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Discovery of molecular switches within the ADX-47273 $mGlu_5$ PAM scaffold that modulate modes of pharmacology to afford potent $mGlu_5$ NAMs, PAMs and partial antagonists

Jeffrey P. Lamb [a,c,†], Darren W. Engers [a,c,d,†], Colleen M. Niswender [a,c,d], Alice L. Rodriguez [a,c,d], Daryl F. Venable [a,c], P. Jeffrey Conn [a,c,d], Craig W. Lindsley [a,b,c,d,*]

[a] Department of Pharmacology, Vanderbilt University Medical Center, Nashville, TN 37232, USA
[b] Department of Chemistry, Vanderbilt University, Nashville, TN 37232, USA
[c] Vanderbilt Program in Drug Discovery, Nashville, TN 37232, USA
[d] Vanderbilt Specialized Chemistry Center(MLPCN), Nashville, TN 37232, USA

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ABSTRACT

This Letter describes a chemical lead optimization campaign directed at a weak $mGlu_5$ NAM discovered while developing SAR for the $mGlu_5$ PAM, ADX-47273. An iterative parallel synthesis effort discovered multiple, subtle molecular switches that afford potent $mGlu_5$ NAMs, $mGlu_5$ PAMs as well as $mGlu_5$ partial antagonists.

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The metabotropic glutamate receptor subtype 5 ($mGlu_5$) has become a prominent molecular target for a number of CNS pathologies.[1,2] mGlu5 negative allosteric modulators (NAMs) are being actively pursued for anxiety, pain, Parkinson's disease, cocaine addiction and Fragile X Syndrome, while $mGlu_5$ positive allosteric modulators (PAMs) are under development for the treatment of schizophrenia.[3–9] The prototypical $mGlu_5$ allosteric ligand is MPEP (**1**),[10] a NAM, and many allosteric ligands, both PAM and NAM, bind at the MPEP-site.[1–10] Recently, we reported on the discovery of molecular switches in a series of MPEP-site phenylethynyl pyrimidines in which incorporation of a single methyl group in either the 3- or 4-position converted an $mGlu_5$ partial antagonist lead **2** (IC_{50} = 486 nM, 71% partial) into either a NAM **3** (IC_{50} = 7.5 nM) or PAM **4** (EC_{50} = 3.3 μM, 4.2-fold shift), respectively (Fig. 1).[11] Further SAR identified additional, subtle molecular switches that afforded centrally penetrant and in vivo active $mGlu_5$ NAMs and PAMs.[12] After these key findings, we began to take note of pharmacology switches, and identified these in multiple $mGlu_5$ allosteric modulator scaffolds.[13,14] Interestingly, our initial SAR work in the $mGlu_5$ PAM ADX-47273 **5** series in 2009 produced potent PAMs, such as **6** (EC_{50} = 240 nM, 14-fold shift), and ago-PAMs such as **7** (EC_{50} = 170 nM, 20-fold shift), but only one weak NAM **8** (IC_{50} = 8.7 μM).[15] This was the first indication that pharmacology switching is possible in the ADX-47273 series by replacing an aryl amide, as in **6**, with a cyclobutyl amide in **8**.[15]

While we were exploring this finding, a manuscript appeared in 2010 describing the identification of racemic $mGlu_5$ NAM **9**, closely related to our NAM **8**, from an HTS screen, and the parallel synthesis of over 1300 analogs.[16] However, within this manuscript, there is little discussion of the impact of stereochemistry and *no* mention of pharmacology switching. Here, we present our SAR study, developed though an iterative parallel synthesis approach, that afforded potent $mGlu_5$ PAMs, NAMs and partial antagonists from subtle modifications to the ADX-47273 scaffold.

Our initial library evaluated two dimensions: stereochemistry at the 3-postion and replacement for the 2-pyridyl moiety while holding the cyclobutyl amide constant. In our earlier work in the ADX-47273 series,[15] the (*S*)-stereochemistry at the 3-position was essential for $mGlu_5$ PAM activity, and it was important to ascertain the stereochemical bias, if any, to produce NAMs. In the event, (*S*)-**10** was converted to the methyl ester **11**, followed by acylation to yield **12**. Saponification provides **13**, which is then

* Corresponding author.
E-mail address: craig.lindsley@vanderbilt.edu (C.W. Lindsley).
† These authors contributed equally to this work.

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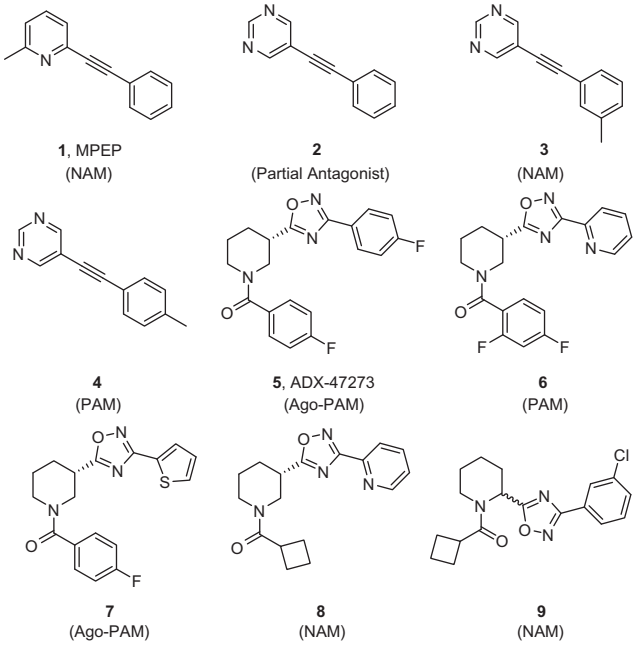
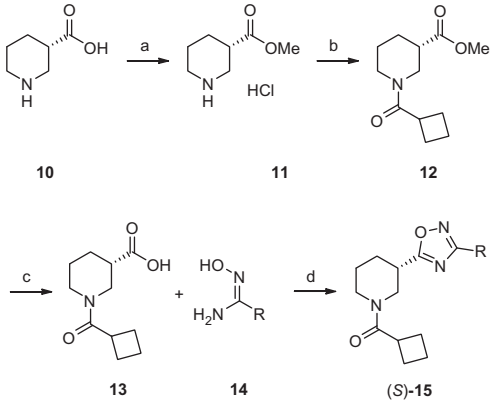


Figure 1. Structures of selected MPEP-site allosteric ligands that display a range of $mGlu_5$ pharmacology with subtle modifications.

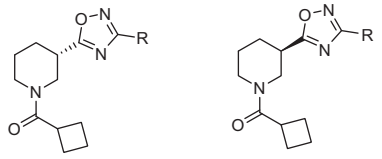
coupled to various (*Z*)-*N*'-hydroxylimidamides **14** and refluxed to deliver analogs (*S*)-**15** (Scheme 1). The analogous (*R*)-**15** congeners were made via the same scheme except (*R*)-**10** was used.

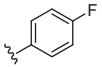
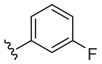
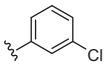
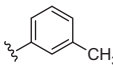
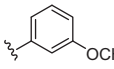
As shown in Table 1, the stereochemical preference we identified in our earlier PAM work in this series carried over into the NAM pharmacology with the (*S*)-enantiomer preferred, that is, (*S*)-**15e** (IC_{50} = 0.2 μM) versus (*R*)-**15e** (IC_{50} = 3.1 μM). Significantly, 3-substituted aryl congeners (*S*)-**15e–f**, proved most enlightening, affording submicromolar $mGlu_5$ NAMs, with in the case of (*S*)-**15e**, an ~41-fold increase in potency over **8**.[15] These data led us to consider if there is stereochemical bias for pharmacological mode of action within the **9** scaffold. Thus we prepared small, enantiopure libraries of analogs (*S*)-**20** and (*R*)-**20**, from either (*S*)-**16** and (*R*)-**16**, respectively, and evaluated them in our $mGlu_5$ assays (Scheme 2). As shown in Table 2, this effort found that both enantiomers afford comparable activity and mode of pharmacology. This library provided an efficacious submicromolar PAM (*S*)-**20c** (EC_{50} = 730 nM, 71% Glu Max) as well as several submicromolar NAMs ((*S*)- and (*R*)-**20e–f**) which also afforded a full blockade of the EC_{80}, and in the case of (*S*)-**20f**, an 77 nM NAM. Based on these data, our next round of library synthesis employed both the **20e** NAM scaffold and the **20c** PAM scaffold, and focused on evaluating other amide moieties beyond the cyclobutyl amide. These analogs **21** and **22** were readily prepared following a variation of Scheme 2.



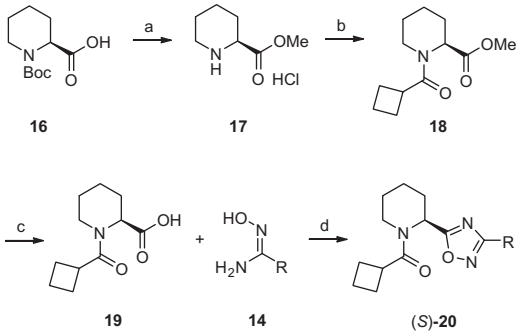
Scheme 1. Reagents and conditions: (a) $SOCl_2$, MeOH (99%), cylcobutane carbonyl chloride, DIEA, DCM (96%); (c) LiOH, THF, H_2O (95%); (d) EDCI, HOBt, DIEA, dioxane, reflux, 24 h (45–59%).

Table 1
Structures and activities of analogs (*S*)-**15** and (*R*)-**15**



Compd	R	Pharmacology	IC_{50}[a] (μM)	EC_{50}[a] (μM)	Glu Max[a] (%)
(*S*)-**15a**		NAM	9.3	NA	67
(*R*)-**15a**		Inactive	—	—	—
(*S*)-**15b**		Inactive	—	—	—
(*R*)-**15b**		Inactive	—	—	—
(*S*)-**15c**		NAM	>10	NA	33
(*R*)-**15c**		NAM	9.9	NA	19
(*S*)-**15d**		NAM	2.4	NA	31
(*R*)-**15d**		NAM	>10	NA	60
(*S*)-**15e**		NAM	0.2	NA	2.4
(*R*)-**15e**		NAM	3.1	NA	18
(*S*)-**15f**		NAM	0.7	NA	2.5
(*R*)-**15f**		NAM	4.7	NA	14
(*S*)-**15g**		NAM	1.8	NA	2.1
(*R*)-**15g**		NAM	>10	NA	54

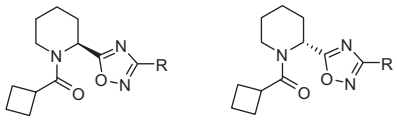
[a] Average of at least three independent determinations. NA, not applicable.



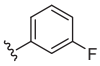
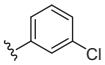
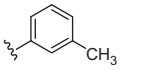
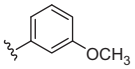
Scheme 2. Reagents and conditions: (a) $SOCl_2$, MeOH (99%), cylcobutane carbonyl chloride, DIEA, DCM (95%); (c) LiOH, THF, H_2O (95%); (d) EDCI, HOBt, DIEA, dioxane, reflux, 24 h (40–55%).

The library of **20e** analogs, **21a–i**, afforded both NAMs and partial antagonists,[17] with no evidence of PAM activity (Table 3). Interestingly, the three and five-membered saturated ring amides **21a** and **21c**, afforded partial antagonists, while the four and six-membered saturated ring amides **21b** and **21d** afforded full non-competitive antagonists (NAMs). In contrast, the library of **20c** analogs, **22a–i**, afforded predominantly PAMs and ago-PAMs. For example, **22a** proved to be a potent (EC_{50} = 78 nM, 70% Glu Max) $mGlu_5$ PAM, more potent than the previous PAMs **6** and **7** we

Table 2
Structures and activities of analogs (*S*)-**20** and (*R*)-**20**

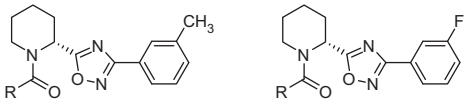


(*S*)-**20** (*R*)-**20**

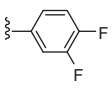
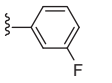
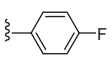
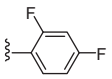
Compd	R	Pharmacology	IC_{50}[a] (μM)	EC_{50}[a] (μM)	Glu Max[a] (%)
(*S*)-**20a**		NAM	2.6	NA	11
(*R*)-**20a**		NAM	3.5	NA	7
(*S*)-**20b**		NAM	10	NA	33
(*R*)-**20b**		Inactive	—	—	—
(*S*)-**20c**		PAM	NA	0.7	71
(*R*)-**20c**		PAM	NA	0.6	37
(*S*)-**20d**		NAM	0.9	NA	6
(*R*)-**20d**		NAM	10	NA	38
(*S*)-**20e**		NAM	0.5	NA	3
(*R*)-**20e**		NAM	0.3	NA	6
(*S*)-**20f**		NAM	0.08	NA	1.8
(*R*)-**20f**		NAM	0.3	NA	0.5

[a] Average of at least three independent determinations. NA, not applicable.

Table 3
Structures and activities of analogs **21** and **22**



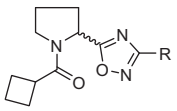
21 **22**

Compd	R	Pharmacology	IC_{50}[a] (μM)	EC_{50}[a] (μM)	Glu Max[a] (%)
21a		Part. Antag.	0.28	NA	16
21b		Ago-PAM	NA	0.08	70
21b/20c		NAM	0.37	NA	5.8
22b/20e		PAM	NA	0.65	38
21c		Part. Antag.	0.6	NA	24
22c		Ago-PAM	NA	0.31	80
21d		NAM	2.5	NA	9.2
22d		PAM	NA	3.5	58
21e		NAM	0.73	NA	0.9
22e		NAM	10	NA	34
21f		NAM	10	NA	46
22f		PAM	NA	1.4	93
21g		NAM	4.7	NA	16
22g		PAM	NA	0.46	59
21h		NAM	10	NA	47
22h		PAM	NA	2.3	91
21i		Inactive	—	—	—
22i		PAM	NA	2.9	95

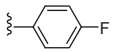
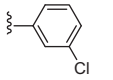
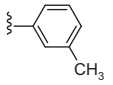
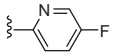
[a] Average of at least three independent determinations. NA, not applicable.

developed in the ADX-47273 series.[15] In addition, we observed an interesting trend here with the 3- and 5-membered saturated ring amides **22a** and **22c** affording ago-PAMs, while the 4- and 6-membered saturated ring amides **22b** and **22d** displaying pure PAM activity. Again, very subtle perturbations to the core scaffold engender opposing modes of $mGlu_5$ pharmacology.

Table 4
Structures and activities of analogs **23**

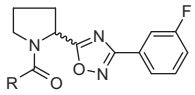


23

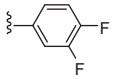
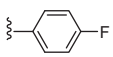
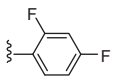
Compd	R	Pharmacology	IC_{50}[a] (μM)	EC_{50}[a] (μM)	Glu Max[a] (%)
23a		PAM	NA	1.5	64
23b		PAM	NA	2.7	72
23c		NAM	10	NA	25
23d		NAM	2.4	NA	34
23e		Inactive	NA	NA	NA
23f		Inactive	NA	NA	NA
23g		Inactive	NA	NA	NA

[a] Average of at least three independent determinations. NA, not applicable.

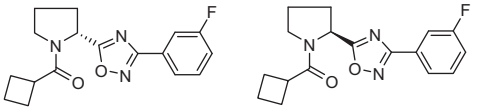
Table 5
Structures and activities of analogs **24**



24

Compd	R	Pharmacology	IC_{50}[a] (μM)	EC_{50}[a] (μM)	Glu Max[a] (%)
24a/ 23a		PAM	NA	2.9	68
24b		PAM	NA	1.5	63
24c		PAM	NA	1.0	75
24d		PAM	NA	10	8
24e		PAM	NA	0.43	60
24f		PAM	NA	3.7	62
24g		PAM	NA	2.6	47
24h		PAM	NA	3.6	59
24i		PAM	NA	10	61

[a] Average of at least three independent determinations. NA, not applicable.



(*R*)-**23a** (*S*)-**23a**

$mGlu_5$ EC_{50} = 530 nM, 68.4% Glu Max

$mGlu_5$ EC_{50} 7,000 nM, 57.3% Glu Max

Figure 2. Resolved enantiomers of **23a**. All the $mGlu_5$ PAM activity resides in the (*R*)-enantiomers, (*R*)-**23a**.

At this point, we elected to examine the impact of contracting the piperidine ring to a pyrrolidine ring while maintaining the original cyclobutyl amide and surveying a diverse group of substituents on the oxadiazole ring. This initial library employed racemic proline to afford racemic analogs **23**, following a variation of Scheme 2. As shown in Table 4, this modification afforded inactive compounds, weak NAMs (IC_{50}s ~10 μM) and two low micromolar PAMs (**23a** and **23b**). Based on these data, we made a second generation library holding constant the 3-fluorobenzene moiety of **23a**, and surveyed a diverse collection of amide moieties to replace the cyclobutyl group. As shown in Table 5, this effort afforded predominantly pure PAMs **24** with a range of potencies and efficacies. To address the role of stereochemical preference, we separated racemic **23a** into pure enantiomers (*S*)-**23a** and (*R*)-**23a** by chiral SFC. In this case, (*R*)-**23a** is a potent $mGlu_5$ PAM (EC_{50} = 530 nM) while (*S*)-**23a** is a very weak PAM (EC_{50} = 7000 nM) (Fig. 2). Note, this is the opposite stereochemical preference observed within the 3-piperidinyl-based $mGlu_5$ PAMs **5**–**7**.[15]

In summary, an iterative parallel synthesis optimization approach for our weak $mGlu_5$ NAM **8**, identified multiple regioisomeric and stereochemical 'molecular switches' that modulated modes (NAM, partial antagonist, PAM, ago-PAM) of $mGlu_5$ pharmacology. From **8** (IC_{50} = 8.7 μM), potent PAMs (EC_{50} = 78–200 nM) and NAMs (IC_{50} = 77–400 nM), were developed. In many cases, the perturbations in structure were subtle which led to opposing modes of pharmacology and suggests subtle conformational changes within the GPCR either facilitate or prohibit coupling to the G-protein. These data, coupled with our earlier work in a structurally distinct MPEP-site scaffold **2**, suggests that metabolites of MPEP-site allosteric ligands must be characterized, as metabolites may engender opposing modes of $mGlu_5$ modulation. Additional studies with these ligands, as well as their metabolites, are in progress and will be reported in due course.

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