



Synthesis and SAR of a novel metabotropic glutamate receptor 4 (mGlu₄) antagonist: Unexpected ‘molecular switch’ from a closely related mGlu₄ positive allosteric modulator

Thomas Utley^{a,b}, Dustin Haddenham^a, James M. Salovich^{a,b,c}, Rocio Zamorano^{a,b}, Paige N. Vinson^{a,b}, Craig W. Lindsley^{a,b,c,d}, Corey R. Hopkins^{a,b,c,d,*}, Colleen M. Niswender^{a,b,*}

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

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

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

^d Department of Chemistry, Vanderbilt University, Nashville, TN 37232, USA

ARTICLE INFO

Article history:

Received 11 July 2011

Revised 28 September 2011

Accepted 30 September 2011

Available online 8 October 2011

Keywords:

Metabotropic glutamate receptor 4

mGlu₄

Molecular switch

Antagonist

ABSTRACT

Herein we report the discovery and SAR of a novel antagonist of metabotropic glutamate receptor 4 (mGlu₄). The antagonist was discovered via a molecular switch from a closely related mGlu₄ positive allosteric modulator (PAM). This antagonist (VU0448383) displays an IC₅₀ value of 8.2 ± 0.4 μM and inhibits an EC₈₀ glutamate response by 63.1 ± 6.6%.

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G-protein coupled receptor (GPCR) orthosteric ligands currently represent the largest class of drug molecules. However, over the past several years, modulation of these targets via a novel mechanism, allosteric modulation, has generated much attention and research focus.^{1–3} One phenomenon that has been reported recently is the notion of ‘molecular switching’, referring to allosteric ligands that switch modes of pharmacology upon subtle modifications of the chemical scaffold.⁴ While there have been a number of reports of molecular switches within the Groups I^{5,6} and II⁷ metabotropic glutamate receptors, to the best of our knowledge, this is the first report of a molecular switch within allosteric ligands of the Group III subfamily.

As part of the Molecular Libraries Production Center Network (MLPCN),⁸ we had previously performed a high throughput screen of the NIH Molecular Libraries Small Molecule Repository (MLSMR) collection of compounds to identify muscarinic acetylcholine receptor 4 (M₄)-specific antagonists using a cell line expressing M₄ with a chimeric G protein, G_{qi5}, to induce calcium mobilization. During primary and confirmation screening, VU0219493 was flagged as an M₄ antagonist when screened at a single dose of 10 μM. The compound

was then evaluated for concentration-dependent activity at M₄, a stage which involved counter screening using four cell lines, one of which was a cell line co-expressing metabotropic glutamate receptor 4 (mGlu₄) and G_{qi5}.⁹ The purpose of this counter screen was to control for nonspecific GPCR or G_{qi5}-mediated effects of HTS hits. While VU0219493 failed to confirm as active in these follow-up screens for muscarinic receptors (IC₅₀ >30 μM for both M₄ and M₁), surprisingly, the compound robustly potentiated the mGlu₄/

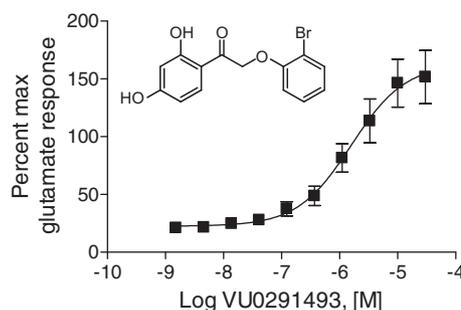


Figure 1. VU0219493 (inset structure) potentiates glutamate-induced calcium stimulation in human mGlu₄/G_{qi5} cells in a concentration-dependent fashion. Data represent the mean ± SEM of three independent experiments performed in triplicate.

* Corresponding authors.

E-mail address: colleen.niswender@vanderbilt.edu (C.M. Niswender).

¹ These authors equally contributed to this work.

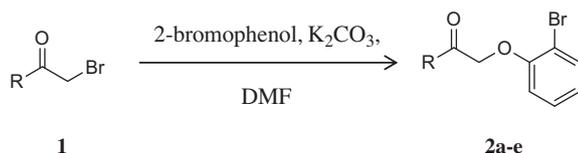
Gq₁₅ calcium response with a potency of $1.4 \pm 0.4 \mu\text{M}$ (Fig. 1). The activity of this hit was also verified in cells co-expressing rat mGlu₄ with GIRK potassium channels¹⁰ ($\text{EC}_{50} = 1.1 \mu\text{M}$) and a $30 \mu\text{M}$ concentration of the compound shifted a concentration-response curve for glutamate ninefold to the left (data not shown).

With a verified positive allosteric modulator (PAM) hit in hand,¹¹ we began a chemical optimization program around this scaffold. SAR exploration was begun by reacting commercially available α -bromoketones with 2-bromophenol to yield the desired compounds. Methylation of the phenolic groups or replacing the phenols with fluorine led to inactive compounds (**2b** and **2c**). The 2,4-dihydroxyphenyl group could be replaced with 2-pyridyl (**2d**; $4.6 \pm 1.4 \mu\text{M}$); however, a significant loss of potency was observed.

In our screening program, we routinely assess compound activity in the presence of glutamate concentrations that generate a response that is either 20% (to detect potentiators) or 80% (to identify antagonists) of a maximal agonist response. Using this strategy, we found that inclusion of a 4-chloro-3-pyridyl group engendered a 'molecular switch' within the scaffold, resulting in a compound with mGlu₄ antagonist activity (**2e**, $7.8 \mu\text{M}$, blockade of the EC_{80} glutamate response by approximately 75%, Table 1).

Based on the activity of **2e**, a brief SAR around the left-hand pyridyl moiety was undertaken (Table 2). Removal of the pyridine nitrogen led to inactive compounds (**3a**, **3b**, and **3e**). In addition, moving the nitrogen around the ring also was unproductive and led to inactive compounds (**3c** and **3d**). Interestingly, the 2-pyridyl compound was an activator (**2d**), but other regioisomers were inactive.

Table 1
Initial SAR for mGlu₄ PAM VU0219493

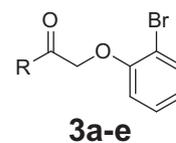


Compd	R	hmGlu ₄ EC ₅₀ (μM) ^a	Efficacy %PHCC
2a		1.4 ± 0.4	79.0 ± 3.1
2b		Inactive	12.1 ± 3.1
2c		Inactive	12.2 ± 4.7
2d		4.6 ± 1.4	81.9 ± 2.7
2e		Antagonist ^b ($\text{IC}_{50} = 7.8$)	%Inhibition 74.4

^a Potentiation EC_{50} and efficacy (% PHCC) are the average of at least three independent determinations performed in triplicate (mean \pm SEM shown in table). The maximal response generated in mGlu₄ CHO cells in the presence of mGlu₄ PAMs varies slightly in each experiment; therefore, PAM data are normalized to a control PAM, PHCC, response obtained in each day's run.

^b For **2e**, potency and efficacy (% inhibition) data are for blockade of an EC_{80} glutamate response.

Table 2
SAR of the pyridyl group



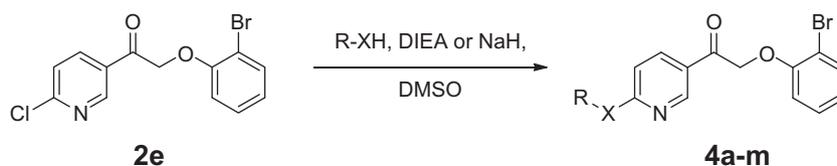
Compd	R	hmGlu ₄ IC ₅₀ (μM) ^a	% Inhibition
2e		8.2 ± 0.4	63.1 ± 6.6
3a		Inactive	-3.1 ± 1.3
3b		Inactive	-6.9 ± 3.0
3c		Inactive	-4.0 ± 6.1
3d		Inactive	-1.5 ± 1.5
3e		Inactive	2.3 ± 1.6

^a Antagonist activity at mGlu₄ is assessed by measuring the ability of a compound to concentration-dependently inhibit an EC_{80} glutamate response. IC_{50} and % inhibition values are the average of at least three independent determinations (mean \pm SEM shown in table). Compounds were ranked as active if they inhibited the EC_{80} response by at least 20%.

A more extensive SAR optimization was undertaken to further evaluate compounds as antagonists of the mGlu₄ receptor (Table 3). The initial antagonist hit was re-synthesized and evaluated in antagonist mode where potency and efficacy were confirmed ($8.2 \pm 0.4 \mu\text{M}$; 63.1% inhibition). Further evaluation by displacement of the 4-chloro, however, was not productive as all compounds were inactive (**4a–h** and **4j–m**) or very weak in their activity (**4i**, $>10 \mu\text{M}$, 25.5% inhibition).

In contrast to the 4-chloro-3-pyridyl portion of the molecule, there was somewhat more tractable SAR on the right-hand aryl portion of the scaffold (Table 4). These compounds were either made in an analogous fashion as shown in Table 1, or, for the biaryl compounds, they were synthesized from compound **2e** via a palladium-mediated Suzuki cross coupling.¹² The ether could not be replaced by a thio-ether (**5a**) or an unsubstituted phenyl derivative (**5b**); however, the 2-bromo could be substituted with a 3-bromo without significant loss in potency or efficacy (**5d**, $7.7 \pm 0.8 \mu\text{M}$; 58.5% inhibition). 2-fluoro (**5c**), 3-methoxy (**5e**), 3-dimethyl amine (**5f**), 4-methyl (**5h**), 3-methyl ester (**5g**) and 4-methoxy (**5i**) groups were not tolerated. In contrast, 4-trifluoromethyl was similar in potency to the original antagonist (**5j**, $4.7 \pm 0.5 \mu\text{M}$; 59.8% inhibition). The SAR of the 2-substituted phenyl derivatives was not predictive and rather shallow. For example, the 2-phenyl and 2-(2-pyridyl) derivatives were active with weak inhibition (**5n**, $>10 \mu\text{M}$; 31.1% inhibition and **5o**, $8.0 \pm 0.9 \mu\text{M}$; 60.0% inhibition, respectively). In contrast, the other two pyridyl regioisomers (**5p** and **5q**), as well as a cyclohexyl or other replacements (**5s**, **5u**, **5v**, and **5x**), were inactive. Several other compounds evaluated exhibited weak antagonist activity (**5t**; $>10 \mu\text{M}$, 56.2% inhibition, **5w**; $>10 \mu\text{M}$; 36.5% inhibition).

Table 3
SAR of substituted pyridine compounds



Compd	R-X	hmGlu ₄ IC ₅₀ (μM) ^a	% Inhibition
2e	Cl	8.2 ± 0.4	63.1 ± 6.6
4a		Inactive	-5.4 ± 6.1
4b		Inactive	-7.3 ± 3.9
4c		Inactive	-7.2 ± 4.8
4d		Inactive	-7.2 ± 2.3
4e		Inactive	-9.9 ± 4.9
4f		Inactive	-2.9 ± 5.8
4g		Inactive	-15.8 ± 6.1
4h		Inactive	2.2 ± 6.0
4i		>10	25.5 ± 9.2
4j		Inactive	-4.8 ± 3.0
4k		Inactive	0.9 ± 5.4
4l		Inactive	14.0 ± 15.5
4m		Inactive	4.3 ± 13.9

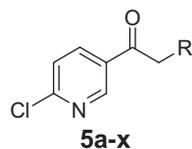
^a Antagonist activity at mGlu₄ is assessed by measuring the ability of a compound to concentration-dependently inhibit an EC₈₀ glutamate response. IC₅₀ and % inhibition values are the average of at least three independent determinations performed in triplicate (mean ± SEM shown in table). Compounds were ranked as active if they inhibited the EC₈₀ response by at least 20%.

To confirm that these compounds were specific to mGlu₄, we counter screened the entire set against the original screening cell line which contains the human M4 muscarinic receptor co-expressed with Gq_{i5}. No compounds significantly affected an EC₂₀ response to the M4 agonist acetylcholine; **5r** and **5s**, which were inactive at mGlu₄, weakly inhibited EC₈₀ responses to ACh by 26% and 23%, respectively. As a further validation that these compounds were inhibitors of mGlu₄, the lead antagonist for this series, VU0448383, was confirmed to be active in the rat mGlu₄ thallium flux assay (Fig. 2). Although this antagonist, and the ser-

ies in general, is of low potency, it is important to note that in our previous reports exploring the structure activity relationships of other mGlu₄ PAM scaffolds (representing approximately 5,000 compounds), we have never observed a similar pharmacology 'switch' for mGlu₄, suggesting that this compound represents a major advance as a tool compound for the study of mGlu₄ biology.

In conclusion, we have shown that slight structural modifications to a novel mGlu₄ PAM scaffold lead to a 'molecular switch', resulting in a novel mGlu₄ antagonist. Specifically, changing the

Table 4



Compd	R ₁	hmGlu ₄ EC ₅₀ (μM) ^a	% Inhibition
5a		Inactive	12.0 ± 12.2
5b		Inactive	-8.7 ± 4.2
5c		Inactive	-10.7 ± 10.2
5d		7.7 ± 0.8	58.5 ± 16.3
5e		Inactive	-2.8 ± 5.0
5f		Inactive	4.4 ± 9.9
5g		Inactive	12.9 ± 13.2
5h		Inactive	16.9 ± 11.0
5i		Inactive	-18.3 ± 12.0
5j		4.7 ± 0.5	59.8 ± 14.6
5l		Inactive	-17.0 ± 3.6
5m		Inactive	-14.8 ± 8.7
5n		>10	31.1 ± 15.8
5o		8.0 ± 0.9	60.0 ± 9.4
5p		Inactive	-3.4 ± 2.4

Table 4 (continued)

Compd	R ₁	hmGlu ₄ EC ₅₀ (μM) ^a	% Inhibition
5q		Inactive	-4.8 ± 6.4
5r		Inactive	7.8 ± 3.3
5s		Inactive	7.4 ± 5.2
5t		>10	56.2 ± 12.7
5u		Inactive	-10.4 ± 9.4
5v		Inactive	-0.1 ± 4.6
5w		>10	36.5 ± 13.7
5x		Inactive	-7.1 ± 9.2

^a IC₅₀ and % EC₈₀ values are the average of at least three independent determinations performed in triplicate (mean ± SEM shown in table). Compounds were ranked as active if they inhibited the EC₈₀ response by at least 20%.

2,4-dihydroxyphenol to a 4-chloro-3-pyridyl moiety resulted in the switch from PAM to antagonist (**2e**). Further modifications of the 4-chloro-3-pyridyl moiety were not tolerated suggesting a shallow binding pocket for that portion of the molecule. The far right side of the molecule did allow for some modification; however, all compounds were shown to be equipotent with **2e**. Although 'molecular switches' have been reported for other mGlu receptors, to our knowledge this is the first report for a Group III mGlu receptor, namely, mGlu₄. Further studies are planned and will be reported in due course.

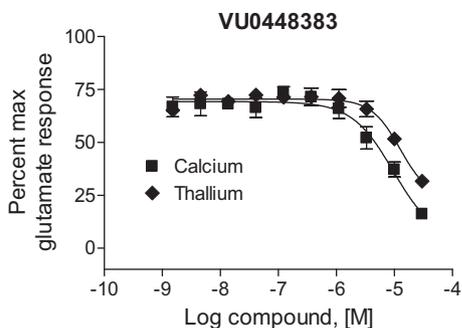


Figure 2. VU0448383 antagonizes glutamate induced responses in human mGlu4/Gq₁₅ cells (calcium assay) and rat mGlu4/GIRK cells (thallium assay) in a concentration-dependent fashion.

Acknowledgments

The authors acknowledge C. David Weaver, Emily Days, and Christopher Farmer of the Vanderbilt High Throughput Screening facility. This work was supported by Grants from the NIH and Michael J. Fox Foundation.

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9. Calcium mobilization assays. FBS, 20 mM HEPES, 100 units/ml penicillin/streptomycin, and 1 mM sodium pyruvate (Plating Medium). The cells were grown overnight at 37 °C in the presence of 5% CO₂. During the day of assay, the medium was replaced with 20 µL of 1 µM Fluo-4, AM (Invitrogen, Carlsbad, CA) prepared as a 2.3 mM stock in DMSO and mixed in a 1:1 ratio with 10% (w/v) pluronic acid F-127 and diluted in Assay Buffer (Hank's balanced salt solution, 20 mM HEPES and 2.5 mM Probenecid (Sigma-Aldrich, St. Louis, MO)) for 45 min at 37 °C. Dye was removed and replaced with 20 µL of Assay Buffer. For concentration-response curve experiments, compounds were serially diluted 1:3 into 10 point concentration response curves and were transferred to daughter plates using the Echo. Test compounds were again applied and followed by EC₂₀ concentrations of glutamate. Test compounds were transferred to daughter plates using an Echo acoustic plate reformatter (Labcyte, Sunnyvale, CA) and then diluted into Assay Buffer. Ca²⁺ flux was measured using the Functional Drug Screening System 6000 (FDSS6000, Hamamatsu, Japan). Baseline readings were taken (10 images at 1 Hz, excitation, 470 ± 20 nm, emission, 540 ± 30 nm) and then 20 µl/well test compounds were added using the FDSS's integrated pipettor. Cells were incubated with compounds for approximately 2.5 min and then an EC₂₀ concentration of glutamate was applied. Curves were fitted using a four point logistical equation using Microsoft XLfit (IDBS, Bridgewater, NJ). Thallium flux assays have been described in detail in Ref. ⁹.
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