EC_{50} for potentiation of an EC_{20} glutamate concentration. All assay signals were normalised to saturating concentrations of (−)-PHCCC (**1**) which was set to 100% Glu max. Accordingly, the % Glu max of test compounds was referenced to **1** at maximal activation. In the assay described[19] **1** yielded an average EC_{50} of 5 μM.

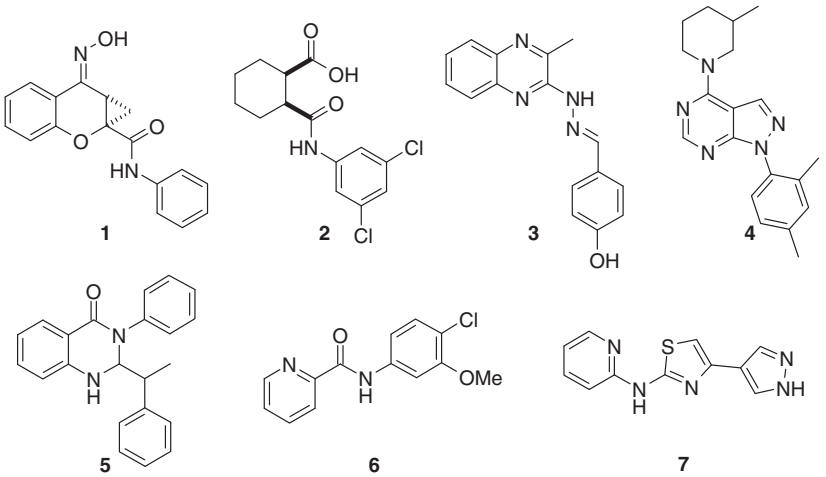
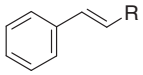
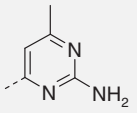
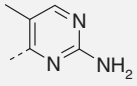
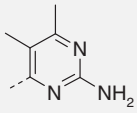
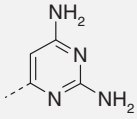
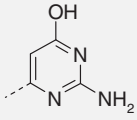
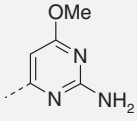
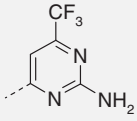
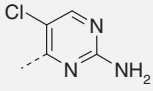
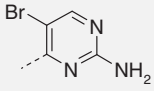
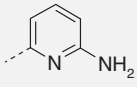
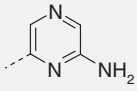


Figure 1. Literature $mGlu_4$ PAMs.

Table 2
Modifications to the pyrimidine



Compound	R	$mGlu_4$ (h) EC_{50} (μM)	% Glu max
18		0.8	99
19		0.2	94
20		1.6	88
21		>30	–
22		>30	–
23		2	106
24		0.6	95
25		1.6	102
26		1.3	94
27		5.8	92
28		15.8	72

The changes to the olefin moiety in **11** are presented in Table 3. Saturation of the double bond to give **29** resulted in a >30-fold drop in potency. Replacement of the olefin in **11** with an alkyne to give **30** also resulted in a drop in potency although it was less dramatic and the compound was active with an EC_{50} ~20 μM (86% Glu max). Cyclopropanation of the double bond to give **31** resulted in loss of potency, however, imposing additional conformation constraint on the scaffold as shown with the benzofuranyl derivative **32** was tolerated. When the size of the bicyclic ring was increased to a benzothiophenyl (**33**) or naphthyl (**34**), then no activity was observed at 30 μM.

All modifications that were made to the phenyl ring (Table 4) led to at least a twofold drop in activity on $mGlu_4$. Introduction of fluorine (**35–37**) in the *ortho*-, *meta*-, or *para*-positions all led to a slight drop in potency but when a cyano group (**38–40**) was introduced in any position a complete loss of activity was observed. This was also the case for the methyl analogues (**41–43**) perhaps indicating steric rather than electronic influences. Replacement of the phenyl ring with isosteric heteroaryl groups (**44–48**) provided compounds with some activity although the 4-pyridyl (**48**) was inactive up to 30 μM.

Table 3
Modifications to the olefin

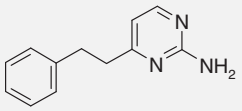
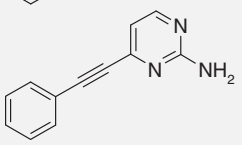
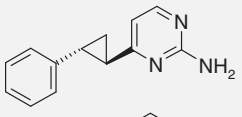
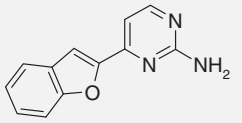
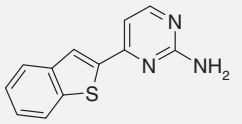
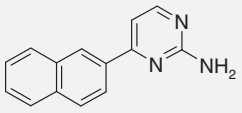
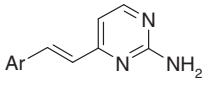
Compound	Structure	$mGlu_4$ (h) EC_{50} (μM)	% Glu max
29		>30	–
30		20	86
31		>30	–
32		4.9	58
33		>30	–
34		>30	–

Table 4
Modifications to the phenyl ring



Compound	Ar	$mGlu_4$ (h) EC_{50} (μM)	% Glu max
35	*o*-F Ph	2.9	101
36	*m*-F Ph	4.5	99
37	*p*-F Ph	4.5	106
38	*o*-CN Ph	>30	–
39	*m*-CN Ph	>30	–
40	*p*-CN Ph	>30	–
41	*o*-Me Ph	>30	–
42	*m*-Me Ph	>30	–
43	*p*-Me Ph	>30	–
44	2-Thienyl	4.1	98
45	2-Furyl	9.4	94
46	2-Pyridyl	11.7	92
47	3-Pyridyl	16.4	55
48	4-Pyridyl	>30	–

The results from our preliminary SAR evaluation of compound **11** suggested that there were limited options for optimisation of the $mGlu_4$ PAM potency. Whilst several compounds with at least comparable activity had been identified following small changes to the pyrimidine ring, we had observed that $mGlu_4$ PAM activity was lost when minor point modifications were made to the four regions of the molecule. This observation of limited SAR within

Table 5
Selectivity profile for compound **11**

Species	Receptor	MOA	EC_{50}/IC_{50} (μM)	% Glu max/% antagonism
Human	$mGlu_4$	PAM	1.0	106
Human	$mGlu_4$	Agonism[a]	>30	—
Human	$mGlu_5$	NAM	>30	—
Human	$mGlu_5$	PAM	>10	—
Human	$mGlu_5$	Agonism[a]	>10	—
Rat	$mGlu_4$	PAM	1.0	110
Rat	$mGlu_5$	NAM	>10	—

MOA, mode of action.
[a] mGlu assay was run in the absence of glutamate.

the field of $mGlu_4$ PAMs has been well documented for other chemical series.[13,15]

In the absence of a significantly superior compound in terms of potency on $mGlu_4$ (i.e., >10-fold more potent), we elected to profile **11** in more detail (Table 5). In the rat $mGlu_4$ PAM in vitro assay the EC_{50} was 1 μM which is comparable to the human assay. The human $mGlu_4$ assay was also run in the absence of glutamate and the EC_{50} was >30 μM suggesting that **11** does not act as an agonist of $mGlu_4$. For our selectivity profiling of **11**, we considered $mGlu_5$ to be the most relevant related target of interest because NAMs of $mGlu_5$ have been reported to show efficacy in animal models of PD.[4,23] Hence, **11** was screened in rat and human $mGlu_5$ assays. In our hands, the IC_{50} of **11** was >30 μM on human $mGlu_5$ and >10 μM on rat $mGlu_5$ and so the window of activity between $mGlu_5$ NAM and $mGlu_4$ PAM is estimated to be >30-fold and >10-fold in human and rat, respectively. The EC_{50} for compound **11** in human $mGlu_5$ PAM or agonist assays was measured as >10 μM. Additionally, **11** was run in a receptor screening panel of 68 targets and no activity was observed at $\geqslant$50% at 10 μM for any of the receptors.[24]

Compound **11** was profiled in several in vitro ADMET assays in order to determine whether it was a suitable candidate for in vivo evaluation in pre-clinical PD rodent models. Permeability was assessed in CaCo-2 cells and **11** was found to have good permeability with no apparent efflux issue (A–B 41.9×10^{-6} cm s^{-1}, B–A 19.6×10^{-6} cm s^{-1}). Additionally the plasma protein binding in rats was measured as 90% bound. The metabolic stability of **11** was assessed in rat and human microsomes and found to be 62% and 83% hepatic blood flow (%Qh) in these species.[25] The limited stability translated into a high in vivo clearance in rats of 75 mL/min/kg (107% Qh) and **11** had a moderate volume of distribution (2.7 L/kg) with a short mean residence time (0.6 h) when dosed at 2 mg/kg via intravenous injection. Compound **11** was orally bioavailable (F = 51%) and 30 min following administration of a 30 mg/kg dose, the plasma concentration was found to be 11.6 μM. Encouragingly, **11** was highly CNS penetrant with a concentration in the brain, also at the 30 min time point, measured as ~33.8 μM (brain:plasma ratio ~2.9). The CSF levels were measured as ~0.7 μM which is in line with the predicted free fraction based on the plasma protein binding data and in the range of the in vitro $mGlu_4$ EC_{50}.

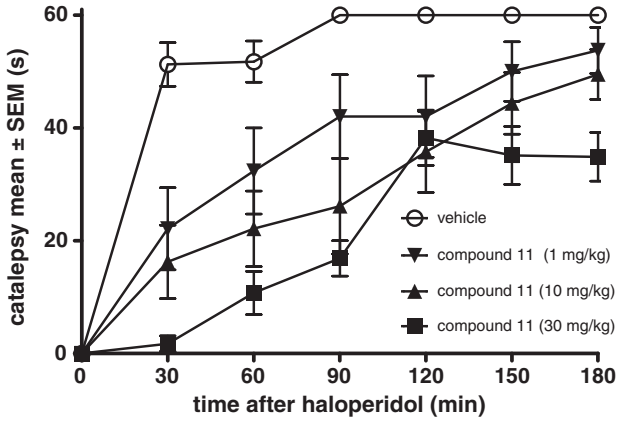


Figure 3. Dose dependent effect of compound **11** in a haloperidol-induced catalepsy rat model.

Although **11** suffered from limited in vitro $mGlu_4$ activity together with rapid in vivo clearance, we reasoned that the high CNS levels (CSF levels in the range of the in vitro EC_{50}) that were achieved in rats following oral administration together with a selectivity of >10-fold over $mGlu_5$ (in the rat assays) qualified **11** as a potential tool compound for an in vivo proof of concept study. We selected the haloperidol-induced catalepsy rat model as our symptomatic model for PD and we administered three doses of **11** (1, 10 and 30 mg/kg po; n = 8 per experimental group) orally 30 min prior to haloperidol (dosed at 2.5 mg/kg ip). Catalepsy was indicated by duration of time (seconds, y-axis) that the rat remains with its forelimbs on a bar (height 8 cm). The cut off time was 60 s.[26] From the results illustrated in Figure 3 compound **11** showed dose dependent efficacy with a reduction in catalepsy observed at all doses. The ED_{50} was estimated as ~1 mg/kg.

To conclude we have identified the $mGlu_4$ PAM 4-((*E*)-styryl)-pyrimidin-2-ylamine **11** from a high-throughput screen. We conducted a preliminary SAR study around **11** and we found that there was only limited scope to modify the structure and improve or indeed maintain the PAM activity on $mGlu_4$. In vivo pharmacokinetic profiling of **11** revealed it to be highly brain penetrant following oral administration and subsequent examination of **11** in the haloperidol-induced catalepsy model revealed a dose dependent effect thus providing further support for stimulation of $mGlu_4$ as a potential treatment for PD. To our knowledge compound **11** represents the first disclosure of an orally available $mGlu_4$ PAM displaying efficacy in a symptomatic PD animal model. Additional in vivo evaluation of the tool compound **11** will be presented in due course.

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19. A CHO-K1 cell line that was engineered to over-express human $mGlu_4$ proteins was used. Activation of $mGlu_4$ in the respective cell line leads to a decrease of intracellular free cAMP due to coupling of these receptors to Gi-proteins and subsequent inhibition of cellular adenylate cyclases. The respective concentration of intracellular cAMP was quantified in order to assess the potency of the compounds of interest. Intracellular cAMP concentrations were determined with the commercially available HitHunter™ cAMP XS+ assay kit (DiscoverRx) with a luminescence read-out. This cAMP assay was performed according to the manufacturer's protocol with slight modifications. Briefly, on the day of the assay culture medium was removed and the cells washed once with Dulbecco's phosphate buffered saline (PBS, PAA). Subsequently, all wash solution was removed and 15 μL of the test compound at the required concentration in an assay 'specific compound solution' (see below) was added to each well of the 384-well assay plate. After incubation for 30 min at 37 °C, 5 μL of XS+ Antibody Reagent and 20 μL of a freshly prepared mixture of XS+ ED/LYSIS/CL Substrate Working Solution were added (all kit components) resulting in a volume of 40 μL. This was followed by an incubation of the assay plate for 1 h at room temperature. Subsequently, 20 μL of XS+ EA Reagent (kit component) were added to each well followed by an incubation of 3 h at room temperature. Finally, luminescence was read on a standard luminescence reader (Novostar, BMG Labtech). In case the potential to positively modulate $mGlu_4$ (positive modulator assay) was assayed the 'specific compound solution' consisted of compound at the concentration of interest, 5 μM Forskolin and 15 μM Glutamate (equivalent to EC_{20} in this assay) in PBS. In case the pure stimulation of $mGlu_4$ was assayed (agonist assay) the 'specific compound solution' consisted of compound at the concentration of interest and 5 μM Forskolin in PBS. Test compounds and positive controls were assayed for dose–response at eight concentrations in triplicate. Serial dilutions in 100% DMSO were made (at 100-fold of final concentration) and starting and final concentrations covered a concentration range of three log units. In order to generate the required assay concentrations in the 'specific compound solutions' the 100% DMSO solutions were diluted in two steps down to 1% DMSO. A valid EC_{50} value was calculated from a minimum of three independent compound dilutions. Dose–response curves were evaluated according to a 'one binding site' equation in XLfit 4.1 software (Model 204; $y = A + (B - A)/(1 + (10\text{^}((C - x) * D)))$; $C \rightarrow \log(EC_{50})$ value; $D \rightarrow$ slope factor) and values for EC_{50} and Hill slope were calculated. Parameters A (top) and B (bottom) were fixed.
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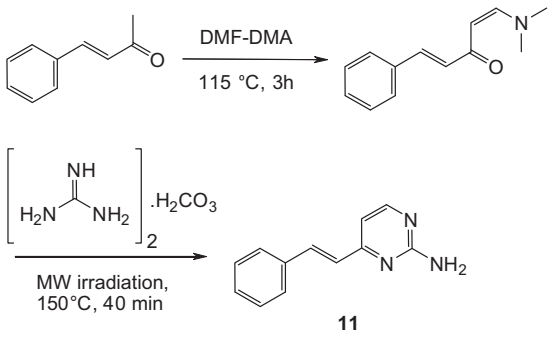
21. Recently compounds structurally related to SIB-1893, MTEP and **11** have been described as $mGlu_5$ ligands that can switch between positive, negative and partial modulators. See: Sharma, S.; Kedrowski, J.; Rook, J. M.; Smith, R. L.; Jones, C. K.; Rodriguez, A. L.; Conn, P. J.; Lindsley, C. W. *J. Med. Chem.* **2009**, *52*, 4103.
22. Synthesis of compound **11**:



11

23. $mGlu_5$ Antagonists have been shown to have efficacy in anti-Parkinsonian animal models. See, for example: (a) Dekundy, A.; Pietraszek, M.; Schaefer, D.; Cenci, M. A.; Danysz, W. *Brain Res. Bull.* **2006**, *69*, 318; (b) Rylander, D.; Iderberg, H.; Li, Q.; Dekundy, A.; Zhang, J.; Li, H.; Baishen, R.; Danysz, W.; Bezard, E.; Cenci, M. A. *Neurobiol. Dis.* **2010**. doi:10.1016/j.nbd.2010.05.001.
24. The receptor panel included adenosine A_{2A}, $5HT_{1A}$ and adrenergic α2A receptors which have all been reported to be potential targets for PD therapy.
25. Compound **11** did not inhibit cytochrome P450 enzymes (3A4, 2D6, 2C9, 2C19) up to a concentration of 50 μM.
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