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Efficacy switching SAR of mGluR5 allosteric modulators: Highly potent positive and negative modulators from one chemotype

Anette Graven Sams [a], Gitte Kobberøe Mikkelsen [a], Robbin M. Brodbeck [b], Xiaosui Pu [b], Andreas Ritzén [a,*]

[a] Discovery Chemistry and DMPK, H. Lundbeck A/S, Ottiliavej 9, DK-2500 Valby, Denmark
[b] ST-1 Glutamate Biology, Lundbeck Research USA, Inc., 215 College Road, Paramus, NJ 07652-1431, USA

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ABSTRACT

A series of metabotropic glutamate 5 receptor (mGluR5) allosteric ligands with positive, negative or no modulatory efficacy is described. The ability of this series to yield both mGluR5 PAMs and NAMs with single-digit nanomolar potency is unusual, and the underlying SAR is detailed.

Glutamate is the principal excitatory neurotransmitter in the mammalian central nervous system, where it mediates its effects through interaction with ionotropic and metabotropic receptors. The metabotropic glutamate receptors (mGluRs) are class C GPCRs and eight subtypes are known. They are divided into three groups based on their preferred G protein coupling and pharmacological responses to various ligands: 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] These receptors contain two distinct domains; a large extracellular domain which binds glutamate at the orthosteric binding site, and a heptahelical transmembrane domain, which has been found to bind a variety of ligands at one or more allosteric binding sites.[2] Receptor activation by glutamate binding can be positively or negatively modulated or be left unaffected by the binding of ligands to an allosteric site.

The mGluR5 has been suggested to be involved in a number of disease states, and both inhibition and activation of this receptor could have therapeutic benefits. The anxiolytic effect of the mGluR5 negative allosteric modulator (NAM) fenobam **1** (see Fig. 1) has been demonstrated in a phase II clinical trial,[3] and mGluR5 NAMs have been proposed as treatment of, for example, fragile X mental retardation,[4–6] Parkinson's disease[7–9] and gastroesophageal reflux disease.[10,11] Activation of mGluR5 has been postulated to ameliorate both positive symptoms and cognitive deficits in schizophrenia.[12–18]

We previously reported the discovery of a novel class of mGluR5 positive allosteric modulators (PAMs), exemplified by compound **2**.[19] Interestingly, we have now observed the phenomenon of efficacy switching in this compound series, from positive to negative modulation or even allosteric bindning without modulation,[20] resulting from small structural variations. We propose the term neutral allosteric binder (NAB) to denote a compound that binds to an allosteric site with no effect on receptor signaling. Such compounds can only be identified by a combination of functional and binding assays. Four new analogs of **2** were prepared by methods similar to those reported previously, see Table 1. Even from this small sample of compounds, it was observed that efficacy switching from PAM to NAM and NAB results from variations of either the R or the left-hand side Ar moieties. Similar efficacy switching has been observed for other allosteric modulators of mGluR5, both MPEP-derived[21] and with other scaffolds.[22–26]

Seeking to find more potent PAMs and NAMs, we hypothesized that locking the amide in the bioactive conformation would reduce the binding entropy penalty of the ligand thereby increasing its potency. Because the bioactive conformation was not known to us, we substituted the global energy minimum conformation and introduced a conformational restraint into scaffold **2** by cyclization. The SAR at both ends of the scaffold was elaborated, both in terms of potency and efficacy. To this end, a parallel synthesis

* Corresponding author.
E-mail address: arit@lundbeck.com (A. Ritzén).

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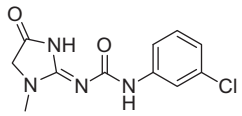
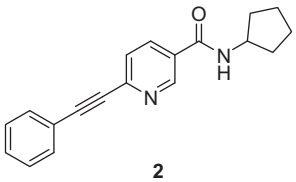


Fenobam, **1**

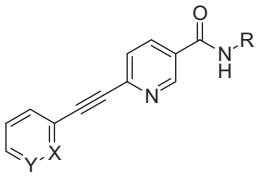


2

Figure 1. Structures of the mGluR5 NAM fenobam **1** and PAM **2**.

protocol was devised for the preparation of the compounds in Table 2, see Scheme 1. To prepare the intermediates **14a–d**, ethyl 2-chloronicotinate **7** was vinylated in a Stille reaction to yield **8**.[27] This intermediate was reacted with ammonium chloride and acetic acid, or with primary amines to directly yield the cyclized products **9** or **10b–d**, while **10a** was obtained from methylation of **9** using NaH and MeI. Next, treatment with *m*-CPBA gave the N-oxides **11a–d**, which were transformed into the 2-chloro-dihydronaphthyridinones **12a–d** through reaction with $POCl_3$. A Sonogashira coupling reaction with trimethylsilylacetylene

Table 1



2: R = cyclopentyl, X = Y = CH
3: R = Me, X = Y = CH
4: R= *i*-Pr, X = Y = CH
5: R = cyclopentyl, X = N, Y = CH
6: R = cyclopentyl, X = CH, Y = N

	Efficacy	IC_{50}[a] (nM)	I_{max}[a] (%)	EC_{50}[a] (nM)	E_{max}[a] (%)	K_i[b] (μM)
2	PAM			19 ± 2	106 ± 10	3.3 ± 1.6
3	NAB					15 ± 6
4	PAM			62 ± 11	61 ± 9	7.1 ± 2.2
5	NAM	227 ± 26	90 ± 3			7.6 ± 1.1
6	PAM			>10000	68 ± 14	>32

[a] FLIPR functional assay, see Supplementary data for details. Data given as mean value ± SEM of four to five experiments. I_{max} refers to percent inhibition of mGluR5 L-Glu EC_{80} response at the highest tested concentration of the NAM (10 μM). E_{max} refers to percent stimulation (relative to the response at saturating L-Glu concentration in the absence of modulator) of mGluR5 L-Glu EC_{20} response at the highest tested concentration of the PAM (10 μM).

[b] [^{3}H]-MPEP binding assay, see Supplementary data for details. Data given as mean value ± SEM of four experiments.

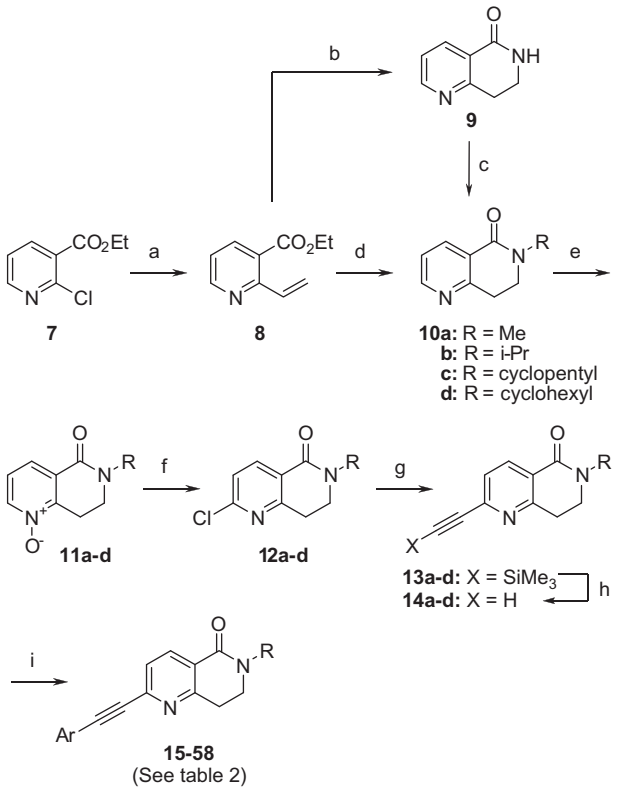
Table 2

Compound	R	Ar	Efficacy	IC_{50}[a] (nM)	I_{max}[a] (%)	EC_{50}[a] (nM)	E_{max}[a] (%)	K_i[b] (μM)
15	Me	Phenyl	NAM	30 ± 1	58 ± 3			0.76 ± 0.09
16	Me	*o*-Tolyl	NAM	776 ± 409	80 ± 3			>32
17	Me	*m*-Tolyl	NAM	2.9 ± 0.4	86 ± 2			0.82 ± 0.60
18	Me	*p*-Tolyl	NAM	1401 ± 409	68 ± 5			7.45 ± 2.24
19	Me	2-Chlorophenyl	NAM	916 ± 223	70 ± 3			7.90 ± 1.27
20	Me	3-Chlorophenyl	NAM	22 ± 17	84 ± 3			0.25 ± 0.15
21	Me	4-Chlorophenyl	NAM	352 ± 161	73 ± 5			6.15 ± 2.63
22	Me	3-Methoxyphenyl	NAM	293 ± 76	88 ± 2			3.66 ± 0.88
23	Me	2-Pyridyl	NAM	184 ± 29	92 ± 2			6.24 ± 2.52
24	Me	3-Pyridyl	NAB					27.38 ± 4.22
25	Me	2-Thienyl	NAM	451 ± 165	49 ± 6			2.17 ± 0.98
26	*i*-Pr	Phenyl	NAM	706 ± 245	60 ± 3			1.28 ± 0.45
27	*i*-Pr	*o*-Tolyl	NAM	810 ± 171	76 ± 4			>32
28	*i*-Pr	*m*-Tolyl	NAM	4.8 ± 1.4	88 ± 1			0.51 ± 0.25
29	*i*-Pr	*p*-Tolyl	PAM			1012 ± 380	28 ± 7	20.18 ± 6.99
30	*i*-Pr	2-Chlorophenyl	NAM	2517 ± 690	61 ± 5			5.68 ± 0.67
31	*i*-Pr	3-Chlorophenyl	NAM	5.7 ± 2.5	86 ± 2			0.10 ± 0.03
32	*i*-Pr	4-Chlorophenyl	NAM	4228 ± 808	61 ± 6			27.14 ± 4.46
33	*i*-Pr	3-Methoxyphenyl	NAM	111 ± 14	88 ± 1			2.95 ± 0.72
34	*i*-Pr	2-Pyridyl	NAM	27 ± 4	89 ± 2			2.74 ± 1.31
35	*i*-Pr	3-Pyridyl	NAB					19.55 ± 7.39
36	*i*-Pr	2-Thienyl	NAM	45 ± 3	78 ± 2			1.93 ± 0.71
37	Cyclopentyl	Phenyl	PAM			5.9 ± 2.5	109 ± 15	0.89 ± 0.23
38	Cyclopentyl	*o*-Tolyl	PAM			239 ± 55	107 ± 13	18.79 ± 4.34
39	Cyclopentyl	*m*-Tolyl	PAM			7.5 ± 3.2	97 ± 13	0.67 ± 0.09
40	Cyclopentyl	*p*-Tolyl	PAM			4816 ± 1006	97 ± 18	>32
41	Cyclopentyl	2-Chlorophenyl	PAM			393 ± 65	115 ± 13	>32
42	Cyclopentyl	3-Chlorophenyl	PAM			33 ± 11	87 ± 4	5.23 ± 1.71
43	Cyclopentyl	4-Chlorophenyl	PAM			979 ± 206	98 ± 14	>32
44	Cyclopentyl	3-Methoxyphenyl	PAM			462 ± 77	25 ± 3	>32
45	Cyclopentyl	2-Pyridyl	NAM	199 ± 48	80 ± 2			1.77 ± 0.63
46	Cyclopentyl	3-Pyridyl	PAM			748 ± 170	68 ± 10	>32
47	Cyclopentyl	2-Thienyl	PAM			12 ± 3	103 ± 13	2.84 ± 0.50
48	Cyclohexyl	Phenyl	PAM			40 ± 5	122 ± 13	12.43 ± 6.65
49	Cyclohexyl	*o*-Tolyl	PAM			466 ± 18	62 ± 10	>32
50	Cyclohexyl	*m*-Tolyl	PAM			36 ± 16	54 ± 6	3.21 ± 2.52
51	Cyclohexyl	*p*-Tolyl	NAB					27.15 ± 4.45
52	Cyclohexyl	2-Chlorophenyl	PAM			924 ± 369	68 ± 8	>32
53	Cyclohexyl	3-Chlorophenyl	PAM			171 ± 81	47 ± 7	12.02 ± 6.55
54	Cyclohexyl	4-Chlorophenyl	[c]					>32
55	Cyclohexyl	3-Methoxyphenyl	NAB					23.15 ± 4.88
56	Cyclohexyl	2-Pyridyl	NAM	518 ± 128	74 ± 2			2.24 ± 0.76
57	Cyclohexyl	3-Pyridyl	PAM			387 ± 106	73 ± 7	15.61 ± 5.35
58	Cyclohexyl	2-Thienyl	PAM			13 ± 3	112 ± 14	0.72 ± 0.24

[a] FLIPR functional assay, see Supplementary data for details. Data given as mean value ± SEM of four to five experiments. I_{max} refers to percent inhibition of mGluR5 L-Glu EC_{80} response at the highest tested concentration of the NAM (10 μM). E_{max} refers to percent stimulation (relative to the response at saturating L-Glu concentration in the absence of modulator) of mGluR5 L-Glu EC_{20} response at the highest tested concentration of the PAM (10 μM).

[b] [^{3}H]-MPEP binding assay, see Supplementary data for details. Data given as mean value ± SEM of four experiments.

[c] Inactive in functional assays and without affinity for the MPEP binding site.



Scheme 1. Reagents and conditions: (a) vinyl–$SnBu_3$, $Pd(PPh_3)_2Cl_2$, 2,6-di-*t*-Bu-4-Me-phenol, DMF, 55 °C, 90%; (b) NH_4Cl, AcOH, H_2O, reflux, 32%; (c) NaH, MeI, DMF, 91%; (d) RNH_2, 50 °C, 68–74%; (e) *m*-CPBA, $CHCl_3$, 5–20 °C, 88–99%; (f) $POCl_3$, 120 °C, 12–27%; (g) TMS-acetylene, $Pd(dppf)Cl_2$, CuI, DMF, NEt_3, 80–98%; (h) NaOH, MeOH, H_2O, 64–85%; (i) ArI, $(PPh_3)_2PdCl_2$, CuI, NEt_3 or *i*-Pr_2NEt, THF, 60 °C.

afforded the intermediates **13a–d**, which were transformed into **14a–d** by desilylation under basic conditions. These building blocks were coupled with eleven different aryl halides under Sonogashira conditions in a library format to yield the desired target compounds. Functional and binding data are summarized in Table 2, and functional data is presented graphically in Figure 2.

A very clear trend towards NAM efficacy for the lower alkyl R groups and PAM efficacy for the cycloalkyl R groups was observed. The efficacy SAR of the Ar group was in most cases dominated by that of the R group, but the 2-pyridyl and 3-pyridyl groups were in some cases able to override the influence of the R group. Interestingly, the effects of these two Ar groups were opposite, with 2-pyridyl tending to turn PAMs into NAMs, for example, **37** versus **45** and **48** versus **56**, while 3-pyridyl turned NAMs into NABs, see **15** versus **24** and **26** versus **35**. Similar but less pronounced was the influence of 3-methoxyphenyl, which lowered the maximum efficacy of a PAM in one case (**37** vs **44**) and turned a PAM into a NAB in another (**48** vs **55**). The data in Table 1 suggests that this trend for the Ar group also holds for the original series of analogues of **2**, while the R group does not seem to play the same role there.

Gratifyingly, both NAMs and PAMs with functional IC_{50} or EC_{50} below 10 nM were identified, for example, **17**, **28**, **31**, **37** and **39**. For the NAMs, there was little or no difference in potency between R = Me and R = *i*-Pr, but the Ar group had a very large influence with *m*-tolyl and 3-chlorophenyl yielding the most potent compounds. Among the PAMs, the preferred R group was cyclopentyl and for the Ar group, Ph, *m*-tolyl and 2-thienyl gave the most potent compounds, while *p*-tolyl and 4-chlorophenyl gave the lowest potency. In particular, for **54** the EC_{50} was above the detection limit of the assay, and this compound also did not displace [^{3}H]-MPEP at 32 μM, the highest concentration tested.

In order to detect NABs, and to establish that the compounds bind to the same site on the receptor as the prototypical NAM MPEP, all compounds were also tested in a radioligand displacement binding assay. In general, binding K_i values were much higher than the functional potency (IC_{50} or EC_{50}), and not perfectly correlated. This discrepancy has been observed in the literature[28] but it is to the best of our knowledge not completely understood. Our working hypothesis is that the discrepancy is based, in part, on partially overlapping but not identical binding sites between our test compounds and [^{3}H]-MPEP. The use of an alternative radioligand closer to the structure of the test compounds would likely clarify this debate. At the highest concentration tested (32 μM) many, but not all, compounds completely displaced [^{3}H]-MPEP. Based on Hill slopes near unity and the lack of evidence to the contrary, we believe that all compounds would achieve full displacement if high enough concentrations were to be tested.

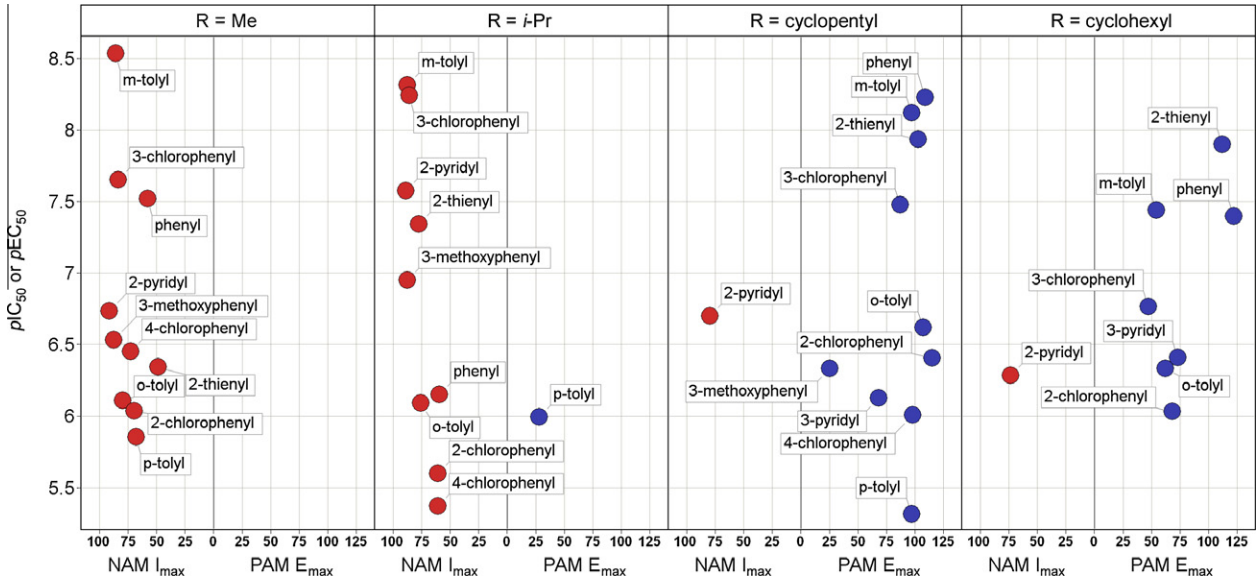


Figure 2. Functional response of compounds **15**–**58**. NAMs are shown in red; PAMs in blue. For explanation of I_{max} and E_{max}, see legend of Table 1.

The ability of a chemical series to yield both mGluR5 PAMs and NAMs of such high potency is unusual although not unprecedented.[25] Most reported cases of efficacy switching within one chemotype have provided high potency NAMs with a few examples of moderately potent PAMs.[22–24] In one case, optimization from a NAM screening hit to a moderately potent PAM was possible.[26] In general, there is a scarcity of high potency mGluR5 PAMs in the literature, and the two most potent such compounds in the present report, **37** (EC_{50} = 5.9 nM) and **39** (EC_{50} = 7.5 nM), are among the most potent mGluR5 PAMs reported to date.[29]

The clear and predictable efficacy SAR trend of the present series dominated by the R group appears to be unusual for allosteric modulators of the mGluR5 receptor. In contrast, literature precedence has often highlighted the unpredictable nature of efficacy switching as a function of small structural changes.[25,26]

In conclusion, we have discovered an improved series of allosteric mGluR5 modulators that provided both PAMs and NAMs of excellent potency. The efficacy and potency SAR has been outlined.[30]

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Supplementary data

Supplementary data (these data include experimental details for the synthesis of compounds **3–58** and descriptions of functional and binding assays) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.03.103.

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