Published in final edited form as:

Bioorg Med Chem Lett. 2010 September 1; 20(17): 5175–5178. doi:10.1016/j.bmcl.2010.07.007.

Synthesis and SAR of novel, 4-(phenylsulfamoyl)phenylacetamide mGlu₄ positive allosteric modulators (PAMs) identified by functional high-throughput screening (HTS)

Darren W. Engers^{a,b}, Patrick R. Gentry^{a,b}, Richard Williams^{a,b}, Julie L. Bolinger^a, C. David Weaver^{a,b}, Usha N. Menon^a, P. Jeffrey Conn^{a,b}, Craig W. Lindsley^{a,b,c}, Colleen M. Niswender^{a,b,*}, and Corey R. Hopkins^{a,b,*}

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

^bVanderbilt Program in Drug Discovery, Nashville, TN, 37232, USA

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

Abstract

© 2010 Elsevier Ltd. All rights reserved.

*Corresponding authors. Tel +1 615 343 4303; fax: +1 615 343 3088. colleen.niswender@vanderbilt.edu (C.M.N.). Tel.: +1 615 936 6892; fax: +1 615 936 4381. corey.r.hopkins@vanderbilt.edu (C.R.H.).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain

VU0364439

EC₅₀ = 19.8 nM

Herein we disclose the synthesis and SAR of a series of 4-(phenylsulfamoyl)phenylacetamide compounds as mGlu₄ positive allosteric modulators (PAMs) that were identified via a functional HTS. An iterative parallel approach to these compounds culminated in the discovery of VU0364439 (**11**) which represents the most potent (19.8 nM) mGlu₄ PAM reported to date.

L-Glutamic acid (glutamate) is the primary excitatory neurotransmitter in synaptic pathways in the CNS.¹ There are two major classes of glutamate receptors, divided based upon the mechanism of activation. Ionotropic glutamate receptors (iGlu's) are ligand-gated ion channels, whereas metabotropic glutamate receptors (mGlu's) do not form an ion channel pore, but rather are a type of G protein-coupled receptor (GPCR).^{2, 3} The mGlu's are members of the group C family of GPCRs and are themselves categorized into three families based on their receptor structure and physiological activity: Group I (mGlu₁ and mGlu₅), Group II (mGlu₂ and mGlu₃) and Group III (mGlu₄, mGlu₆, mGlu₇ and mGlu₈). This last group of mGlu's (Group III) has historically been the least studied, mainly due to a dearth of selective ligands, especially allosteric modulators (modulators that act via a site other than the orthosteric site).^{4, 5} We have been interested in one particular Group III receptor, mGlu₄, due to the potential therapeutic benefits of activation of this receptor in disease states such as epilepsy⁶⁻⁸ and Parkinson's disease.^{4, 5}

Until recently, the most well characterized mGlu₄ positive allosteric modulator (PAM) has been the compound PHCCC^{9, 10}, **1**, a partially selective mGlu₄ potentiator. However, more recently, our laboratories and others have expanded the list of novel probes for mGlu₄ positive allosteric modulation (Figure 1).¹¹⁻¹⁹ With the exception of VU0361737¹⁵, **2**, these disclosed mGlu₄ PAMs have been deficient in their CNS penetration, limiting their usefulness for in vivo studies.

A functional high-throughput screening (HTS) campaign initiated at Vanderbilt to identify novel mGlu₄ PAMs has led to the identification of many of these previously reported

ligands (Figure 1). In addition to the compounds already reported, there were a number of 4-(phenylsulfamoyl)phenylacetamides, represented by **4** (Figure 2). Based on our previous results, we first changed the 2-furyl amide of **4** to the 2-pyridyl amide, **5**, and a 30-fold increase in potency was observed. At this point we embarked on a lead identification program to determine if this structural class of compounds would be a tractable lead for our mGlu₄ PAM program.

The initial SAR centered around the right-side sulfonamide portion of the molecule. To that end, compounds were synthesized in a three step protocol starting with the commercially available 4-nitrophenylsulfonyl chloride. Thus, the sulfonyl chloride was reacted with an appropriate amine (pyridine, DCM; 67–97%) yielding **7**. Next, the nitro compound was reduced to the aniline (H₂, Pd/C or Ra-Ni; >95%) which was then acylated with picolinyl chloride giving the desired compounds, **8a–n**, in high overall yield.

The SAR analysis of the compounds is outlined in Table 1. The pharmacological assay utilized to assess mGlu₄ PAM activity (calcium mobilization¹¹) shows day-to-day variability in the maximal PAM response; for this reason, all data have been normalized to the % response of the control PAM, PHCCC, to compare relative efficacy. Changing from the 2,4,6-trimethylphenyl sulfonamide, **5**, to the less sterically hindered phenyl sulfonamide, **8a**, provided a compound that was equipotent (159 nM vs. 177 nM) and exhibited similar maximal response. Evaluating other aryl substituents led to the 2-chlorophenyl sulfonamide, **8b**, which provided a dramatic increase in potency (27 nM, 88.2%); one of the most potent mGlu₄ PAMs reported to date. However, going from the 2-chloro to 2-fluoro, **8c**, resulted in an erosion of potency (27 nM vs. 206 nM), but minimal effect on maximal potentiation. Further substitution around the phenyl ring resulted in less active (**8d**, 325 nM, 87.6%), or inactive compounds (**8e** and **8f**) with slight modification. Going away from arylsulfonamides to alkyl or cycloalkyl sulfonamides resulted in reduced potency and maximal effect (**8g–8n**). Dialkylation of the sulfonamide led to reduced activity (to inactive) and much reduced max values (**8l–8n**).

Next, we focused on our best compound (2-chlorophenylsulfonamide, **8b**) and evaluated alternative amide moieties. Based on our previous work, we chose a small heteroaryl subset for evaluation. Small substitution on the pyridyl ring was tolerated (**9a**, 6-fluoro, 122 nM, 90.0%); however, a larger group led to an inactive compound (**9b**, 6-methoxy, inactive). The other 6- and 5-membered heterocycles were also well tolerated (**9c–9e**). Next, having evaluated cycloalkylamines as the sulfonamide portion, we wanted to evaluate simple methylated compounds. Utilizing the active 2-pyrimidine and 2-thiazole amides, the N-Me arylsulfonamides were synthesized and evaluated and there was a ~5- to 10-fold loss of potency, as well as a dramatic decrease in maximal response, as compared to PHCCC (**9f**, 990 nM, 31.1%; **9g**, 686 nM, 25.4%).

Lastly, modifications were made to the internal phenyl ring in an attempt to improve potency and maximal response. 3-Methoxy substitution, **10**, on both the internal phenyl ring and sulfonamide ring was well-tolerated (132 nM, 112.1%) and gave a compound equipotent to the lead compound, **5**. Substituting a 3-chloro in the internal phenyl ring gave a compound equipotent to our best compound, **8b**, (**11**, 19.8 nM, 102.3%). Moving the chloro to the 2-position (relative to the amide, **12**) led to an inactive compound suggesting that steric bulk ortho to the acetamide is not tolerated. These substituents could potentially hinder the NH group from making a key interaction in the binding site. Rearranging the orientation of the sulfonamide and chloro group led to a 36-fold loss of potency (**13**, 731 nM, 54.9%). Removal of the NH group altogether and replacement with a methylene group also led to a much less potent compound (**14**, 237 nM, 46.3%). Finally, reversal of the methylene and sulfone moieties led to a compound with much decreased potency and

maximal response (**15**, 2170 nM, 46.5%) suggesting the need for the NH and orientation of the sulfone directly attached to the internal phenyl ring.

Having a number of very potent and efficacious compounds in hand, we next wanted to evaluate in vitro pharmacokinetic (PK) properties. The compounds were evaluated for their metabolic stability in both human and rat microsomes, as well as their protein binding in both species.¹⁵ As can be seen in Table 4, these compounds all exhibited poor microsomal stability in both human and rat. Although the initial screening hit, **4**, showed good stability in RLM (93.3% remaining); all other compounds tested were very unstable with less than 30% remaining after the incubation period of 60 min. Metabolite ID analysis showed oxidation of both the internal phenyl ring, as well as oxidation of the pyridine ring (data not shown). In addition, with the exception of compounds **4**, **9g** and **10**, these compounds were highly protein bound in the rat, with even less free fraction observed in the human. Due to these disappointing in vitro PK results, these compounds were not progressed further into in vivo testing.

In summary, we report a series of small molecule mGlu₄ positive allosteric modulators identified through a functional HTS lead. These compounds represent a series of 2-pyridylamide-4-phenylsulfonamide compounds that possess excellent in vitro potency (VU0364439, **11**, 19.8 nM) and maximal response relative to the control PAM, PHCCC. Unfortunately, these compounds possess less than ideal PK properties preventing their use as in vivo tools; however, we anticipate that these compounds will inform the mGlu₄ community with more in vitro tool compounds.

Acknowledgments

The authors thank Qingwei Luo and Rocío Zamorano for technical assistance with pharmacology assays and Emily L. Days, Tasha Nalywajko, Cheryl A. Austin and Michael Baxter Williams for their critical contributions to the HTS portion of the project and Matt Mulder, Chris Denicola and Sichen Chang for the purification of compounds utilizing the mass-directed HPLC system. This work was supported by the National Institute of Mental Health, National Institute of Neurological Disorders and Stroke, the Michael J. Fox Foundation, the Vanderbilt Department of Pharmacology and the Vanderbilt Institute of Chemical Biology.

References and notes

1. Niswender CM, Conn PJ. *Annu. Rev. Pharmacol. Toxicol* 2010;50:295. [PubMed: 20055706]
2. Conn PJ, Pin J-P. *Ann. Rev. Pharmacol. Toxicol* 1997;37:205. [PubMed: 9131252]
3. Conn, PJ.; Patel, J. *The Metabotropic Glutamate Receptors*. Totowa, NJ: Humana Press; 1994.
4. Hopkins CR, Lindsley CW, Niswender CM. *Future Med. Chem* 2009;1:501. [PubMed: 20161443]
5. Lindsley CW, Niswender CM, Engers DW, Hopkins CR. *Curr. Top. Med. Chem* 2009;9:949. [PubMed: 19754407]
6. Muhle H, von Spiczak S, Gaus V, Kara S, Helbig I, Hampe J, Franke A, Weber Y, Lerche H, Kleefuss-Lie AA, Elger CE, Schreiber S, Stephani U, Sander T. *Epilepsy Res* 2010;89:319. [PubMed: 20338729]
7. Ngomba RT, Ferraguti F, Badura A, Citraro R, Santolini I, Battaglia G, Bruno V, De Sarro G, Simonyi A, van Luijckelaar G, Nicoletti F. *Neuropharmacology* 2008;54:344. [PubMed: 18022649]
8. Pitsch J, Schoch S, Gueller N, Flor PJ, van der Putten H, Becker AJ. *Neurobiol. Dis* 2007;26:623. [PubMed: 17446080]
9. Maj M, Bruno V, Dragici Z, Yamamoto R, Battaglia G, Inderbitzin W, Stoeckl N, Stein T, Gasparini F, Vranesic I, Kuhn R, Nicoletti F, Flor PJ. *Neuropharmacology* 2003;45:895. [PubMed: 14573382]
10. Marino MJ, Williams DL Jr, O'Brien JA, Valenti O, McDonald TP, Clements MK, Wang R, DiLella AG, Kinney GG, Conn PJ. *Proc. Nat. Acad. Sci* 2003;100:13668. [PubMed: 14593202]

11. Niswender CM, Johnson KA, Weaver CD, Jones CK, Xiang Z, Luo Q, Rodriguez AL, Marlo JE, de Paulis T, Thompson AD, Days EL, Nalywajko T, Austin CA, Williams MB, Ayala JE, Williams R, Lindsley CW, Conn PJ. *Mol. Pharmacol* 2008;74:1345. [PubMed: 18664603]
12. Niswender CM, Lebois EP, Luo Q, Kim K, Muchalski H, Yin H, Conn PJ, Lindsley CW. *Bioorg. Med. Chem. Lett* 2008;18:5626. [PubMed: 18793851]
13. Williams R, Johnson KA, Gentry PR, Niswender CM, Weaver CD, Conn PJ, Lindsley CW, Hopkins CR. *Bioorg. Med. Chem. Lett* 2009;19:4967. [PubMed: 19640716]
14. Williams R, Niswender CM, Luo Q, Le U, Conn PJ, Lindsley CW. *Bioorg. Med. Chem. Lett* 2009;19:962. [PubMed: 19097893]
15. Engers DW, Niswender CM, Weaver CD, Jadhav S, Menon UN, Zamorano R, Conn PJ, Lindsley CW, Hopkins CR. *J. Med. Chem* 2009;52:4115. [PubMed: 19469556]
16. Bolea, C. PCT WO2009/010454. 2009. p. 67
17. Bolea, C.; Celanire, S. PCT WO2009/010455. 2009. p. 115
18. McCauley, JA.; Butcher, JW.; Hess, JW.; Liverton, NJ.; Romano, JJ. PCT WO 2010/033350. 2010. p. 64
19. McCauley, JA.; Hess, JW.; Liverton, NJ.; McIntyre, CJ.; Romano, JJ.; Rudd, MT. PCT WO 2010/033349. 2010. p. 66
20. Compounds **1** (PHCCC), **2** (VU0361737), and **3** (VU0155041) are now available from TOCRIS bioscience (Cat. Nos. 1027, 3707, and 3248, respectively).
21. Leister W, Strauss K, Wisnoski D, Zhao Z, Lindsley C. *J. Comb. Chem* 2003;5:322. [PubMed: 12739949]
22. All new compounds were characterized by LCMS and/or ¹H NMR and found to be in agreement with their structures (>95% purity).

(-)-PHCCC, **1**

VU0361737, **2**

VU0155041, **3**

Figure 1.
Recently reported mGlu₄ PAMs.²⁰

Figure 2.
HTS Hit (VU0130734, **4**) to lead (VU0361591, **5**)

Figure 3. Concentration-dependent potentiation of glutamate in mGlu₄/Gqi5 CHO cells by 5. In the absence of an EC₂₀ concentration of glutamate, 5, does not elicit receptor activation.

Scheme 1.

Synthesis of 4-sulfamoylphenylpicolinamides, **8a-n**. All library compounds were purified by mass-directed prep LC where required.^{21, 22}

Table 1

SAR analysis of sulfonamide substitution.

Compound	R ¹	R ²	EC ₅₀ (hmGlu)
5	2,4,6-trimethylphenyl	H	159
8a	Phenyl	H	177
8b	2-Chlorophenyl	H	27
8c	2-Fluorophenyl	H	206
8d	2,5-Difluorophenyl 4-Chloro-3-	H	325
8e	trifluoromethylphenyl 3-Chloro-4-	H	inactive
8f	fluorophenyl	H	inactive
8g	Cyclohexyl	H	1030
8h	Cyclopropyl	H	>10,000
8i	Cyclobutyl	H	>5,000
8j	Cyclopropylmethyl	H	>5,000
8k	2-methoxyethyl	H	>10,000

Compound	R ¹	R ²	EC ₅₀ (hmGlu)
81			372

Compound	R ¹	R ²	EC ₅₀ (μM)	hmGlu	inactiv
8m

Compound	R ¹	R ²	EC ₅₀ (μM)	hmGlu
8n				inactive

^a Compounds were tested at a 30 μM final concentration. Data represent the average of triplicate determinations performed on one day. For % PHCCC, the maximal response elicited by compound was divided by the response of the control PAM, PHCCC, on that same day.

Table 2

Heteroarylamine evaluation, **9a–g**.

Compound	R	R ¹	EC ₅₀ (nM) hmGlu ₄ ^a
9a		H	122
9b		H	inactive

Compound	R	EC ₅₀ (nM)	
		R ¹	hmGlu ₄ ^a
9c		H	478

Compound	R	R ¹	EC ₅₀ (nM) hmGlu ₄ ^a
9d		H	77.4

Compound	R	R ¹	EC ₅₀ (nM) hmGlu ₄ ^a
9e		H	269

Compound	R	EC ₅₀ (nM)	
		R ¹	hmGlu ₄ ^a
9f		Me	990

Compound	R	EC ₅₀ (nM)	
		R ¹	hmGlu ₄ ^a
9g		Me	686

^aCompounds were tested at a 30 μ M final concentration. Data represent the average of triplicate determinations performed on one day. For % PHCCC, the maximal response elicited by compound was divided by the response of the control PAM, PHCCC, on that same day.

Table 3

Further SAR of 2-pyridylamide-4-phenylsulfamoyl compounds.

Compound	Structure	EC ₅₀ (nM) hmGlu ₄ ^a , 11	% PH
10		132	112
11		19.8	102
12		inactive	8.1
13		731	54.5
14		237	46.5

Compound	Structure	EC ₅₀ (nM) hmGlu ₄ ^{a,11}	% PH
15		2170	46.3

^a Compounds were tested at a 30 μ M final concentration. Data represent the average of triplicate determinations performed on one day. For % PHCCC, the maximal response elicited by compound was divided by the response of the control PAM, PHCCC, on that same day.

Table 4

In Vitro Pharmacokinetic Evaluation of Select Compounds.

Compound	HLM (% remaining)	RLM (% remaining)	PPB (r) (% free)	PPB (h) (% free)
4	nd	93.3	8.2	2.2
5	nd	25.8	0.3	0.1
8b	0.02	0.02	0.3	0.1
9d	0.84	1.2	0.1	0.1
9f	2.5	3.3	2.7	1.7
9g	0.1	0.08	37	0.5
10	1.3	0.05	12.7	0.3
11	0.63	0.02	0.1	0.1
13	11.9	0.05	nd	nd