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Optimization of an ether series of mGlu₅ positive allosteric modulators: Molecular determinants of MPEP-site interaction crossover

Jason T. Manka^{a,b,c}, Paige N. Vinson^{a,b}, Karen J. Gregory^{a,b,i}, Ya Zhou^{a,b,c}, Richard Williams^{a,b,c}, Kiran Gogia^{a,b}, Emily Days^{a,e}, Satya Jadhav^{a,b}, Elizabeth J. Herman^{a,b}, Hilde Lavreysen^f, Claire Mackie^g, José M. Bartolomé^h, Gregor J. Macdonald^f, Thomas Steckler^f, J. Scott Daniels^{a,b,c}, C. David Weaver^{a,e}, Colleen M. Niswender^{a,b,c}, Carrie K. Jones^{a,b,c,j}, P. Jeffrey Conn^{a,b,c}, Craig W. Lindsley^{a,b,c,d}, and Shaun R. Stauffer^{a,b,c,d,*}

^aDepartment of Pharmacology, Vanderbilt University Medical Center, Nashville, TN 37232, USA

^bVanderbilt Center for Neuroscience Drug Discovery, Vanderbilt University Medical Center, Nashville, TN 37232, USA

^cVanderbilt Specialized Chemistry Center for Probe Development (MLPCN), Nashville, TN 37232, USA

^dDepartment of Chemistry, Vanderbilt University, Nashville, TN 37232, USA

^eVanderbilt Institute of Chemical Biology, Vanderbilt University, Nashville, TN 37232, USA

^fNeuroscience, Janssen Research and Development, Turnhoutseweg 30, B-2340 Beerse, Belgium

^gCREATE ADME/Tox, Janssen Research and Development, Turnhoutseweg 30, B-2340 Beerse, Belgium

^hNeuroscience Medicinal Chemistry, Janssen Research and Development, Jarama 75, 45007-Toledo, Spain

ⁱDrug Discovery Biology, MIPS & Dept. of Pharmacology, Monash University, Parkville, VIC, Australia

^jTennessee Valley Healthcare System, U.S. Department of Veterans Affairs, Nashville, TN 37212, USA

Abstract

We report the optimization of a series of non-MPEP site metabotropic glutamate receptor 5 (mGlu₅) positive allosteric modulators (PAMs) based on a simple acyclic ether series. Modifications led to a gain of MPEP site interaction through incorporation of a chiral amide in conjunction with a nicotinamide core. A highly potent PAM, **8v** (VU0404251), was shown to be efficacious in a rodent model of psychosis. These studies suggest that potent PAMs within topologically similar chemotypes can be developed to preferentially interact or not interact with the MPEP allosteric binding site.

Keywords

Metabotropic glutamate receptor 5; mGlu₅; Positive allosteric modulator (PAM); Non-MPEP

The development of positive allosteric modulators (PAMs) of mGlu₅^{1–4} in an effort to test the NMDA hypofunction⁵ for schizophrenia has provided robust preclinical validation in multiple psychosis and cognition models.^{6–12} Allosteric mechanisms of receptor modulation may provide several advantages over orthosteric-based strategies, including increased receptor subtype selectivity, improved chemical tractability, and reduced receptor sensitization.^{13–15} Recent studies using the mGlu₅ MPEP-site PAM CDPPB (Fig. 1, 1)¹⁶ have shown that, upon sub-chronic dosing, tolerance develops in sleep architecture endpoints in rats. In parallel, mGlu₅ expression levels are reduced, although modest and transiently as noted upon removal of drug. This observation raises interesting mechanistic questions in regard to sensitization when administering a PAM, including whether or not these effects are metabolite mediated (e.g., ‘molecular switches’),^{9,17–21} receptor residence time dependent, allosteric site dependent, or inherent to a particular scaffold.

In an attempt to understand differential binding site-dependent properties of mGlu₅ PAMs in mGlu₅ signaling, we sought to identify structurally diverse PAMs that had the potential to interact with allosteric sites other than the classical MPEP site (Fig. 1, 1–4).^{1–5,8–14,16} Currently, the in vivo profile of a true non-MPEP site mGlu₅ PAM has yet to be identified.

Poor physicochemical properties and a lack of adequate potency against the rat mGlu₅ receptor have prevented the non-MPEP PAM CPPHA (**5**) from being pursued in preclinical animal models.^{22,23} Fortunately, through an HTS campaign in these labs, a second non-MPEP site PAM, VU0357121 (**6**),²⁴ was identified as a potential starting point for non-MPEP site PAM optimization; however, VU0357121 (**6**) itself was found not suitable for further testing in vivo.²⁵ PAM **6** shares the common amide backbone structure found in CPPHA (**5**). Alternatively, the ether linkage of **6** can be envisioned as an acetylene replacement (Figs. 1 and 2) related to PAMs like **3** (VU0360172).¹⁰ Interestingly, despite their structural resemblances and similar functional EC₅₀s in calcium mobilization assays expressing the rat mGlu₅ receptor, VU0357121 (**6**) was not observed to displace the MPEP-site ligand [³H]methoxyPEPy.²⁴ Although discovered and optimized within separate academic and lead optimization campaigns, we were intrigued to understand if through directed SAR we might identify the major molecular determinants responsible for governing MPEP versus non-MPEP allosteric site interactions of these respective mGlu₅ PAMs.^{26,27} Here, we describe the synthesis and SAR of a series of non-MPEP and MPEP site mGlu₅ PAMs based on a simple acyclic ether scaffold and propose the major molecular determinants required for MPEP-site interaction by examining hybrid molecules containing variations on the western ether group, core aromatic ring, and amide sub-structure (Fig. 2).

Ethers **7–8** were prepared using one of two three step sequences (Scheme 1) starting from either 4-bromo methylbenzoate (phenyl analogs **7**) or from methyl 6-chloronicotinate (nicotinamide analogs **8**). Saponification of **9**, followed by amide coupling to give intermediate **10** and a late stage etherification using either S_NAr or Cu-mediated Ullman conditions provided ethers **7–8**. Alternatively, etherification can be committed early in the synthesis to provide intermediate **11**, which upon ester hydrolysis and amide coupling allowed for late stage incorporation of diverse amides to furnish final benzamide or nicotinamide ethers **7–8**.

Initial efforts focused on holding the *n*-butyl ether tail of **6** (VU0357121) constant while changing the core structure as either phenyl or nicotinamide while surveying a number of

eastern amides (selected analogs, Table 1). Functional EC₅₀s in calcium mobilization assays using HEK293 cells expressing the rat mGlu₅ receptor were employed in addition to single concentration evaluation of [³H]methoxyPEPy binding to evaluate MPEP site interaction.¹⁰

Similar to the non-MPEP site PAM CPPHA (**5**),²³ SAR was extremely flat, with this first generation library (over 48 analogues per scaffold) affording no improvements in PAM activity over **6**. Within the phenyl core series, cycloalkyls **7a**, **7c** resulted in a >15-fold loss in functional activity. Heterocycles, including close structural analogs of **6**, such as trisubstituted pyridyl amide **7b**, proved to be deleterious for activity. Capping the NH amide of **6** to afford the *N*-methyl congener **7d** also had a negative impact on activity and revealed a strict requirement for the secondary amide. Interestingly, the observed amide SAR contrasts with previous SAR within the phenyl acetylene templates in which both secondary and tertiary amides were equally tolerated.^{10,20,21,28} The nicotinamide core modification to give **8a** resulted in 22-fold loss in activity versus **6**; however, the cyclohexyl replacement **8b** was comparable in activity to **8a** within the context of the nicotinamide core. Overall, amongst the *n*-butyl ethers examined, little MPEP site interaction was observed; although perhaps a weak trend for interaction with cyclohexyl derivatives **7a** and **8b** was observed (12–27% displacement).

In parallel, studies were conducted focusing on modification of the western ether tail (Table 2, **8d–k**), examining alkyl group tether length, branching, and incorporating western aromatic moieties similar to the pendant aryl groups utilized within the known acetylenic PAM chemotypes.^{10,20,21,28} In order to mitigate potential plasma esterase activity for secondary anilido amides^{29,30} such as **6** and **8a**, the western ether survey was conducted using a cyclohexyl amide in conjunction with the more polar nicotinamide core heterocycle (Table 2). Among the analogs tested, three showed submicromolar PAM activity, including the homolog pentyl ether **8d** which was fivefold more potent versus **8b** (EC₅₀ = 449 nM), cyclopentylmethyl ether **8j** (EC₅₀ = 446 nM), and the benzyl ether **8k** (EC₅₀ = 34 nM). Shortening of the tail to propyl analog **8e** resulted in complete loss of potentiation, indicating that pentyl was the optimal chain length for potency. Branching at the alpha carbon was unproductive (**8f**); however, branching one additional carbon removed at the beta carbon modestly improved potency twofold (racemic **8g**, EC₅₀ = 1076 nM). On the basis of this result, ring constraints were incorporated. The cyclopropyl methyl derivative **8i** unfortunately proved inactive; however, the cyclopentyl methyl ether **8j** maintained good potency (EC₅₀ = 446 nM). A limited binding study was conducted for two of the submicromolar PAMs **8j** and **8k**. The benzyl derivative **8k** with the highest functional activity showed weak MPEP site interaction with 21% displacement at 10 μM, indicative of an estimated >100-fold divergence from its functional activity. Collectively, these data suggest a similar mechanism of receptor activation for **8k** and **6**, involving an allosteric site of interaction overlapping and/or outside the MPEP site. PAM **8k** can be envisioned as a close structural analog of the acetylene based PAM **3** (VU0360172); however, in contrast to **8k**, acetylene PAM **3** has a reported K_i of 195 nM. With **8k** in hand a subsequent study was conducted to further optimize functional activity. In addition we hoped to address metabolic instability of both the eastern and western lipophilic substructures based upon soft-spot analysis from rat microsomal incubations which indicated extensive oxidative metabolism.

To this end, we focused on a number of sterically encumbered amide groups in conjunction with simple fluorine substitution of the western phenyl ring in an attempt to improve stability whilst maintaining good PAM potency (Table 3, **8l–v**). Relative to **8k** fluorine substitution was tolerable at the 3-position (**8l**, EC₅₀ = 29 nM). In contrast, fluorine substitution at the 4-position (**8m**) resulted in a sevenfold loss in activity. Similar western phenyl SAR (H vs 3-fluorine) was also observed within the tetrahydropyranyl and cyclobutanyl amides (**8n–8q**). Although oxidative metabolism in vitro was reduced with the

incorporation of either a tetrahydropyran or oxetane ring system (data not shown), these initial eastern heterocycle modifications were not as effective in retaining potentiator activity, as only modest PAM activity was observed (tetrahydropyran **8o**, EC₅₀ = 1604 nM). Highly hindered substituents proximal to the amide NH, such as *tert*-butyl amide **8s** or cyclobutyl amide **8q**, were far more effective affording PAMs with activity routinely under 500 nM (EC₅₀s 136–300 nM). Identification of enantiospecific switches is a desirable property to have within a lead modulator scaffold, providing enhanced specificity for the target and simultaneously the potential for enhanced mGlu selectivity. Extending a cycloalkyl one carbon from the amide and incorporating a stereogenic center bearing an alpha methyl group led to enantioselective PAM **R-8t**.²⁷ The chiral trifluorocyclopropyl derivative **8u**, a lower MW analog of **R-8t**, was found to also have an EC₅₀ < 100 nM and was relatively stable metabolically in vitro (data not shown). The chiral (*R*)-*N*-(3,3-dimethylbutan-2-yl)nicotinamide **8v** furnishes a highly potent PAM with an impressive EC₅₀ of 7.2 nM. PAM **8v** represents an twofold increase in activity over cyclohexyl analog **R-8t** within our high expressing rat receptor HEK cell line, and is one of the most potent mGlu₅ PAMs reported to date. Examination of **8v** in a lower expressing human mGlu₅ receptor cell line³¹ revealed a ~10-fold rightward shift in potency (70 nM) with no detected agonism activity for compound alone (Fig. 3). With the exquisite activity of **8v** (VU0404251), we opted to examine its in vitro and in vivo DMPK profile further (Fig. 3). Interestingly, PAM **8v** showed full displacement of the MPEP-site radioligand [³H]-methoxyPEPy with a K_i of 153 ± 23 nM, suggestive of a competitive interaction for the MPEP site. Although limited to single point binding and functional EC₅₀ data, it appears that within the alkyl amide sub-series (**7a**, **8b**, and **8k**) that the nicotinamide core modification in conjunction with the optimized chiral eastern amide of **8v** are more critical determinants for the MPEP site crossover interaction and not the identity of the western ether tail group. Additional analogs and binding studies within the series are needed to more fully support this hypothesis.³²

Point mutation studies examining the glutamate concentration–response curve (CRC) fold-shift to further illuminate the putative allosteric binding site and critical residues for modulator binding and cooperativity determinants have proven useful using a number of well established mGlu₅ tool compounds.^{24,33,34} Unfortunately for some chemotypes, including VU0357121 (**6**),²⁴ conclusions from mutagenesis and binding studies may be ambiguous due to overlapping sites or cooperativity between sites/residues in the presence of novel PAMs. Analysis of MPEP site sensitive mutants (P654F, T780A, A809V Fig. 4) and CPPHA sensitive mutant (F585I Fig. 4) on the fold-shift of the glutamate CRC in the presence of 1 μM of **8v** indicate a profile similar to VU0357121 (**6**). MPEP-site point mutations reduced the ability of **8v** to shift the glutamate concentration–response curve (Fig. 4) as compared to the effect at the wild-type rat mGlu₅ receptor, where 1 μM of **8v** elicits a fivefold shift of the glutamate CRC. Collectively, the binding and point mutation studies outlined here demonstrate a consistent hypothesis involving **8v** interacting at the MPEP site.

While **8v** lacked optimal solubility (Fassif <20 μg/mL) and free fraction (rat, human plasma protein binding 99%), it possessed more than adequate potency and selectivity for potential proof-of-concept studies in vivo for the series. Results from non-crossover in vivo pharmacokinetic (PK) studies in rats (1 mg/kg IV, 10 mg/kg PO 20% β-CD) revealed a CL_p of 13.7 mL/min/kg and a terminal half-life (*t*_{1/2}) of 5 h. Snapshot oral PK drug concentrations in portal vein, plasma, and brain (0.5, 1.0, 3.0, 6.0 h) revealed a moderate first-pass effect (*E*_{hep} 0.41) with relatively high volume of distribution (*V*_{ss} = 2.3 L/kg) and good systemic exposure (plasma_{AUC} = 23 μM h). Due to high plasma protein binding, the relative CNS penetration of VU0404251 (**8v**) was low (brain_{AUC} = 1.2 μMh, b/p = 0.05); however, absolute brain levels (*C*_{max} = 435 nM, *T*_{max} = 30 min) were considered sufficient for further evaluation. Encouraged by these results, a high oral dose of VU0404251 (**8v**) was

administered and examined for its effects on the reversal of amphetamine-induced hyperlocomotion in rats (Fig. 5), a model predictive of antipsychotic activity.^{7,8} PAM VU0404251 (**8v**) showed robust effects in reversal of hyperlocomotion, achieving an estimated terminal unbound brain concentration of ca. 74 nM, a concentration which is ca ten fold above the rat in vitro EC₅₀ of VU0404251 (**8v**).³⁵

In summary, we have explored hybrid PAMs of VU0360172 and VU0357121 focusing on understanding the molecular determinants responsible for MPEP versus non-MPEP allosteric site interactions. Based upon radioligand, functional, and single point mutation studies within this series, we have demonstrated through subtle chemical modification, that potent PAMs within similar chemotypes can be developed to preferentially interact or not interact with the MPEP allosteric binding site.³⁶ From these efforts, a highly potent non-acetylenic PAM VU0404251 (**8v**) was identified and shown to be efficacious in vivo at a single high dose. Future studies to definitively understand the observed structure-function/affinity relationship and the identification of true non-MPEP modulators of mGlu₅ with utility in vivo are in progress. Efforts within this and related series uncovering potentiators of mGlu₅ with enhanced solubility and unbound free fraction will be reported in due course.

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MPEP Site In Vivo Tools Non-MPEP Site

Figure 1.
Representative MPEP site (**1–4**) and non-MPEP site (**5** and **6**) mGlu₅ PAMs.

Figure 2.
MPEP interacting PAM VU0360172 (**3**), non-MPEP PAM VU0357121 (**6**), and proposed hybrid molecules **7-8**.

8v, VU0404251

Figure 3.
In vitro profile for **8v** (VU0404251).

rmGlu₅ EC₅₀ = 7.2±0.3 nM, 83% glu max
hmGlu₅ EC₅₀ = 70±12 nM 86% glu max
[³H]-methoxyPEPy Ki = 153 ± 23 nM
mGlu_{1-4,6-8} > 10 μM
Glutamate Fold Shift (h,r): 10, 8.2
rat *f_u* brain = 1%

Figure 4.
Fold-shifts studies using **8v** (VU0404251).

Figure 5. Reversal of amphetamine-induced hyperlocomotion with mGlu₅ PAM **8v** (VU0404251) at a dose of 100 mg/kg p.o. (vehicle 20% β -CD).

Scheme 1.

Synthesis of analogs **7–8**. Reagents and conditions: (a) LiOH, MeOH, THF, 95% (b) HNR^1R^2 , HATU, DIPEA, DMF, 60–95% (c) R^3OH , NaH, DMF, 30–75% (d) R^3OH , CuI, TMP, Cs_2CO_3 , toluene, 20–55%, (d) All compounds were purified by silica-phase automated chromatography or mass-directed prep RP-HPLC where required.

Table 1

Structures and activities of alkyl ethers **7a–d** and **8a–c**

Compd	X	Amide	Rat pEC ₅₀ ^a (±SD)	EC ₅₀ (nM)	[³ H]MeOPEPy %displacement ^b
7a	CH		6.22 (±0.16)	607	12
7b	CH		<5.0	>10,000	0
7c	CH		5.29 (±0.25)	5172	6.5
7d	CH		Inactive	Inactive	NT
8a	N		6.14 (±0.19)	725	5

Compd	X	Amide	Rat pEC ₅₀ ^a (±SD)	EC ₅₀ (nM)	[³ H]MeOPEPy %displacement ^b
8b	N		5.60 (±0.19)	2491	27
8c	N		5.72 (±0.09)	1901	9.5

NT = not tested.

^a pEC₅₀ are the average of three independent determination and represent a coefficient of variation (CV) <0.05.

^b Determined at 10 μM test compound, average of two determinations.

Table 2Structures and activities of nicotinamide ethers **8d–k**

Compd	Ether	Rat pEC ₅₀ ^a (±SD)	EC ₅₀ (nM)	[³ H]MeOPEPy%displacement ^b
8d		6.35 (±0.10)	449	NT
8e		Inactive	Inactive	NT
8f		Inactive	Inactive	NT
8g		5.97 (±0.10)	1076	NT
8h		5.94 (±0.03)	1139	NT
8i		Inactive	Inactive	NT
8j		6.35 (±0.19)	446	0
8k		7.47 (±0.33)	34	21

NT = not tested.

^apEC₅₀ are the average of three independent determination and represent a coefficient of variation (CV) <0.05.^bDetermined at 10 μM test compound, average of two determinations.

Table 3Structures and activities of nicotinamide benzyl ethers **8k–v**

Compd	X	Amide	Rat pEC ₅₀ ^a (±SD)	EC ₅₀ (nM)
8k	H		7.47 (±0.33)	34
8l	3-F		7.54 (±0.26)	29
8m	4-F		6.60 (±0.14)	251
8n	H		5.42 (±0.36)	3810
8o	3-F		5.79 (±0.38)	1604
8p	H		6.53 (±0.32)	297
8q	3-F		6.52 (±0.33)	300
8r	3-F		Inactive	Inactive
8s	3-F		6.87 (±0.24)	136
S-8t	3-F		6.78 (±0.16)	167
R-8t	3-F		7.82 (±0.38)	15
8u	3-F		7.07 (±0.09)	83
8v (VU0404251)	3-F		8.14 (±0.30)	7.2

^apEC₅₀ are the average of three independent determination and represent a coefficient of variation (CV) <0.10.