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Discovery and SAR of a novel series of non-MPEP site $mGlu_5$ PAMs based on an aryl glycine sulfonamide scaffold

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ABSTRACT

Herein we report the discovery and SAR of a novel series of non-MPEP site metabotropic glutamate receptor 5 ($mGlu_5$) positive allosteric modulators (PAMs) based on an aryl glycine sulfonamide scaffold. This series represents a rare non-MPEP site $mGlu_5$ PAM chemotype.

Allosteric modulation of metabotropic glutamate receptor subtype 5 ($mGlu_5$), with positive allosteric modulators (PAMs) is an increasingly popular approach for selective receptor activation.[1–5] Targeting NMDA hypofunction,[6] as opposed to classical hyperdopaminergia,[7] $mGlu_5$ PAMs have provided robust preclinical validation in multiple schizophrenia and cognition models.[8–15] Recently, $mGlu_5$ PAMs have been reported representing diverse chemotypes (Fig. 1);[16] however, the majority of these, such as **1**–**6**, bind at the MPEP (an $mGlu_5$ negative allosteric modulator, or NAM) binding site,[1–5,8–14] and in vivo efficacy has yet to be demonstrated for a non-MPEP site PAM, such as **7**[17,18] or **8**.[19] Moreover, a new phenomenon has emerged where very subtle structural changes, that is, a 'molecular switch', to multiple MPEP-site PAMs can modulate either the mode of pharmacology (PAM to NAM) or mGlu subtype selectivity, raising concerns over the pharmacology of metabolites in vivo.[11,13,20–22] Importantly, this phenomenon of 'molecular switches' has not been observed with the two known non-MPEP site PAM chemotypes, CPPHA (**7**)[17,18] and VU0357121 (**8**),[19] though the SAR has proven to be far steeper than the MPEP site congeners. Thus, our lab has been focused on the identification and optimization of additional non-MPEP site $mGlu_5$ PAMs to ascertain the presence or absence of 'molecular switches', and to determine if non-MPEP site ligands afford the same in vivo efficacy profile as MPEP-site PAMs.

Herein, we describe the synthesis and SAR of a novel series of potent, non-MPEP site $mGlu_5$ PAMs based on an aryl glycine sulfonamide scaffold identified in a functional HTS.[12]

A high-throughput functional screen, employing a triple-add calcium mobilization assay of 160,000 compounds, identified 1400 confirmed $mGlu_5$ PAMs, and over 60 with EC_{50}s below 500 nM in our high-expressing rat $mGlu_5$ HTS cell line.[12] From this, we identified VU0034403 (**9**), an unprecedented $mGlu_5$ PAM chemotype with potent (rat EC_{50} = 98 nM, 48% Glu Max, 7.9-fold shift) ago-PAM activity (Fig. 2 top panel), and amenable to chemical optimization via an iterative library approach. Significantly, **9** was selective versus the other mGluRs ($mGlu_{1-4,6-8}$ >10 μM), and afforded minimal displacement of [^{3}H]-methoxyPEPy (K_i >10 μM, Fig. 2 bottom panel), suggesting that **9** binds at a site distinct from the MPEP site.

Synthesis of first generation analogs **13** proceeded smoothly through a five step process (Scheme 1) surveying diversity at the

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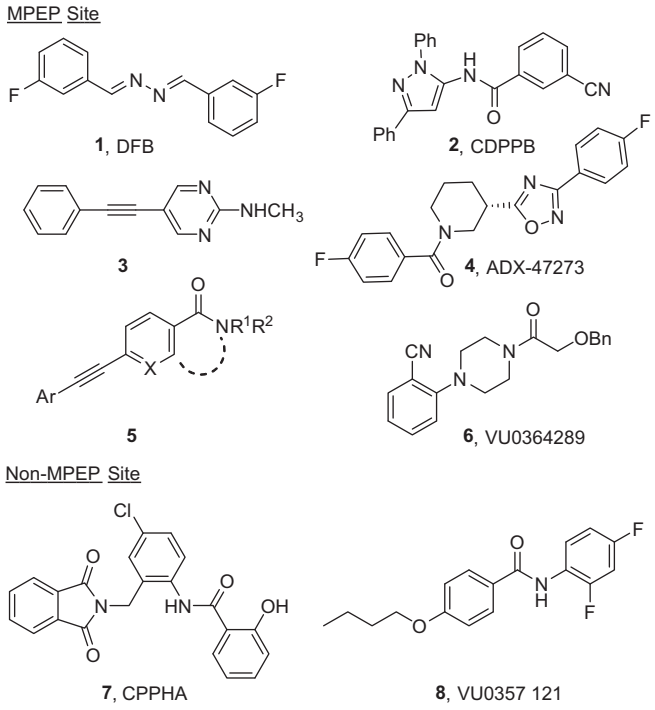


Figure 1. Representative MPEP site (**1**–**6**) and non-MPEP site (**7** and **8**) $mGlu_5$ PAMs.

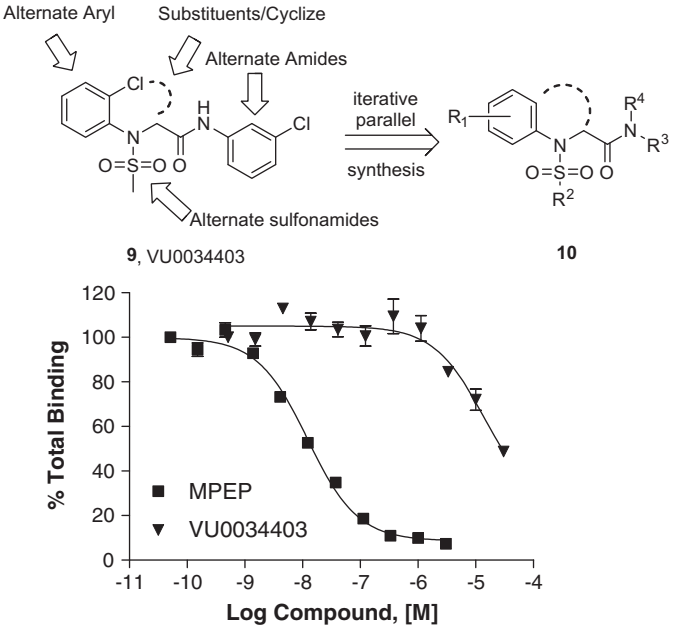
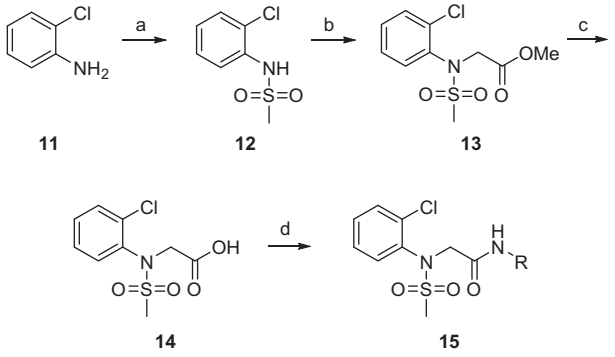


Figure 2. Top panel: Structure of non-MPEP $mGlu_5$ PAM HTS hit VU0034403 (**9**) and the chemical optimization plan leading to analogs **10**. Bottom panel: Radioligand displacement assay [^{3}H]-methoxyPEPy using VU0034403 and MPEP.

Eastern amide moiety. Beginning with 2-Cl aniline **11**, sulfonylation delivered **12** in quantitative yield. Alkylation of **12** with methyl bromoacetate provided ester **13**, which was saponified to give acid **14**. A two-step amide coupling procedure, employing a diverse collection of amines (aromatic, benzylic, 1°, 2°, aliphatic, with basic and acidic moieties) generated analogs **15** in isolated yields ranging from 26% to 68%.

While it was important to employ the high expressing rat $mGlu_5$ cell line in the HTS to ensure we identified even weak $mGlu_5$ PAMs, the chemical optimization program transitioned to human $mGlu_5$ cell lines with expression levels more closely aligned with native expression.[12,23] Potency was maintained for freshly prepared **9** in the rat cell-line while maximum efficacy increased (rat EC_{50} = 78 nM, 74% Glu Max), and Table 1 shows data for selected analogs **15** in both rat and human $mGlu_5$ cell lines. Against the human receptor, the potency of HTS lead **9** was tenfold less potent relative to the rat receptor cell line with an EC_{50} of 780 nM.



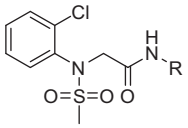
Scheme 1. Library synthesis of analogs **15**. Reagents and conditions: (a) $MeSO_2Cl$, pyridine, CH_2Cl_2, 99%, (b) (i) NaH, DMF, (ii) methyl bromoacetate, DMF, 94–98%, (c) LiOH, MeOH, THF, 99%, (d) PS-HOBt, HATU, 2,6-lutidine, DMF, RR'NH, CH_2Cl_2, 26–68%. All library compounds were purified by mass-directed prep LC where required.

Similar to the non-MPEP PAM CPPHA (**7**), SAR was extremely flat, with this first generation library affording very few active PAMs, and a ~threefold difference between rat and human potency. Of the ~50 analogs tested, only four showed good PAM activity, and these are all closely related phenyl **15a** (rat EC_{50} = 72 nM, 61% Glu Max), and fluoro-pyridine congeners **15b**–**d** (rat EC_{50}s 1.7–4.5 μM, 64–72% Glu Max). Basic and acidic analogs were inactive, as were 3° amide congeners including a constrained indoline. In general cyclic aliphatic amides were weakly active and acyclic amides were inactive. Like **9**, **15a** possessed strong allosteric agonist activity on rat $mGlu_5$ (an ago-PAM) which precluded the determination of an accurate fold shift measurement; however, the fold-shift is estimated to be minimal (~1.2). Within this series no ago-PAM activity was detected in the lower expressing human $mGlu_5$ cell line. Based upon previous studies wherein receptor expression level was found to impact allosteric agonist activity[23] we believe this phenomenon to be expression level dependent rather than due to a species difference. While **15a** possessed the potency, selectivity ($mGlu_{1-4,6-8}$ >10 μM) and free fraction (rat plasma protein binding, F_u = 0.13) required for in vivo studies in rats for a non-MPEP PAM ([^{3}H]-methoxyPEPy K_i >10 μM), **15a** suffered poor metabolic stability (⩽4% remaining after 15 min).[24] The pyridyl congener **15b** displayed an equivalent extent of plasma protein binding (rat F_u = 0.17, human F_u = 0.09) and improved metabolic stability in rat liver microsomes (39% remaining after 15 min); however, the rat $mGlu_5$ potency was not optimal for in vivo studies.

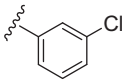
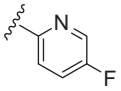
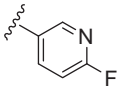
The next library iteration surveyed branching at the α-position with alkyl substituents. Analogs were easily prepared (Scheme 2), beginning with alkylation of **12** using the appropriate ethylbromoacetate (Me, Et, and n-Pr branched) and saponification to afford acid intermediates **16a**–**c**. Amide formation was performed as previously described to generate racemic analogs **17**. The methyl derivative **17a** was active (rat EC_{50} = 68 nM), but displayed diminished efficacy (43% Glu Max). Larger alkyl groups (Et **17b** and n-Pr **17c**) were weak (**17b** rat EC_{50} = 3.3 μM) or inactive (**17c** rat EC_{50} >10 μM).

Based on these data, we then introduced a cyclic constraint between the α-position and the 2-Cl moiety to generate a racemic

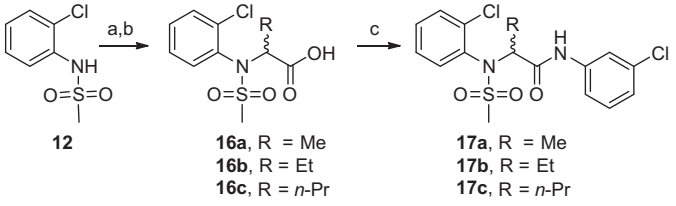
Table 1
Structures and activities of analogs **15**



15

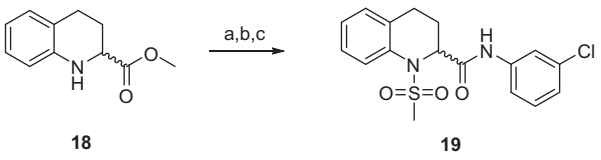
Compd	R	rmGlu$_5$ pEC$_{50}$/EC$_{50}$	% Glu Max	hmGlu$_5$ pEC$_{50}$/EC$_{50}$[a]	% Glu Max
9		7.11 0.08	74	6.11 0.78	63
15a		7.14 0.07	61	5.86 1.38	77
15b		5.43 3.72	66	NT	NT
15c		5.76 1.74	64	<5.0 >10	42
15d		5.35 4.47	72	<5.0 >10	35

[a] pEC$_{50}$ are the average of three independent determinations and represent a coefficient of variation (CV) <0.1, EC$_{50}$ units are in μM; NT = not tested.



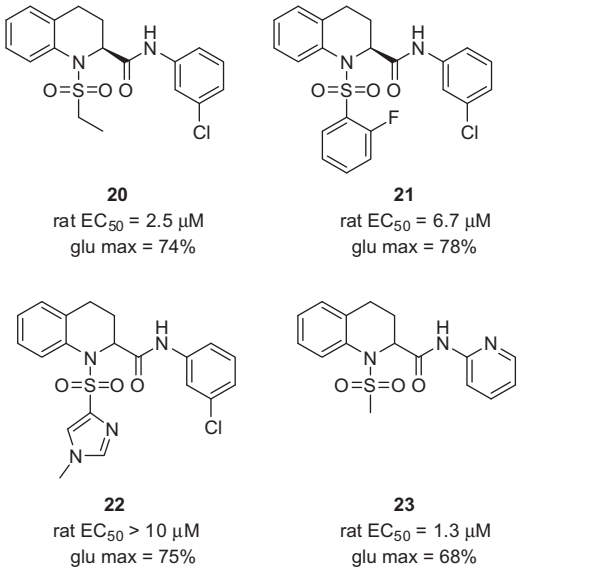
12 **16a**, R = Me; **16b**, R = Et; **16c**, R = *n*-Pr; **17a**, R = Me; **17b**, R = Et; **17c**, R = *n*-Pr

Scheme 2. Library synthesis of analogs **17**. Reagents and conditions: (a) NaH, RBr, DMF, 17-quant.% (b) LiOH, MeOH, THF, 99%, (c) PS-HOBt, HATU, 2,6-lutidine, DMF, (e) m-ClPhNH$_2$, CH$_2$Cl$_2$, 73%. All library compounds were purified by mass-directed prep LC where required.



18 **19**

Scheme 3. Synthesis of racemic tetrahydroisoquinoline derivative **19**. Reagents and conditions: (a) MeSO$_2$Cl, pyridine, CH$_2$Cl$_2$, 99%, (b) LiOH, MeOH, THF, 99%, (d) PS-HOBt, HATU, 2,6-lutidine, DMF, (e) *m*-ClPhNH$_2$, CH$_2$Cl$_2$, 74%.

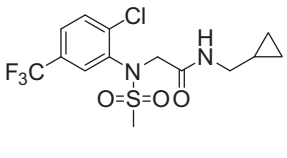


20 rat EC$_{50}$ = 2.5 μM, glu max = 74%; **21** rat EC$_{50}$ = 6.7 μM, glu max = 78%; **22** rat EC$_{50}$ > 10 μM, glu max = 75%; **23** rat EC$_{50}$ = 1.3 μM, glu max = 68%

Figure 3. Structures of non-MPEP mGlu$_5$ PAM (*S*)-tetrahydroisoquinoline analogs **20**–**22**.

tetrahydroisoquinoline derivative **19** (Scheme 3). In the high expressing HTS rat mGlu$_5$ cell line, this provided a moderately potent and efficacious PAM (EC$_{50}$ = 1.0 μM, 60% Glu Max). Based on the commercial availability of enantiopure (*R*)- and (*S*)-**18**, subsequent libraries (consisting of 80 analogs) explored stereochemistry as well as diversity at both the sulfonamide and the amide moieties following the chemistry outlined in Scheme 3. Enantioselective mGlu$_5$ potentiation was observed, with the (*S*)-enantiomer generally >10-fold more potent than the (*R*)-enantiomer. Overall little improvement in potency was observed, SAR remained relatively flat, and metabolic stability remained an issue. Figure 3 highlights representative racemic and (*S*)-enantiomer analogs **20**–**23**. Although efficacy would appear to be overall improved within the tetrahydroisoquinoline series as illustrated by these examples (68–78%), they displayed weak potency in the micromolar range.

In parallel, additional libraries were focused on identifying alternative aryl R$_1$ moieties (Fig. 2) and non-aromatic amides R$_2$ in an attempt to improve metabolic stability while maintaining acceptable potency and efficacy following the routes outlined in Schemes 1 and 2. Similar to CPPHA **7**, SAR was shallow, with only ~10% of compounds assayed displaying mGlu$_5$ PAM activity. Of these, VU0400100 (**24**), an analog with an additional 5-CF$_3$ on the western 2-ClPh ring and an eastern cyclopropyl methyl amide, was studied extensively (Fig. 4). PAM **24** was moderately potent on both human and rat mGlu$_5$, did not displace [^{3}H]-methoxyPEPy,



24, VU0400100

rmGlu$_5$ EC_{50} = 398 nM, 61% glu max
hmGlu$_5$ EC_{50} = 4.5 μM, 70% glu max
[^{3}H]-methoxyPEPy (K_i > 30 μM)
mGlu$_{1\text{-}4,6\text{-}8}$ > 10 μM
in vitro CL_{HEP} (h, r): 15, 53 mL/min/kg
plasma F_u (human, rat): 0.10, 0.13
rat brain F_u = 0.10

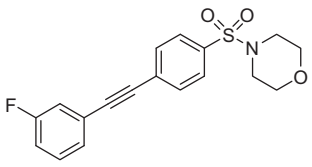
Figure 4. Structure, pharmacological, and DMPK profile of VU0400100, **24**, a non-MPEP site mGlu$_5$ PAM.

was highly selective versus the other mGlus and displayed significantly reduced plasma protein binding (rat, human) and nonspecific binding (F_u, 0.10) in rat brain homogenate binding. Moreover, **24** lacked significant activity in a Ricerca radioligand binding panel of 68 GPCRs, ion channels and transporters (<50% inhibition at 10 μM) and **24** was devoid of functional activity in a panel of ion channels relevant to cardiovascular safety (hERG, Ca, and Na, IC_{50} >10 μM).

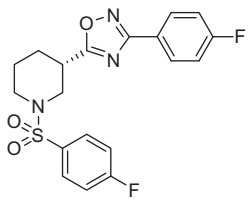
Consistent with the in vitro predicted clearance (Fig. 4), rat pharmacokinetics indicated **24** suffered extensive first-pass hepatic metabolism (E_H, 0.98) following an oral administration, as significantly lower exposure was observed in systemic supply relative to hepatic portal vein blood supply (AUC_{HPV}:AUC_{plasma}, 52). Exposures were improved with intraperitoneal or subcutaneous dosing routes, achieving CNS exposure relative to the systemic circulation (AUC_{brain}:AUC_{plasma}, 0.4) sufficient to suggest brain penetration is possible for this class of non-MPEP based mGlu$_5$ PAMs.

Currently, in vitro studies are underway to establish if the pan-P450 inactivator, 1-aminobenzotriazole (ABT), will have an impact on the clearance of **24** in rat microsomes.[25] If sufficient reduction of P450-mediated clearance of **24** is observed in vitro, it may be possible to enhance exposure in vivo and establish proof-of-concept in rat behavioral models with a non-MPEP site mGlu$_5$ PAM by administering **24** to rats that receive an oral dose of ABT.

It is interesting to note that to our knowledge this is a rare example of a chemical series of mGlu$_5$ PAMs containing a sulfonamide moiety. Analogs of **9** wherein the sulfonamide was replaced with *N*-alkyl, *N*-aryl or a *N*-acetyl moiety were devoid of PAM activity. These observations prompted us to replace the amide moiety in the known mGlu$_5$ MPEP-site PAM series **4** and **5** (Fig. 1) with sulfonamides (Fig. 5). While potency was diminished at least an order of magnitude relative to the direct amide counterpart scaffolds (**4** and **5**, Fig. 1),[12,15] the sulfonamide congeners **25** and **26** were weak to moderately active PAMs in the high expressing rat cell line, suggesting additional avenues for optimization. Within the human cell line the acetylene PAM **25** was inactive and the Addex analog **26** a weak PAM. Competition binding studies using membranes isolated from rmGlu$_5$ demonstrated no displacement of [^{3}H]-methoxyPEPy with up to 30 μM **25** or **26**. Although more potent analogs are needed to establish an allosteric binding site for



25
rmGlu$_5$ EC_{50} = 457 nM, glu max = 55%
hmGlu$_5$ Inactive



26
rmGlu$_5$ EC_{50} = 3.4 μM, glu max = 84%
hmGlu$_5$ > 10 μM, glu max = 43%

Figure 5. Structures and activities of representative MPEP-site mGlu$_5$ PAMs with the amide moiety replaced with the analogous sulfonamide linker.

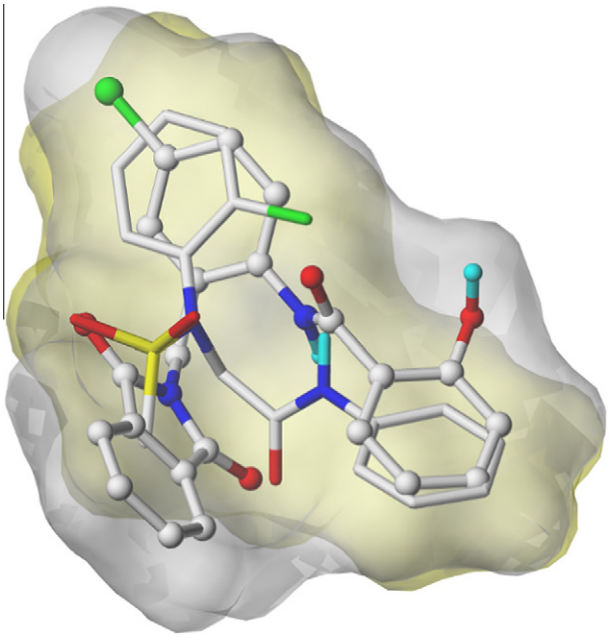


Figure 6. Suflex-Sim overlay of CPPHA (**7**, grey surface, ball and stick) and **15a** (gold surface, capped sticks).

these hybrid PAMs, the activity observed suggests that it may be possible to identify additional sulfonamide containing mGlu$_5$ PAMs.

In addition to the above exercise preparing sulfonamide hybrids using known MPEP-site mGlu$_5$ PAM scaffolds we have also undertaken a computational evaluation of low energy, ligand-based conformational ensembles of MPEP and non-MPEP ligands. Mutual flexible low energy shape-based alignment of sulfonamide **15a** with CPPHA (**7**) using the Surflex-Sim algorithm suggests a preferred fit for CPPHA (**7**) versus other MPEP modulators (Fig. 6).[26] Studies are ongoing to develop a comprehensive model based upon this approach as an entry point to identify additional novel non-MPEP modulators.

Significantly, this is only the third known non-MPEP site mGlu$_5$ PAM chemotype, and like CPPHA (**7**) and VU0357121 (**8**), SAR was steeper than that of MPEP site PAMs. Importantly, no 'molecular switches' were observed within this non-MPEP site series.[16] While DMPK properties currently preclude in vivo studies, key PAMs within this series are valuable tools for detailed in vitro pharmacological and electrophysiology studies. VU0400100 (**24**) has now been declared MLPCN probe ML332 and is freely available upon request.[27] Additional studies and refinements are in progress and will be reported in due course.

Acknowledgments

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