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Design and synthesis of noncompetitive metabotropic glutamate receptor subtype 5 antagonists

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Abstract—A series of diaryl amides was designed and synthesized as novel nonethynyl mGluR5 antagonists. The systematic variation of the pharmacophoric groups led to the identification of a lead compound that demonstrated micromolar affinity for the mGluR5. Further optimization resulted in compounds with improved binding affinities and antagonist profiles, in vitro.
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L-Glutamate is a major excitatory neurotransmitter in the central nervous system (CNS), which acts through the ligand-gated ionotropic glutamate receptor or through the G-protein coupled receptors (GPCR) called metabotropic glutamate receptors (mGluR).[1] The mGluR5 belongs to the Group I subclass and is coupled to the phosphoinositide/Ca^{2+} pathway, which mainly mediates the excitatory effects of glutamate.[1–3] Recently, investigation into the role of mGluR5 in drug abuse has led to speculation that this may be a new target for medication development. For example, studies using either an mGluR5 antagonist or mGluR5 knockout mice showed reduced locomotor stimulant effects induced by cocaine.[4] Moreover, evidence that mGluR5 is involved in the rewarding effects of morphine, nicotine, and ethanol has also been reported.[5] Thus, development of selective mGluR5 antagonists may provide a novel nondopaminergic strategy toward the discovery of drug abuse medications. Additionally the mGluR5 has recently been implicated in anxiety and depression thus these antagonists might provide new therapeutic agents toward the treatment of these CNS disorders.[3]

The first noncompetitive mGluR5 antagonists **1** and **2** were identified through a high throughput functional assay, which subsequently led to the discovery of compounds **3** and **4** (Fig. 1).[6] These potent ethynyl-based compounds have served as important tools to investigate the role of mGluR5 in CNS pathophysiology and drug abuse. However, cross target activity and in vitro metabolism may limit further development of these alkynes as medications.[7] In order to further explore SAR at mGluR5 and potentially avoid these confounds to in vivo investigation, we report herein the design and synthesis of a series of diaryl amides and their close analogues, and results of in vitro binding and functional evaluation at mGluR5.

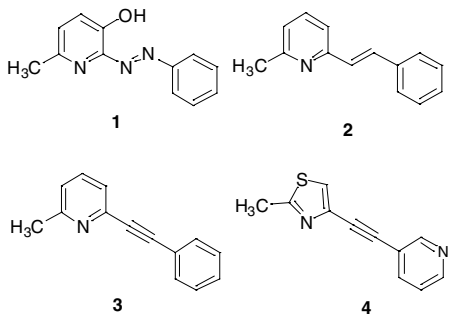

Figure 1. Noncompetitive antagonists of mGluR5: SIB-1757 **1**, SIB-1893 **2**, MPEP **3**, and MTEP **4**.

Keywords: Metabotropic glutamate receptor subtype 5; mGluR5; Noncompetitive antagonists.
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A molecular modeling study was initiated to visualize potential ligand–receptor interactions. The X-ray crystal structure of bovine rhodopsin was used as a template for the homology modeling of the transmembrane region of the mGluR5.[8,9] Based on site-directed mutagenesis data and the binding affinities of MPEP (**3**), the ligand binding site was predicted (Fig. 2).[10] The binding site consists of two hydrophobic regions, wherein the aromatic rings interact, with a linker in between, to position these groups in proper orientation. Thus, a simple ligand design strategy was employed to first explore the SAR around these binding sites by varying rings 'a' and 'b' (Fig. 2). In the course of our studies, others have reported novel mGluR5 antagonists that have replaced the alkyne linker with additional functional groups and heterocyclic rings.[11] In this study, we focused on the amide linkage, to determine if (1) it could mimic the biological activity of the alkyne and (2) for ease of synthesis that allowed the preparation of a larger set of compounds to develop SAR.

A series of compounds was synthesized as shown in Schemes 1–3, wherein 6-methyl picolinaldehyde **5** was refluxed with aniline and then reduced with LAH (Scheme 1) to provide compound **6**.

Benzamide analogues of type **9** and **10** were synthesized by reacting respective acid chlorides with 2-amino-4-methyl thiazole **7** and 2-amino 6-methyl pyridine **8**, respectively (Scheme 2). The appropriately substituted benzamide **10**, like **10a**, was either reduced with LAH to give compound **11a** or, for example, compound **10p** and **10n** were subjected to palladium-catalyzed Suzuki

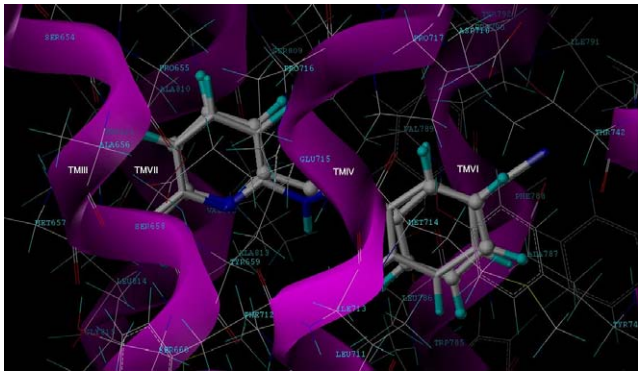
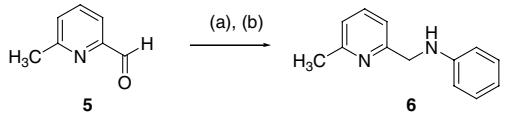


Figure 2. Homology model of mGluR5 for ligand design.



Scheme 1. Synthesis of compound **6**. Reagents and conditions: (a) aniline, methanol, reflux 24 h; (b) LAH, THF, 0 °C 30 min.

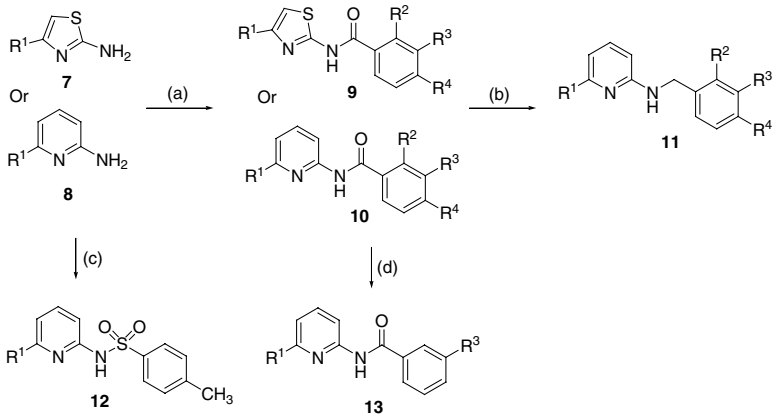
or Heck coupling reactions, respectively, to obtain compounds **13a** and **13b**; similarly compound **10s** was hydrogenated in the presence of 10% Pd/C. The debenzylated compound was then treated with 2-bromophenylethylbromide to give compound **13c**.

The piperidine-substituted compounds **17** were synthesized by Fmoc protection of the DL-pipecolic acid followed by amide synthesis under a modified Schotten–Baumann condition and deprotection of the Fmoc group by treatment with 4-aminomethyl piperidine. The deprotected amide **16** was then reacted with various arylalkyl bromides to obtain compounds **17** (Scheme 3).[12]

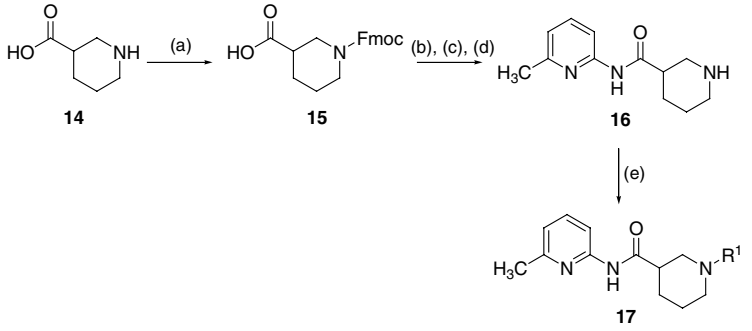
These compounds were evaluated for binding affinity at mGluR5 in a rat brain membrane preparation using [^{3}H]MPEP as the radioligand.[13] Most structural variations were not well tolerated as many compounds in the initial series were inactive, at a concentration of 100 μM (data not shown). Representative structures and binding data are shown in Table 1 and compared to reference compounds **1** and **2**. In general, the compounds with variation in the linker between the aromatic rings 'a' and 'b' (Fig. 2) were inactive (e.g., **6a**, **11a**, and **12a** in Table 1).

Substitution with the amide bond linker however provided some compounds (**9a** and **10a**) that showed moderate affinity at mGluR5 with a slope nearing unity suggesting competitive displacement of the radioligand. There was a considerable loss in affinity when the 6-methyl group in ring 'a' (**10b**) was eliminated. Comparison of activities for compounds 2-naphthyl (**10d**), which was inactive and 1-naphthyl (**10c**), which is moderately active, suggested that there is limited steric tolerance in this region. Substitution at the 3′ position of the aromatic ring 'b' improved the binding affinity substantially (e.g., **10f** IC_{50} = 1.73 μM), however substitution at 2′ (**10e**) and 4′ (**10g**) positions completely lost activity. A comparable activity of compound **10f** to parent compounds **1** and **2** provided strong support to further explore the SAR around this new lead compound.

From these initial data a focused set of compounds with 3′-substitution in the ring 'b' with the amide linker was synthesized, and representative structures and binding data are shown in Table 2. In addition, the active compounds from this set were evaluated in a functional assay measuring the inhibition of agonist-induced phosphoinositide hydrolysis at mGluR5 in CHO cells and are compared to reference compounds **3** and **4**.[14,15] Replacement of the pyridyl 'a' ring with thiazole, analogous to MTEP (**4**), was well tolerated as compound **9d** was as active as compound **10i**. Replacing the 6-methyl group in ring 'a,' which could be a metabolic liability, with a Cl atom (**10q**) maintained the affinity. Substitution with the electronegative CN (**10o**), Br (**10p**) or I (**10n**) at the 3′ position in ring 'b' yielded the most active compounds (IC_{50} = 0.33–1.08 μM) in this series with moderate potency as antagonists in the functional assay (IC_{50} = 5–11 μM). However, substitution with bulky CF_3 (**10k**), SO_2CH_3 (**10l**) or OSO_2CH_3 (**10m**) reduced the binding affinity

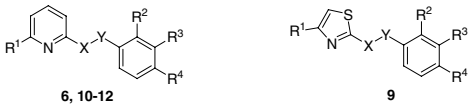


Scheme 2. Synthesis of compounds **9**–**13**. Reagents and conditions: (a) acid chloride, pyridine, rt, 1 h; (b) LAH, THF, reflux 2 h; (c) *p*-TsCl, toluene, 1,4-dioxane, reflux 4 h; (d) phenyl boronic acid, $Pd(PPh_3)_4$, 2 M aq Na_2CO_3, toluene, ethanol, reflux 2 h, **13a**; styrene, $Pd(OAc)_2$, *o*-tolyl phosphine, TEA, acetonitrile, 100 °C, 20 h, **13b**; 10% Pd/C, H_2, MeOH; 2-phenylethylbromide, anhyd K_2CO_3, DMF, 50 °C, 24 h, **13c**.



Scheme 3. Synthesis of compound **17**. Reagents and conditions: (a) Fmoc-Cl, 10% w/v Na_2CO_3, 1,4-dioxane, rt, 2 h; (b) $SOCl_2$, DCM, cat. DMF, rt, 3 h; (c) 2-amino-6-methyl pyridine, aq $NaHCO_3$, $CHCl_3$, rt, 1 h; (d) 4-aminomethyl piperidine, $CHCl_3$, rt, 12 h; (e) 37% HCHO, HCOOH, reflux, 3 h **17a**; arylalkyl bromides, anhyd K_2CO_3, DMF, 80 °C, 2 h, **17b**,**c**; *p*-TsCl, TEA, DCM, 0–5 °C, 1 h, **17d**.

Table 1. Representative structures and in vitro activities of compounds



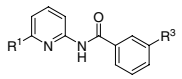
Compound	R^1	X	Y	R^2	R^3	R^4	Binding affinity IC_{50} (μM)
6a	CH_3	CH_2	NH	H	H	H	NA[a]
11a	CH_3	NH	CH_2	H	H	H	NA
12a	CH_3	NH	SO_2	H	H	CH_3	NA
9a	CH_3	NH	CO	H	H	H	65.04 ± 2.87
10a	CH_3	NH	CO	H	H	H	35.00 ± 1.00
10b	H	NH	CO	H	H	H	95.39 ± 6.26
10c	CH_3	NH	CO	1-Naphthyl[b]		H	62.76 ± 1.74
10d	CH_3	NH	CO	H	2-Naphthyl[c]		NA
10e	CH_3	NH	CO	Cl	H	H	NA
10f	CH_3	NH	CO	H	Cl	H	1.73 ± 0.10[d]
10g	CH_3	NH	CO	H	H	Cl	NA
1	—	—	—	—	—	—	1.17 ± 0.07
2	—	—	—	—	—	—	1.08 ± 0.06

[a] Inactive at 100 μM.
[b] Ring 'b' (Fig. 2) is 1-naphthyl.
[c] Ring 'b' (Fig. 2) is 2-naphthyl.
[d] In functional assay 66% inhibition at 10 μM.

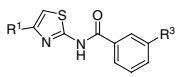
at mGluR5. Additionally substitution with large hydrophobic groups (styryl **13b**, *O*-benzyl, **10s**, etc.) substantially reduced binding affinity.

To further explore the importance of aromaticity in ring 'b' and to potentially access an additional binding domain for added interactions, which might improve

Table 2. Representative structures and in vitro activities of compounds with 3′-substitution in the 'b' ring



10, 13



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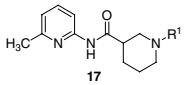
Compound	R^1	R^3	IC_{50} (μM)	
			Binding affinity	Functional activity
9b	CH_3	3-Pyridyl[a]	NA[b]	NT[c]
9c	CH_3	Cl	5.49 ± 0.23	15.00 ± 7.00
9d	CH_3	OCH_3	9.10 ± 0.39	NT
10h	CH_3	3-Pyridyl[a]	NA	NT
10i	CH_3	OCH_3	9.76 ± 0.43	46.00 ± 18.00
10j	CH_3	CH_3	1.84 ± 0.14	5.90 ± 1.10
10k	CH_3	CF_3	18.68 ± 1.11	NT
10l	CH_3	SO_2CH_3	NA	NT
10m	CH_3	OSO_2CH_3	83.57 ± 3.90	NT
10n	CH_3	I	1.08 ± 0.07	10. 80 ± 3.30
10o	CH_3	CN	0.33 ± 0.02	5.33 ± 1.20
10p	CH_3	Br	0.65 ± 0.02	5.48 ± 0.73
10q[d]	Cl	Cl	1.53 ± 0.06	12.70 ± 1.69
10r	CH_3	OPh	56.97 ± 3.26	NT
10s	CH_3	OCH_2Ph	33.04 ± 1.66	NT
13a	CH_3	Ph	7.30 ± 0.39	18.30 ± 1.52
13b	CH_3	*trans*-CH=CHPh	NA	NT
13c	CH_3	$O(CH_2)_2Ph$	23.55 ± 1.37	NA
3	—	—	0.013 ± 0.001	0.039 ± 0.008[15]
4	—	—	NT	0.462 ± 0.112[15]

[a] Ring 'b' (Fig. 2) is 3-pyridyl.
[b] Inactive at 100 μM.
[c] Not tested.
[d] Synthesized from 6-chloro-2-aminopyridine.

binding affinity, saturated 3-piperidinyl amides were synthesized. However, these compounds were inactive, thus suggesting a very limited tolerance in this binding region of the receptor (Table 3).

In summary, a putative ligand-binding site at the transmembrane domain region based on the bovine rhodopsin crystal structure aided in the design of a novel series of potential mGluR5 antagonists. The SAR in this series of diarylamides generally corresponded to those reported for the ethynyl and pyridyl amide analogues[16] but unlike most earlier reports, several amide-linked compounds in the present series showed moderate activity as mGluR5 antagonists. Of note, we observed that these amide analogues generally showed a ~10-fold loss in functional potency as antagonists, as compared to their binding affinities at mGluR5, which has not been described for the ethynyl analogues reported to date.[15,16] Although this may simply be due to a difference in conditions between these two in vitro assays, the possibility exists that changes in conformation, at the receptor protein level, may be affecting these differences. The novel series of compounds described in this communication provide structurally distinct probes to investigate the role of mGluR5 in CNS disorders. The SAR developed herein has lead to the design of a new set of potential mGluR5 antagonists for in vivo investigation that will be reported in due course.

Table 3. Representative structures and in vitro activities of piperidine 3-carboxamides



17

Compound	R^1	Binding affinity[a]
17a	CH_3	NA[b]
17b	CH_2Ph	14
17c	CH_2-*m*-ClPh	31
17d	SO_2-*p*-Tol	31

[a] Percentage inhibition at 100 μM.
[b] Not active.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bmcl.2006.04.032.

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12. All compounds were purified by flash chromatography and characterized by spectroscopic and microanalytical techniques. The final products were crystallized as the HBr salts for biological evaluation. The spectral data supported the assigned structures, for example, *N*-(6-methylpyridin-2-yl)biphenyl-3-carboxamide hydrobromide **13a**, mp 218–220 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.47 (s, 3H), 6.93–9.95 (d, J = 7.6 Hz, 1H), 7.37–7.41 (m, 1H), 7.45–7.49 (m, 2H), 7.54–7.58 (t, J = 7.6 Hz, 1H), 7.61–7.63 (m, 2H), 7.63–7.67 (t, J = 7.6 Hz, 1H), 7.76–7.79 (m, 1H), 7.87–7.90 (dt, J = 7.6, 1.2 Hz, 1H), 8.13–8.14 (t, J = 2.0 Hz, 1H), 8.20–8.22 (d, J = 8.4 Hz, 1H), 8.62 (br s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 24.46, 111.20, 119.72, 126.06, 126.22, 127.41, 128.07, 129.12, 129.42, 131.02, 135.12, 138.93, 140.15, 142.14, 150.92, 157.07, 165.67; IR (neat, cm^{-1}) 3283.80, 3059.10, 1676.40, 1577.80; GC–MS (EI) *m*/*z* 288 (M^+); Anal. ($C_{19}H_{16}N_2O$·HBr) C, H, N. The spectral and analytical data for all other compounds are reported in Supporting information.
13. Rat membranes were prepared each day using a partially thawed frozen rat brain which was homogenized using a Brinkman Polytron (setting 6 for 10 s) in 10 mL/brain of ice-cold 10 mM Tris–HCl, pH 7.0. Membranes were then centrifuged twice for 10 min each at 30,000*g*. After the second centrifugation, the membranes were suspended in 240 mL/brain of ice-cold 50 mM Tris–HCl, pH 7.0, containing 1% bovine serum albumin. The mGluR5 binding sites were labeled using [^{3}H]MPEP (1 nM). The [^{3}H]MPEP was in a protease inhibitor cocktail consisting of 4 μg/mL leupeptin, 2 μg/mL chymostatin, and 10 μg/mL bestatin. The binding assays were carried out in 12 × 75 mm polystyrene tubes that were pre-filled with 50 μL of drug, 100 μL of 100 mM NaCl, and 100 μL of radioligand. The drugs were made up in ice-cold 50 mM Tris–HCl, pH 7.0, with 1% BSA. The radioligand was displaced by 10 concentrations of test drug three times. The experiment was initiated with the addition of 750 μL of the prepared membranes. Samples were incubated in a final volume of 1 mL, for 2.5 h (steady-state) at 4 °C. After incubation, the samples were filtered with a Brandel cell harvester over Whatman GF/B filters presoaked in wash buffer (ice-cold 10 mM Tris–HCl, pH 7.0) containing 0.5% poly(ethylenimine). The nonspecific binding was determined using 10 μM MPEP. Typical total and nonspecific cpms observed for the binding assays were 4000 and 800, respectively. The IC_{50} and slope factor (N) were obtained by using the program MLAB.
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