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4-Aryl piperazine and piperidine amides as novel mGluR5 positive allosteric modulators

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ABSTRACT

Positive allosteric modulation of metabotropic glutamate receptor 5 (mGluR5) is regarded as a potential novel treatment for schizophrenic patients. Herein we report the synthesis and SAR of 4-aryl piperazine and piperidine amides as potent mGluR5 positive allosteric modulators (PAMs). Several analogs have excellent activity and desired drug-like properties. Compound **2b** was further characterized as a PAM using several in vitro experiments, and produced robust activity in several preclinical animal models.

Metabotropic glutamate receptors (mGluRs) are a class of G-protein coupled receptors (GPCRs) that bind glutamate in order to control neurotransmitter release and post-synaptic excitatory neurotransmission. The mGluRs are divided into three groups: group I consisting of mGluR1 and mGluR5, group II consisting of mGluR2 and mGluR3, and group III consisting of mGluR4, mGluR6, mGluR7, and mGluR8.[1] As a member of GPCR family C, the mGluRs contain a large (>500 amino acid) extracellular agonist binding domain in the terminal end of the receptor. Thus, unlike the other GPCR families that contain the agonist binding site within the 7-transmembrane (7TM) spanning region or extracellular loops, mGluR agonists associate indirectly to the transmembrane domains.[1]

Potentiation of the NMDA (*N*-methyl D-aspartate) receptor has been proposed as an alternative antipsychotic therapy based on clinical observations.[2] However, direct agonist activation of NMDA receptors often causes undesired neurotoxicity. Therefore, indirect activation through GPCRs such as the mGluR5 has been pursued instead.[3] The desire to produce potent and selective activators of mGluR5 for use as antipsychotic agents has driven efforts toward development of positive allosteric modulators (PAMs).[4] Interaction with the less conserved allosteric receptor sites on mGluRs should lend itself to greater subtype selectivity compared to competitive binding at the orthosteric agonist binding domain.[5] Additionally, allosteric activation would serve to only enhance the activity of the endogenous agonist glutamate, thus tempering the overall physiological response and reducing the risk of severe side effects.[5]

There are several known structurally distinct types of mGluR5 PAMs (Fig. 1). Difluorobenzaldazine (DFB),[6] MRZ 3573[7] and 3-cyano-*N*-(1,3-diphenyl-1*H*-pyrazol-5-yl) benzamide (CDPPB)[8] are mainly considered tool compounds, due to their lack of CNS

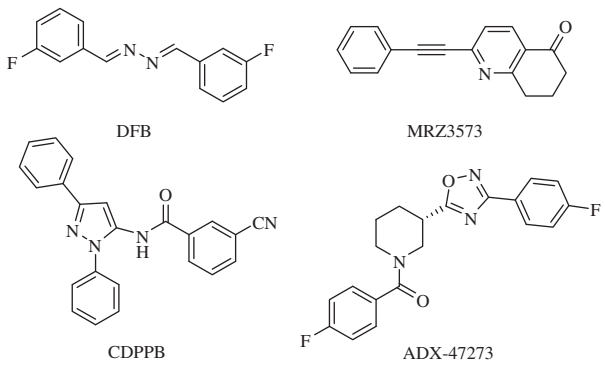

Figure 1. Structure of selected known mGluR5 PAMs.

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drug-like properties. ADX-47273, with its improved physical properties, has been reported to have in vivo efficacy in a number of preclinical antipsychotic and cognition models.[9] We now report our efforts in this area through the discovery of novel 4-aryl piperazine and piperidine amides as mGluR5 PAMs.[10]

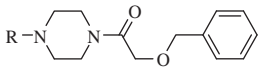
Among several structurally distinct chemical assets available from a high-throughput screening (HTS) campaign to identify mGluR5 PAMS, we chose to further explore substituted acetamides of 4-aryl piperazines and piperidines **1–3**. The synthetic routes to access these amides[11] are summarized in Scheme 1. Fortunately, a number of 4-aryl piperazines were readily available from commercial sources. Otherwise, they could be easily prepared via Buchwald–Hartwig cross coupling between appropriate aryl halides **5** and Boc-protect piperazine **6**. Suzuki coupling between appropriate aryl boronic acids **7** and triflate **8**, followed by catalytic hydrogenation of the trisubstituted double bond and removal of Boc protective group led to the preparation of a wide variety of 4-aryl piperidines **10**. 2-(Benzyloxy) acetic acid was commercially available, while 2-(pyridin-4-ylmethoxy) acetic acid was prepared following known literature conditions.[12] Direct amide coupling reactions between carboxylic acids and piperazine **9** or piperidine **10** afforded the desired final compounds **1–3**. Alternatively, piperazine was converted to the corresponding 2-chloroacetamide **11**, which was used to couple with suitable alcohols to better explore SAR around right hand side (RHS) aryl groups.

The primary assay used to assess project SAR was a calcium influx assay in HEK293 cell line stably expressing the d isoform of human mGluR5 receptor using a fluorometric imaging plate reader (FLIPR) format. PAM activity was assessed by measuring potentiation of the EC_{20} response of L-glutamate in the presence of test compounds.[13]

We started our investigation by evaluating the impact of changes to the left hand side (LHS) aryl moiety directly attached to the nitrogen atom in piperazine (Table 1). Unsubstituted phenyl compound **1a** was weakly active (7.1 μM) as a mGluR5 PAM. *meta* Substitution (**1c**) was briefly explored, and was found to not improve PAM activity. In general, phenyl rings with halogen groups at *ortho*- or *para*-positions led to more active compounds, with *para* substitution having more profound impact. Mono-chlorination at the *para* position (**1d**) gave ~20-fold increase in PAM activity, while 2-chlorophenyl (**1b**) and 4-fluorophenyl (**1e**) led to comparable but smaller gains in activity. Halogen substitution at both *ortho* and *para*-positions on the phenyl ring (**1f–1h**) was also tolerated.

Due to the relatively high lipophilicity of these compounds, most had high clearance (CL_{int}) as measured in human liver microsomes. The attempts to incorporate polar groups into the LHS aryl to lower lipophilicity were met with limited success. Replacement with a 4-chloro-2-pyridyl group (**1i**) led to a slight

Table 1
SAR of 4-aryl piperidine and piperazine amides **1a–i**



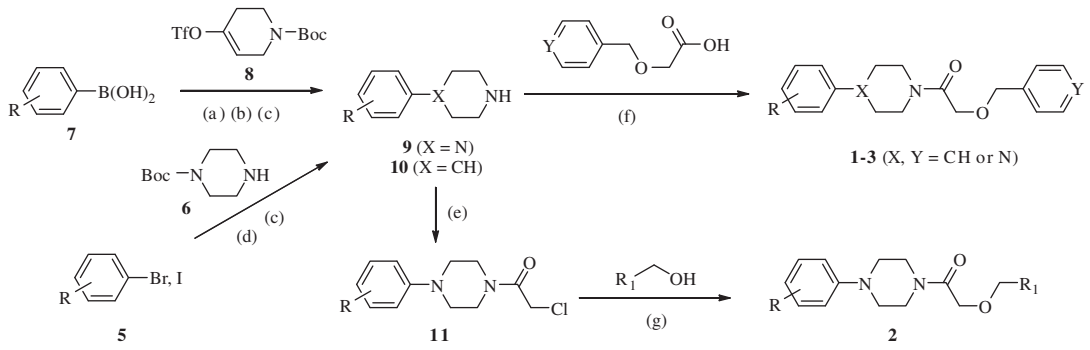
Compd	R	EC_{50}[a] (μM)	*C* log *P*	hCL_{int}[b]
1a	Ph	7.1 ± 1.6	3.4	nd[c]
1b	2-Cl Ph	1.1 ± 0.1	4.2	540
1c	3-Cl Ph	6.7 ± 0.2	4.2	310
1d	4-Cl Ph	0.35 ± 0.04	4.2	290
1e	4-F Ph	1.1 ± 0.2	3.7	110
1f	2,4-DiCl Ph	1 ± 0.2	5.0	450
1g	2,4-DiF Ph	0.30 ± 0.07	3.9	580
1h	2-Cl, 4-F Ph	0.27 ± 0.04	4.4	530
1i	4-Cl-2-Pyridyl	0.57 ± 0.02	3.2	130
1j	4-Pyridyl	>25	2.4	nd[c]

[a] Potentiation of the EC_{20} response to L-glutamate in HEK293 cells expressing human mGluR5 was measured using a Ca++ influx assay. EC_{50}'s are reported as the mean ± SEM ($n \geqslant 2$) and without SEM where $n = 1$.
[b] Human liver microsomal intrinsic clearance (hCLint) was measured as μl/min/mg protein according to the standard liver microsomal stability assay protocol.
[c] nd = not determined.

drop in activity and modest gain in metabolic stability. However, further attenuation of lipophilicity with a 4-pyridyl group (**1j**) resulted in complete loss of activity, suggesting that the lipophilic LHS aryl group was important for potent modulation.

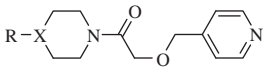
We next replaced the RHS phenyl ring with a 4-pyridyl group to explore the impact on mGluR5 PAM activity and the metabolic stability (Table 2). Compound **2a**, with a 4-chlorophenyl ring as the LHS group, showed moderate PAM activity (EC_{50} 1.4 μM). However, the metabolic stability of **2a** was much better than its close analog **1d** (17 and 290 μl/min/mg, respectively). Addition of halogen atoms or a methyl group at the *ortho* position of the LHS phenyl ring led to compounds (**2b–2e**) with further improved PAM activity and slightly higher clearance. 2,4-Dichlorophenyl analog **2e** was one of the most potent compounds with an EC_{50} = 180 nM. Surprisingly, less lipophilic compounds containing a 2,4-difluorophenyl (**2f**) or 4-chloro-2-pyridyl (**2g**) as the LHS aryl groups were about 10 times less potent than their close analogs in Table 1 (**1g** and **1i**, respectively). Based on these observations, there appeared to be orthogonal SAR present between PAM activity and metabolic stability. Most compounds that had good mGluR5 PAM activity in the piperazine amide chemical series also seemed to exceed desired lipophilicity (*c* Log *P* >2.5). Conversely, metabolic stability was mostly improved by attenuating the lipophilicity.

Several close analogs (**3a–3c**), resulting from replacement of the piperazine ring directly attached to LHS aryl group with a piperidine ring, showed comparable PAM activity and moderate



Scheme 1. Synthesis of 4-aryl piperazine/piperidine amides. Reagents and conditions: (a) $Pd(PPh_3)_2Cl_2$, DME/EtOH/H_2O; (b) PtO_2, H_2, EtOH; (c) HCl, 1,4-dioxane; (d) Buchwald coupling or aromatic substitution; (e) $ClCH_2COCl$, TEA, DCM; (f) EDCI, HOBt, DIPEA, DCM; (g) NaH, THF, rt.

Table 2
SAR of 4-aryl piperidine **2a–2g**, and piperazine amides **3a–3f**



Entry	R	X	EC_{50}[a] (μM)	*C* log *P*	hCL_{int}[b]
2a	4-Cl Ph	N	1.3 ± 0.1	2.7	17
2b	2-Cl, 4-F Ph	N	0.55 ± 0.05	3.0	29
2c	2-F, 4-Cl Ph	N	0.60 ± 0.05	3.0	38
2d	2-Me, 4-Cl Ph	N	0.24 ± 0.04	3.2	68
2e	2,4-Di Cl Ph	N	0.18 ± 0.03	3.5	76
2f	2,4-Di F Ph	N	4.6 ± 1.3	2.4	22
2g	4-Cl-2-Pyridyl	N	4.2 ± 0.2	1.7	4
3a	4-Cl Ph	CH	0.68 ± 0.26	2.8	41
3b	2-Cl, 4-F Ph	CH	0.37 ± 0.11	2.9	56
3c	2,4-Di-Cl Ph	CH	0.39 ± 0.08	3.5	96
3d	4-Cl Ph	C(F)	0.8 ± 0.7	2.8	45
3e	4-Cl Ph	C(Me)	2.1 ± 0.6	3.3	nd[c]
3f	2-Cl, 4-F Ph	C(F)	6.6 ± 1.4	3.0	nd[c]

[a] See footnote a in Table 1 for assay details.
[b] See footnote b in Table 1 for Cl_{int} details.
[c] nd = not determined.

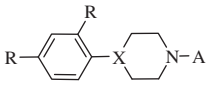
metabolic stability. Fluorination at piperidine C(4) position was tolerated in conjunction with a *para*-chlorophenyl substitution as in **3d** (EC_{50} = 800 nM). However, the 2-chloro-4-fluorophenyl analog **3f** showed more modest results. C(4)-Methyl analog **3e** led to measurable, but significantly reduced activity.

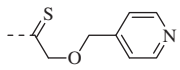
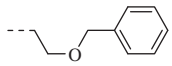
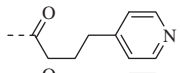
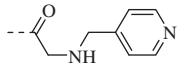
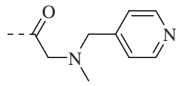
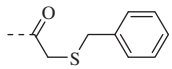
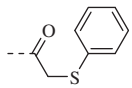
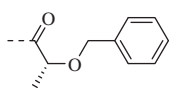
The role of the linker region connecting N(1) on the piperidine and piperazine compounds to Ar-2 was investigated through a series of changes represented by analogs **4a–4h** (Table 3). First, we explored changes to the N(1) amide group. Somewhat surprisingly, conversion of the amide to the thioamide analog **4a** resulted in a near total loss of activity. Additionally, the removal of the amide carbonyl gave basic piperazine **4b,** which proved to be detrimental to PAM activity. These results suggest the specific elements of polarity, basicity and conformational constraint imparted by the amide in the parent series are critical to modulator activity. Likewise, a number of attempts were made to replace the ether oxygen of the linker with other groups including CH_2, NH, NCH_3, and S (compounds **4c–4g**) resulting in >10-fold loss in potency. This sensitivity to the conformational integrity of the ether tether was further substantiated by the complete loss of potency brought about by the simple methyl substitution in **4h**.

We next shifted our efforts to examine the influence of RHS heterocycles on activity and metabolic stability, fixing the 2,4-dichlorophenyl group as the LHS substituent (Table 4). Among three pyridyl groups studied, 2- and 4-pyridyl groups gave relatively more active compounds **2e** and **2h**, while 3-pyridyl **2i** led to moderate activity. Substitution on the pyridine ring did not result in any meaningful improvement of activity (examples not shown). Introduction of pyrimidine or pyrazine rings did improved metabolic stability, but generally resulted in weakly active compounds (**2j–2l**). Interestingly, among three possible regioisomers from introduction of a thiazole ring (**2m–2o**), the 4-thiazole isomer (**2m**) was the most active (290 nM). Thiophene analog **2p** or cyano substituted thiophene **2q** were tolerated for PAM activity, but showed poor metabolic stability.

Based on their in vitro profiles, several compounds were selected to be further characterized, and the results from compound **2b** are summarized as below. As expected for PAMs, it had no

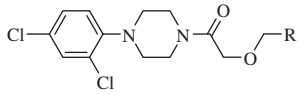
Table 3
SAR of linker region (**4a–4h**)



Entry	A	R	X	EC_{50}[a] (μM)
4a		2-Cl, 4-F	CH	>10.6
4b		Cl	N	>25
4c		Cl	N	5.3 ± 2.4
4d		Cl	N	8.5 ± 5.5
4e		Cl	N	>25
4f		Cl	N	7.1 ± 2.5
4g		Cl	N	>25
4h		Cl	N	>25

[a] See footnote a in Table 1 for assay details.

Table 4
SAR of RHS aryl in piperazine amides **2e–2q**



Compd	R	EC_{50}[a] (μM)	hCl_{int}[b]
2e		0.18 ± 0.03	76
2h		0.31 ± 0.29	94
2i		1.09 ± 0.07	210
2j		5.2 ± 0.9	4
2k		2.8 ± 0.2	17
2l		18 ± 2	16
2m		0.29 ± 0.08	62
2n		1.28 ± 0.03	73
2o		>10.3	80
2p		0.31 ± 0.10	360
2q		0.21 ± 0.04	110

[a] See footnote a in Table 1 for assay details.
[b] See footnote b in Table 1 for Cl_{int} details.

intrinsic agonist activity at the mGluR5 receptor. The radio-tracer ^{3}H-MPEP was used in a competitive binding experiment to measure the affinity of **2b** for the allosteric binding site of mGluR5 in rat cortical membranes. The K_i of **2b** was determined to be 2.37 μM and it completely displaced ^{3}H-MPEP which indicated that these compounds were competing for the same allosteric binding site. As a comparison, CDPPB was found to be a much better binder (K_i ~0.25 μM) under our experimental conditions. PAM **2b** was also screened for agonist, antagonist and modulator activities against all other mGluRs family members, and displayed excellent selectivity, without showing any activity at concentrations up to 25 μM.

Analog **2b** was also profiled extensively in various in vitro and in vivo ADME assays. It exhibited high plasma clearance in rat (55 mL/min/kg), a moderate volume of distribution (4.8 L/kg), a 7.3 h half-life and oral bioavailability of 41%. Excellent in vivo CNS exposure was observed with a brain/plasma ratio of 4.6. In the dog, it exhibited high plasma clearance (38.5 mL/min/kg), a moderate volume of distribution (3.2 L/kg), a 2.9 h half-life and oral bioavailability of 37%.

Based on the favorable in vivo ADME profile, **2b** was chosen for study in several preclinical behavior models. It showed robust activity in both rat conditioned avoidance response (CAR) assay and mouse reversal of MK-801 induced locomotor activity assay, which will be disclosed in a separate publication soon.

In this Letter, we have summarized our laboratory's attempts to broadly scope the key structural features of a series of mGluR5 PAMs containing either a piperazine or piperidine core. Linker modifications have been proven to be not well tolerated. Small, lipophilic groups such as halogen atoms at *ortho*- or *para*-positions of LHS aryl groups are critical for good PAM activity. Exploration of heterocyclic replacement at RHS provided several interesting leads with balanced metabolic stability and PAM activity. In particular, **2b** represents an opportunity to maintain good mGluR5 potency (EC_{50} = 0.55 μM) with limited metabolic clearance (HLM 29 μl/min/mg). It was further characterized as a selective mGluR5 PAM in several in vitro experiments, and was active in several preclinical antipsychotic animal models.

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10. Just before the submission, we became aware of this publication: Zhou, Y.; Manka, J. T.; Rodriguez, A. L.; Weaver, C. D.; Days, E. L.; Vinson, P. N.; Jadhav, S.; Hermann, E. J.; Jones, C. K.; Conn, P. J.; Lindsley, C. W.; Stauffer, S. R. *ACS Med. Chem. Lett.* **2010**, *1*, 000.
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13. A Ca++ Flux FLIPR assay similar to literature[14] was used to detect mGluR5 positive allosteric modulator (PAM) activity in an HEK293 cell line stably expressing the d isoform of human MGluR5. PAM activity was assessed by measuring potentiation of the EC_{20} response to L-glutamate in the presence of test compound.
The day before the experiment 25,000 cells/well were plated in DMEM containing 10% dialyzed FBS (Hyclone) in 384 well Poly-D-Lysine coated plates (Becton Dickinson). After removal of the plating medium the following day, cells were labeled for 1 h at 37 °C in 4.3 μM Fluo-4 AM (In Vitrogen) containing 10% Pluronic F-127 in assay buffer (HBSS(CellGro), 20 mM HEPES, 1 mM Probenecid (Sigma) , pH 7.4). Cells were washed at rt to remove excess dye prior to addition of test compounds serially diluted from 10 mM in 100% DMSO into assay buffer.
Compounds were assayed for any underlying agonist activity by addition of test compounds to the cells on the FLIPR instrument (first addition: 13 μL test compound to 25 μL assay buffer/well) and the response was recorded for 1 min. Eleven different concentrations were tested for each compound. After (15 min) the 1st addition, positive modulator activity was assayed by challenge with EC_{20} (200–300 nM) L-glutamate (second addition: 14 μL (740–1100 nM), final concentration 200–300 nM L-glutamate) and the response was recorded for 1 min. Positive modulator activity was calculated from the fluorescence max-min data normalized to yield responses for no modulation (EC_{20} response) and full stimulation (10 μM L-glutamate) as 0% and 100% modulation, respectively. Concentration–response data were fitted to the four-parameter logistic equation to estimate compound potency (EC_{50}) and efficacy (E_{max}).
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