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Synthesis and SAR of novel, 4-(phenylsulfamoyl)phenylacetamide $mGlu_4$ positive allosteric modulators (PAMs) identified by functional high-throughput screening (HTS)

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ABSTRACT

Herein we disclose the synthesis and SAR of a series of 4-(phenylsulfamoyl)phenylacetamide compounds as $mGlu_4$ 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) $mGlu_4$ 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 ($mGlu_1$ and $mGlu_5$), Group II ($mGlu_2$ and $mGlu_3$), and Group III ($mGlu_4$, $mGlu_6$, $mGlu_7$, and $mGlu_8$). 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, $mGlu_4$, due to the potential therapeutic implications of this receptor in disease states such as epilepsy[6–8] and Parkinson's disease.[4,5]

Until recently, the most well characterized $mGlu_4$ positive allosteric modulator (PAM) has been the compound PHCCC,[9,10] **1**, a partially selective $mGlu_4$ potentiator. However, more recently, our laboratories and others have expanded the list of novel probes for $mGlu_4$ positive allosteric modulation (Fig. 1).[11–19] With the exception of VU0361737,[15] **2**, these disclosed $mGlu_4$ 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 $mGlu_4$ PAMs has led to the identification of many of these previously reported ligands (Fig. 1). In addition to the compounds already reported, there were a number of 4-(phenylsulfamoyl)phenylacetamides, represented by **4** (Figs. 2 and 3). 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 $mGlu_4$ PAM program.

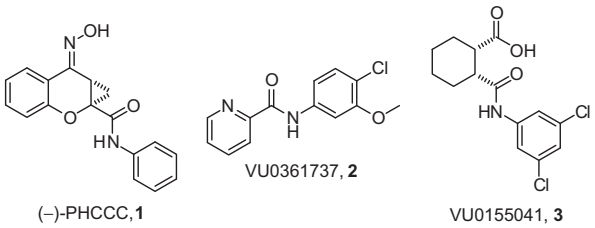

Figure 1. Recently reported $mGlu_4$ PAMs.[20]

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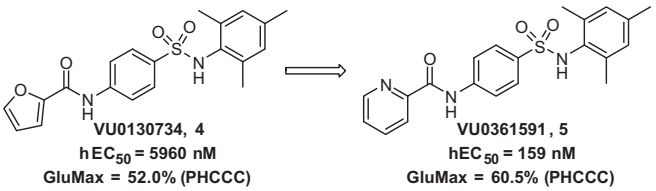


Figure 2. HTS Hit (VU0130734, **4**) to lead (VU0361591, **5**).

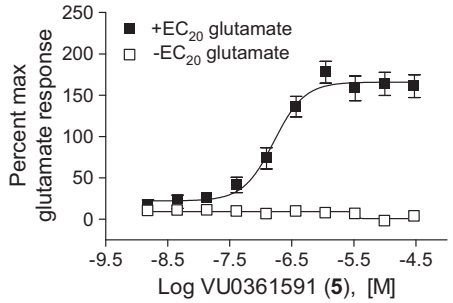
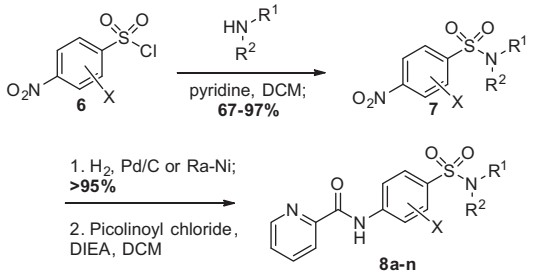


Figure 3. Concentration-dependent potentiation of glutamate in $mGlu_4$/Gqi5 CHO cells by **5**. In the absence of an EC_{20} concentration of glutamate, **5**, does not elicit receptor activation.

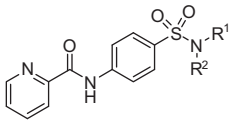
The initial SAR centered around the right-side sulfonamide portion of the molecule. To that end, compounds were synthesized in a three step protocol starting with the commercially available 4-nitrophenylsulfonyl chloride. Thus, the sulfonyl chloride was reacted with an appropriate amine (pyridine, DCM; 67–97%) yielding **7**. Next, the nitro compound was reduced to the aniline (H_2, Pd/C or Ra–Ni; >95%) which was then acylated with picolinyl chloride giving the desired compounds, **8a–n**, in high overall yield (Scheme 1).

The SAR analysis of the compounds is outlined in Table 1. The pharmacological assay utilized to assess $mGlu_4$ PAM activity (calcium mobilization[11]) 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 $mGlu_4$ 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**).



Scheme 1. Synthesis of 4-sulfamoylphenylpicolinamides, **8a–n**. All library compounds were purified by mass-directed prep LC where required.[21,22]

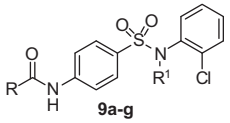
Table 1
SAR analysis of sulfonamide substitution

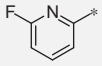
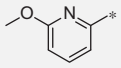


Compound	R^1	R^2	EC_{50} (nM) $hmGlu_4$[a,11]	% PHCCC[a,11]
5	2,4,6-Trimethylphenyl	H	159	60.5
8a	Phenyl	H	177	69.2
8b	2-Chlorophenyl	H	27	88.2
8c	2-Fluorophenyl	H	206	91.7
8d	2,5-Difluorophenyl	H	325	87.6
8e	4-Chloro-3-trifluoromethylphenyl	H	Inactive	19.5
8f	3-Chloro-4-fluorophenyl	H	Inactive	19.8
8g	Cyclohexyl	H	1030	63.1
8h	Cyclopropyl	H	>10,000	23.7
8i	Cyclobutyl	H	>5000	64.4
8j	Cyclopropylmethyl	H	>5000	70.2
8k	2-Methoxyethyl	H	>10,000	22.1
8l			372	35.8
8m			Inactive	15.4
8n			Inactive	12.0

[a] Compounds were tested using a 1:3 serial dilutions starting at 30 μM. 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
Heteroarylamide evaluation, **9a–g**



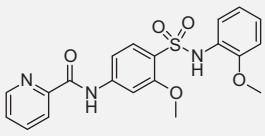
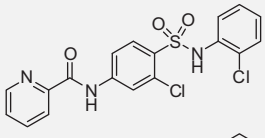
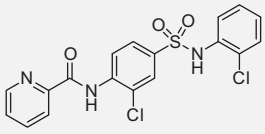
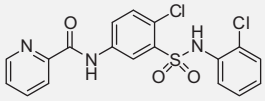
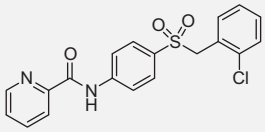
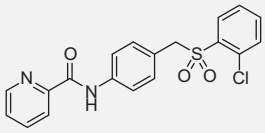
Compound	R	R^1	EC_{50} (nM) $hmGlu_4$[a,11]	% PHCCC[a,11]
9a		H	122	90.0
9b		H	Inactive	8.6
9c		H	478	102.9
9d		H	77.4	79.4
9e		H	269	54.2
9f		Me	990	31.1
9g		Me	686	25.4

[a] Compounds were tested using a 1:3 serial dilutions starting at 30 μM. 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.

Next, we focused on our best compound (2-chlorophenylsulfonamide, **8b**) and evaluated alternative amide moieties (Table 2). 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 six- and five-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 (Table 3). 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.

Table 3
Further SAR of 2-pyridylamide-4-phenylsulfamoyl compounds

Compound	Structure	EC_{50} (nM) $hmGlu_4$[a,11]	% PHCCC[a,11]
10		132	112.1
11		19.8	102.3
12		Inactive	8.1
13		731	54.9
14		237	46.3
15		2170	46.5

[a] Compounds were tested using a 1:3 serial dilutions starting at 30 μM. 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

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 $mGlu_4$ 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 $mGlu_4$ community with more in vitro tool compounds.

Acknowledgments

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