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Octahydropyrrolo[3,4-*c*]pyrrole negative allosteric modulators of mGlu₁

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Abstract

Development of SAR in an octahydropyrrolo[3,4-*c*]pyrrole series of negative allosteric modulators of mGlu₁ using a functional cell-based assay is described in this Letter. The octahydropyrrolo[3,4-*c*]pyrrole scaffold was chosen as an isosteric replacement for the piperazine ring found in the initial hit compound. Characterization of selected compounds in protein binding assays was used to identify the most promising analogs, which were then profiled in P450 inhibition assays in order to further assess the potential for drug-likeness within this series of compounds.

Keywords

Glutamate; GPCR; mGlu₁; Allosteric modulator; CNS; Octahydropyrrolo[3,4-*c*]pyrrole

L-Glutamic acid (glutamate) is the major excitatory neurotransmitter in the mammalian central nervous system (CNS). Activation of both ionotropic and metabotropic glutamate receptors occurs following binding to glutamate. The metabotropic glutamate receptors (mGlu_s) are members of family C within the broader G protein-coupled receptor (GPCR) family. The eight known mGlu_s have been further classified according to their structure, preferred signal transduction mechanisms, and pharmacology (Group I: mGlu₁ and mGlu₅; Group II: mGlu_{2,3}; Group III: mGlu₄₋₈).¹ The majority of these receptors have attracted the attention of researchers as potential therapeutic targets due to their association with a variety of CNS related disorders. Initially, work toward the design of drug-like orthosteric ligands that selectively bind a specific mGlu proved challenging. Perhaps this is not surprising, given that the orthosteric binding site across the mGlu family is highly conserved. A more recent approach that yielded more selective compounds has been the design and development of small molecules that modulate the activity of the receptor, either positively or negatively, through binding to an allosteric site.²

The design of selective small molecule negative allosteric modulators (NAMs) of mGlu₁ has been a fruitful area of research within the mGlu allosteric modulator field.³ Multiple tool compounds have been discovered during recent years, and their evaluation in behavioral models has further established a potential for therapeutic benefit in a number of CNS-related

disorders. Examples include addiction,⁴ anxiety,⁵ epilepsy,⁶ pain,^{5a,7} and psychotic disorders.^{5a,8} Recent publications have also noted a potential role for mGlu₁ inhibition in the treatment of melanoma⁹ and certain types of breast cancer.¹⁰ We recently reported our own initial efforts directed toward the discovery and optimization of structurally novel mGlu₁ NAMs.¹¹ In that Letter we described the discovery and characterization of VU0469650 through an optimization program based on hit compound **1**, which was identified through internal cross screening (Fig. 1).¹² VU0469650 is a potent mGlu₁ NAM as measured in our functional cell based assay, which measures the ability of the compound to block the mobilization of calcium by an EC₈₀ concentration of glutamate in cells expressing human mGlu₁.¹³

The SAR that was developed in the process leading up to the discovery of VU0469650 was primarily focused on evaluation of the amide and heteroaryl portion of the scaffold. Concomitant to that work our attention was also directed toward the design of scaffolds that replaced the piperazine ring of **1** altogether. One piperazine isostere of interest was the octahydropyrrolo[3,4-*c*]pyrrole. This particular ring system has been successfully employed in drug discovery research as an effective replacement for a piperazine ring in the past.¹⁴ Furthermore, the *N*-heteroaryl octahydropyrrolo[3,4-*c*]pyrrole chemotype has proven to be useful for the design of drug-like small molecules that interact with a number of CNS targets (Fig. 2). Examples include the muscarinic acetylcholine receptor M₁ (**2**),^{14a} the orexin receptor type 2 (**3**),¹⁵ the $\alpha 7$ nicotinic acetylcholine receptor (A-582941),¹⁶ and the cannabinoid receptor type 1 (**4**).¹⁷ The investigation of the octahydropyrrolo[3,4-*c*]pyrrole scaffold as a suitable chemotype for the development of novel mGlu₁ NAMs is the subject of this Letter.

As was the case with the piperazine scaffold exemplified by VU0469650, preparation of analogs within this series was relatively straightforward (Scheme 1).¹⁸ For evaluation of SAR around the amide portion of the chemotype (R¹), commercially available **5** was reacted with 2-fluoropyridine under S_NAr conditions to afford **6**. Acidic cleavage of the carbamate protecting group provided amine intermediate **7**, which was readily converted to the target amide compounds **8–19** using established methods. For evaluation of SAR around the aryl portion of the chemotype (R²), commercially available **20** was reacted with 1-adamantoyl chloride to afford intermediate **21**. Removal of the benzyl protecting group was accomplished through a palladium catalyzed hydrogenation.¹⁹ Conversion of amine **22** into target compounds **23–34** was achieved through nucleophilic aromatic substitution reactions with aryl fluorides or Buchwald–Hartwig²⁰ amination reactions with suitable aryl halides.

Direct replacement of the piperazine ring of **1** with the octahydropyrrolo[3,4-*c*]pyrrole group afforded **8**, which proved greater than seven fold more potent than **1** against human mGlu₁ (Table 1). Furthermore, the activity of **8** against rat mGlu₅ was much reduced relative to **1** (8 rmGlu₅ IC₅₀ = 3360 nM; % Glu Max = 1.1). Unfortunately, analogs with alternative amide groups to the 1-adamantyl amide proved much less potent, exhibiting only weak antagonism (**10–13**, **15**, **17**, and **19**) or were inactive (**9** and **16**) up to the top concentration of 30 μ M. Compounds **14** and **18** were exceptions; however, these analogs were sixty and 17-fold less potent than **8**, respectively. We had hoped that the cubyl amide of analog **19** might prove an adequate amide replacement as that group has been noted as a less lipophilic isosteric replacement for the adamantyl group.²¹ Unfortunately, such a modification resulted in a substantial loss of potency at mGlu₁.

Exploration of SAR around the aryl ring of the scaffold proved more fruitful (Table 2). While thiazole **23** and pyrimidines **24** and **25** were only weak antagonists, pyrazine **26** proved more potent, albeit eight fold less than **8**. Fluorination of **8** on the pyridine ring was tolerated with 6-fluoro analog **27** being equipotent to **8** and 5-fluoro analog **28** and 3-fluoro

analog **29** being six fold and 12-fold less potent than **8**, respectively. The 2-cyanophenyl ring (**30**) proved a reasonable alternative to the 2-pyridyl ring (**8**). Desiring to prepare less lipophilic compounds, various pyridine derivatives of **30** were prepared (**31–34**) with analogs **32** and **34** demonstrating moderate potency.

Having explored SAR around the amide and aryl portions of the chemotype with limited success, we turned our attention to a new strategy for replacement of the 1-adamantyl amide with substituted ureas (Scheme 2).²² Reaction of intermediate **7** with 4-nitrophenyl chloroformate afforded carbamate **35**. Treatment of **35** with cyclic secondary amines under microwave irradiation afforded analogs **36–42** (Table 3). This strategy produced three new analogs **38–40** with mGlu₁ IC₅₀ values less than one micromolar. Interestingly, each of these compounds contained similar spirocyclic amine moieties. 2-Azaspiro[4.4]nonane analog **38** was the most potent urea analog and only three fold less potent than **8**.

Having identified several interesting new compounds with good to moderate mGlu₁ activity, we examined them for their propensity to non-specifically bind to rat plasma proteins (Table 4).²³ Since such binding can limit the amount of drug available to interact with the target, absolute functional potency and protein binding are both important factors in the ultimate efficacy of a compound in vivo. The measured fraction unbound in rat plasma proteins generally tracked with the calculated logP values. More lipophilic compounds were more highly bound than less lipophilic compounds; however, pyrazine **26** was an apparent exception to this trend.²⁴ Taking into account both functional potency and fraction unbound in rat plasma, **8** and **38** were deemed two of our most interesting compounds. Examination of the ligand-lipophilicity efficiency (LLE)²⁵ values for these same analogs also places these two analogs among the most drug-like within this set. Given that we are primarily interested in the application of mGlu₁ NAMs for the treatment of CNS disorders, we also chose to further profile these two compounds by measuring their binding to rat brain homogenates. Gratifyingly, both compounds exhibited a greater fraction unbound in rat brain homogenates than VU0469650 ($F_u = 0.016$). These two compounds were also tested at 10 μ M in cell based functional assays for their selectivity against the other members of the mGlu family and both demonstrated good overall selectivity.^{26,27} Compound **8** was chosen as representative of the series and submitted to a commercially available radioligand binding assay panel of 68 clinically relevant GPCRs, ion channels, kinases, and transporters,²⁸ and only four significant responses were noted at a concentration of 10 μ M.²⁹

Wanting to further understand the potential for this chemotype to deliver molecules with drug-like properties and specifically any potential liabilities related to drug–drug interactions, we also profiled **8** and **38** in a cytochrome P450 inhibition assay (Table 5).³⁰ The profile of VU0469650 is pictured alongside for comparison. Both VU0469650 and **8** were moderate inhibitors of CYP3A4. The extent to which a drug inhibits CYP3A4 is a particularly important consideration since this isoform is responsible for the metabolism of approximately half of the drugs in clinical use.³¹ Fortunately, analog **38** proved to have a superior P450 inhibition profile, demonstrating no measurable inhibition up to the top concentration tested (30 μ M). Though the exact reason for this improved profile with **38** has not been conclusively determined, it may be related to the compounds reduced lipophilicity relative to the other two compounds.³² Reducing lipophilicity has been previously noted as a successful strategy for mitigating P450 inhibition in other chemotypes.³³

In conclusion, we have demonstrated that potent and selective mGlu₁ NAM compounds can be prepared within a series of octahydropyrrolo[3,4-*c*]pyrroles that were developed from a piperazine cross screening hit. Key SAR gleaned from this effort included mechanisms for increasing the fraction of unbound compound in the presence of plasma proteins and brain homogenates as well as for reducing P450 inhibition. Future plans for this series include

continued optimization of drug-like properties and subsequent evaluation of pharmacokinetics. Results and observations from such studies will be the subject of future communications.

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27. In the case of **8**, very weak and marginal activity was noted for mGlu₆₋₈ while no activity was observed at mGlu₂₋₄. A full CRC for **8** against mGlu₅ was run (IC₅₀ = 3.36 μ M). In the case of **38**, very weak and marginally significant activity was noted for mGlu₆ and mGlu₈ while no activity was observed at mGlu₂₋₄ and mGlu₇. A full CRC for **38** against mGlu₅ was run (IC₅₀ >10 μ M).
28. LeadProfilingScreen®, Eurofins Panlabs, Inc. (<http://www.eurofinspanlabs.com>)
29. Significant responses are defined as those that inhibited more than 50% of radioligand binding. In the case of **8**, the following significant responses were noted: human opiate κ (60%), human serotonin 5-HT₃ (90%), human sigma σ_1 (95%), and rat Na⁺ channel site 2 (52%).
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Figure 1.
mGlu₁ NAM initial hit **1** and tool compound VU0469650.

Figure 2.
Examples of small molecule octahydropyrrolo[3,4-*c*]pyrroles that interact with CNS targets.

Scheme 1.

Reagents and conditions: (a) R^2F , DIEA, NMP, μ wave, 180–250 °C; (b) HCl, MeOH, dioxanes; (c) R^1COCl , DIEA, CH_2Cl_2 ; (d) R^1CO_2H , HATU, DIEA, CH_2Cl_2 ; (e) ι -adamantoyl chloride, DIEA, CH_2Cl_2 ; (f) H-cube®, Pd/C, MeOH, 80 bar, 80 °C; (g) R^2X (X = Cl, Br, or I), $Pd_2(dba)_3$ or $Pd(OAc)_2$, Xantphos, $NaOtBu$ or Cs_2CO_3 , dioxanes, μ wave, 120 °C or thermal, 100 °C.

Scheme 2.

Reagents and conditions: (a) 4-nitrophenyl chloroformate, CH_2Cl_2 ; (b) HNR^1R^2 , NMP, μwave , 200°C .

Table 1

Amide SAR

Compound	R	mGlu ₁ pIC ₅₀ (±SEM) ^a	mGlu ₁ IC ₅₀ (nM)	% Glu Max (±SEM) ^{a,b}
8		7.07 ± 0.07	85	2.2 ± 0.3
9		<4.5	>30,000	—
10		<5.0 ^c	>10,000	57.7 ± 3.6
11		<5.0 ^c	>10,000	36.5 ± 13.2
12		<5.0 ^c	>10,000	14.9 ± 0.9
13		<5.0 ^c	>10,000	37.9 ± 4.9
14		5.29 ± 0.19	5140	9.3 ± 4.9

Compound	R	mGlu ₁ pIC ₅₀ (±SEM) ^a	mGlu ₁ IC ₅₀ (nM)	% Glu Max (±SEM) ^{a,b}
15		<5.0 ^c	>10,000	29.2 ± 2.6
16		<4.5	>30,000	—
17		<5.0 ^c	>10,000	47.6 ± 3.7
18		5.84 ± 0.18	1430	0.0 ± 1.5
19		<5.0 ^c	>10,000	58.7 ± 4.0

^a Calcium mobilization mGlu₁ assay; values are average of $n = 3$.

^b Amplitude of response in the presence of 30 μM test compound as a percentage of maximal response (100 μM glutamate); average of $n = 3$.

^c Concentration response curve (CRC) does not plateau.

Table 2

Aryl/heteroaryl SAR

Compound	R	mGlu ₁ pIC ₅₀ (±SEM) ^a	mGlu ₁ IC ₅₀ (nM)	% Glu Max (±SEM) ^{a,b}
8		7.07 ± 0.07	85	2.2 ± 0.3
23		<5.0 ^b	>10,000	56.6 ± 8.6
24		<5.0 ^c	>10,000	10.3 ± 7.5
25		<5.0 ^c	>10,000	25.5 ± 5.7
26		6.17 ± 0.21	669	-1.1 ± 1.8
27		7.03 ± 0.08	93	2.3 ± 0.2

Compound	R	mGlu ₁ pIC ₅₀ (±SEM) ^a	mGlu ₁ IC ₅₀ (nM)	% Glu Max (±SEM) ^{a,b}
28		6.32 ± 0.10	478	2.2 ± 1.2
29		6.00 ± 0.14	1000	2.1 ± 1.4
30		6.73 ± 0.07	185	6.9 ± 1.5
31		5.25 ± 0.14	5590	12.2 ± 8.4
32		6.08 ± 0.17	833	0.1 ± 0.5
33		<5.0 ^c	>10,000	36.8 ± 5.1
34		6.26 ± 0.05	553	1.3 ± 1.1

^a Calcium mobilization mGlu₁ assay; values are average of $n = 3$.

^b Amplitude of response in the presence of 30 μM test compound as a percentage of maximal response (100 μM glutamate); average of $n = 3$.

^c CRC does not plateau.

Table 3

Urea SAR

Compound	R	mGlu ₁ pIC ₅₀ (±SEM) ^a	mGlu ₁ IC ₅₀ (nM)	% Glu Max (±SEM) ^{a,b}
36		4.93 ± 0.07	11,900	-7.5 ± 14.6
37		<5.0 ^c	>10,000	23.2 ± 6.5
38		6.53 ± 0.12	297	-0.1 ± 0.6
39		6.11 ± 0.05	769	0.7 ± 0.5
40		6.44 ± 0.08	364	1.5 ± 0.4
41		5.29 ± 0.10	5160	-5.8 ± 3.8
42		5.72 ± 0.04	1910	-1.5 ± 1.1

^a Calcium mobilization mGlu₁ assay; values are average of *n* = 3.^b Amplitude of response in the presence of 30 μM test compound as a percentage of maximal response (100 μM glutamate); average of *n* = 3.^c CRC does not plateau.

Table 4

Protein binding results

Compound	<i>cLogP</i> ^a	mGlu ₁ IC ₅₀ (nM)	LLE ^b	Rat PPB (<i>F_u</i>) ^c	Rat BHB (<i>F_u</i>) ^c
8	4.28	85	2.79	0.029	0.023
26	4.96	669	1.21	0.103	–
27	4.53	93	2.50	0.012	–
28	4.44	478	1.88	0.015	–
30	4.55	185	2.18	0.014	–
34	3.39	553	2.87	0.045	–
38	2.41	297	4.12	0.090	0.051
40	3.73	364	2.71	0.015	–

^a Calculated using ADRIANA.Code (www.molecular-networks.com).

^b LLE (ligand-lipophilicity efficiency) = pIC₅₀–*cLogP*.

^c *F_u* = fraction unbound.

Table 5Inhibition of P450^{a,b}

P450	Compound		
	VU0469650	8	38
CYP3A4	7.0	6.2	>30
CYP2C9	>30	>30	>30
CYP2D6	12.4	24.3	>30
CYP1A2	>30	>30	>30

^a Assayed in pooled human liver microsomes in the presence of NADPH.^b IC₅₀ data in μ M.