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Lead optimization of the VU0486321 series of $mGlu_1$ PAMs. Part 1: SAR of modifications to the central aryl core

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ABSTRACT

This Letter describes the lead optimization of the VU0486321 series of $mGlu_1$ positive allosteric modulators (PAMs). While first generation PAMs from Roche were reported in the late 1990s, little effort has focused on the development of $mGlu_1$ PAMs since. New genetic data linking loss-of-function mutant $mGlu_1$ receptors to schizophrenia, bipolar disorder and other neuropsychiatric disorders has rekindled interest in the target, but the ideal in vivo probe, for example, with good PK, brain penetration and low plasma protein binding, for robust target validation has been lacking. Here we describe the first modifications to the central aryl core of the VU0486321 series, where robust SAR was noted. Moreover, structural variants were identified that imparted selectivity (up to >793-fold) versus $mGlu_4$.

Recently, deleterious non-synonymous single nucleotide polymorphisms (nsSNPS) in the *GRM1* gene, which encodes metabotropic glutamate receptor subtype 1 ($mGlu_1$), were discovered that correlated with a higher incidence of neuropsychiatric disease, including schizophrenia and bipolar disorder.[1,2] Our lab then demonstrated that signalling downstream of these loss-of-function mutant receptors could be partially rescued with $mGlu_1$ PAMs;[3] however, neither the first generation Roche $mGlu_1$ PAM (**1**, Ro 07-11401)[4] nor our chemically distinct series (**2**, VU0483605),[3] derived from an $mGlu_4$ PAM scaffold (**3**, VU0400195, ML182)[5] via 'molecular switches'[6] (Fig. 1), had the requisite PK, CNS penetration and free fraction to serve as the ideal in vivo tool compounds for robust target validation and potential adverse effect liability assessment.[3,7] Subsequent SAR studies in our lab identified key substituents on the phthalimide moiety that engendered improved plasma stability, along with a critical 3-methyl furyl amide that provided **4** (VU0486321), a potent $mGlu_1$ PAM with moderate rat PK (CL_p = 13.3 mL/min/kg, $t_{1/2}$ = 54 min), good free fraction (human F_u = 0.05, rat F_u = 0.03) and excellent CNS penetration (K_p = 1.02).[7] Despite this notable advance, we sought an in vivo proof-of-concept tool compound with a longer half-life and ideally greater selectivity versus $mGlu_4$ (**4** is 35-fold selective). In this Letter, we describe, for the first time, SAR for modifications to the central aryl ring of **4**, and the impact on $mGlu_1$ and $mGlu_4$ PAM activity with analogs **5**.

In order to access analogs **5** and survey the SAR for the two regions depicted in blue in Figure 1, a general five step synthetic route was developed. As shown in Scheme 1, commercial, functionalized *p*-amino nitroarenes/heteroarenes **6** were *bis*-Boc protected to provide **7**, followed by reduction of the nitro moiety to afford **8**. Analogs **8** were then acylated with 3-methylfuran-2-carbonyl chloride to provide congeners **9**. Finally, acid-mediated deprotection liberated the free aniline which was then condensed with various phthalic anhydrides to afford final analogs **5**. The 3-methylfuranyl amide was held constant in this campaign, as it was the lone, standout amide from our earlier efforts,[7] where SAR proved steep.

The first core modification examined focused on an aryl moiety substituted with H, Me, Cl, CF_3 and OMe and F in the 2-position, relative to the phthalimide, analogs of **5** indicated as **10** (Table 1). All of the analogs **10** were potent $mGlu_1$ PAMs with EC_{50}s less than 500 nM, and most under 100 nM, with excellent efficacy (% Glu Max >90), a welcomed departure from the steep SAR that plagued the discovery of **4**.[7] Also, all compounds showed considerable agonism at concentrations above 10 μM, a feature previously observed with parent compound **4**, with the exception of the unsubstituted phenyl ring and 2-trifluoromethyl analogs which had a pure PAM profile. Interestingly, SAR for $mGlu_4$ varied greatly with respect

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0960-894X/

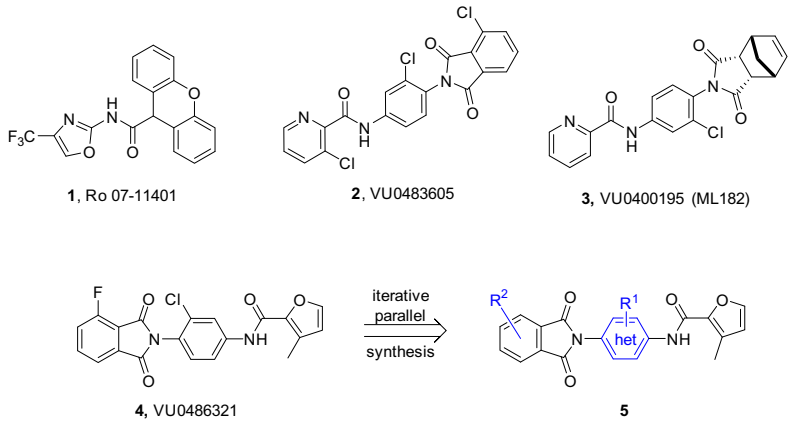
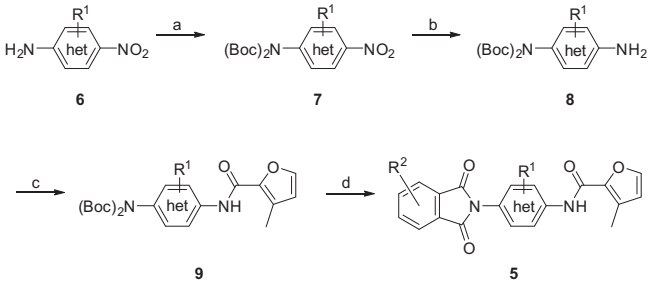


Figure 1. Structures of reported $mGlu_1$ PAMs **1**–**4**, and the novel targeted analogs **5**, to be accessed through iterative parallel synthesis.

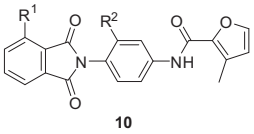


Scheme 1. Reagents and conditions: (a) Boc_2O, DMAP, THF, rt, 59–97%; (b) H_2, Pd/C, EtOH, rt, 94–99%; (c) 3-methylfuran-2-carbonyl chloride, DIEA, DCM, rt, 39–98%; (d) (i) TFA, DCM, rt (ii) phthalic anhydrides, AcOH, reflux, 53–94%.

to $mGlu_4$ PAM potency (EC_{50}s from 260 nM to >10 μM) and efficacy (% Glu Max from 35 to 122). While lack of activity at $mGlu_4$ is key for an $mGlu_1$ PAM in vivo probe, a dual $mGlu_1$/$mGlu_4$ PAM is intriguing in an antipsychotic agent, as $mGlu_4$ PAM activity can alleviate catalepsy, a major adverse event with the standard treatment of care.[8] PAM **10a**, with both an un-functionalized central phenyl core and unsubstituted phthalimide moiety, proved to be not only potent ($mGlu_1$ EC_{50} = 48 nM), but also >208-fold selective versus $mGlu_4$ ($mGlu_4$ EC_{50} >10 μM). All other modifications to either the phenyl core or the phthalimide moiety engendered more significant activity at $mGlu_4$, but $mGlu_1$ was still preferred. Relative to **10a**, the addition of substituents at either R^1 or R^2 provided potent $mGlu_1$ PAMs, but the SAR possessed little discernable texture with electron-donating, electron-withdrawing and/or more lipophilic moieties proving effective. One notable trend was that 2-CF_3 on the phenyl core was less effective, affording a diminution in $mGlu_1$ PAM potency relative to **10a**, providing analogs such as **10o**, with an $mGlu_1$ EC_{50} of 530 nM.

The lack of SAR texture with analogs **10** then led us to explore heterocyclic and bicyclic congeners of **5**, indicated as analogs **11** (Table 2), wherein the phenyl core was replaced with either a pyridine, pyrimidine or naphthalene, while holding the amide constant and surveying substituents on the phthalimide. Here, the SAR possessed texture, with 2-pyridyl analogs **11a**–**d** maintaining good $mGlu_1$ PAM potency (EC_{50}s 35 nM to 387 nM) and a range of selectivity versus $mGlu_4$ (~18- to 52-fold). Installation of a 2-pyrimidinyl core, analogs **11e**–**h**, lost significant activity at $mGlu_1$ (EC_{50}s 200 nM to >5 μM), and selectivity versus $mGlu_4$ was eroded. Indeed, both **11e** and **11h**, were equipotent $mGlu_1$ and $mGlu_4$

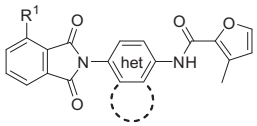
Table 1
Structures and activities for analogs **10**



Compd	R^1	R^2	$hmGlu_1$ EC_{50} (μM)[a] [% Glu Max ± SEM]	$mGlu_1$ pEC_{50} (±SEM)	$hmGlu_4$ EC_{50} (μM) [% Glu Max[b]]	Fold selective versus $mGlu_4$
10a	H	H	0.048 [82 ± 6]	7.32 ± 0.13	>10 [—]	>208
10b	Me	H	0.016 [108 ± 6]	7.79 ± 0.09	0.35 [62]	21.7
10c	Cl	H	0.029 [101 ± 14]	7.54 ± 0.10	0.483 [35]	16.7
10d	F	H	0.044 [96 ± 4]	7.36 ± 0.05	0.382 [34]	8.6
10e	H	F	0.022 [101 ± 4]	7.65 ± 0.14	0.265 [65]	11.9
10f	Me	F	0.026 [107 ± 3]	7.58 ± 0.10	0.287 [102]	11.0
10g	Cl	F	0.028 [100 ± 5]	7.56 ± 0.10	0.264 [93]	9.5
10h	F	F	0.035 [104 ± 6]	7.46 ± 0.12	0.674 [78]	19.3
10i	H	Me	0.089 [105 ± 7]	7.05 ± 0.10	0.885 [75]	9.9
10j	Me	Me	0.021 [100 ± 7]	7.67 ± 0.13	0.422 [122]	19.8
10k	Cl	Me	0.049 [117 ± 10]	7.31 ± 0.20	1.01 [127]	20.5
10m	F	Me	0.049 [95 ± 3]	7.31 ± 0.12	1.10 [86]	22.4
10n	H	CF_3	0.123 [108 ± 6]	6.91 ± 0.11	6.66 [86]	54.1
10o	Me	CF_3	0.530 [117 ± 11]	6.27 ± 0.16	5.76 [109]	10.8
10p	Cl	CF_3	0.106 [105 ± 4]	6.98 ± 0.08	2.47 [120]	23.3
10q	F	CF_3	0.155 [110 ± 8]	6.81 ± 0.11	2.87 [80]	18.5
10r	H	OMe	0.046 [96 ± 3]	7.33 ± 0.08	1.18 [50]	25.6
10s	Me	OMe	0.049 [94 ± 6]	7.31 ± 0.15	0.882 [107]	18
10t	Cl	OMe	0.018 [93 ± 3]	7.74 ± 0.14	0.647 [103]	36.1
10u	F	OMe	0.144 [103 ± 6]	6.84 ± 0.10	1.93 [73]	13.4

[a] Calcium mobilization $mGlu_1$ assays, values are average of three (n = 3) independent experiments performed in triplicate.
[b] Glu Max is expressed as % of PHCCC response, which is used as a normalization control as the % max values vary on a daily basis for the $mGlu_4$ assay.

Table 2
Structures and activities for analogs **11**



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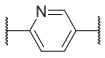
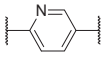
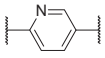
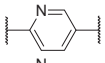
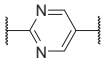
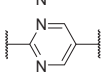
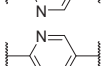
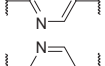
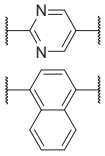
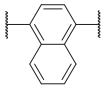
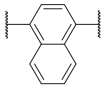
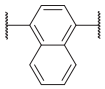
Compd	R^1	Het/bicycle	$hmGlu_1$ EC_{50} (μM)[a] [% Glu Max ± SEM]	$mGlu_1$ pEC_{50} (±SEM)	$hmGlu_4$ EC_{50} (μM) [% Glu Max[b]]	Fold Selective versus $mGlu_4$
11a	H		0.387 [80 ± 4]	6.41 ± 0.11	>10 [—]	>25.8
11b	Me		0.095 [105 ± 6]	7.02 ± 0.12	5.00 [100]	52.7
11c	Cl		0.035 [91 ± 8]	7.46 ± 0.09	1.113 [44]	31.9
11d	F		0.198 [93 ± 3]	6.70 ± 0.08	3.55 [19]	17.9
11e	H		4.61 [89 ± 4]	5.33 ± 0.22	4.67 [23]	1.01
11f	Me		0.751 [111 ± 5]	6.12 ± 0.22	4.47 [104]	5.95
11g	Cl		0.208 [84 ± 5]	6.68 ± 0.09	2.53 [45]	12.2
11h	F		5.33 [99 ± 6]	5.27 ± 0.19	>10 [24]	>1.88
11i	H		0.020 [99 ± 8]	7.69 ± 0.14	0.353 [54]	17.4
11j	Me		0.016 [88 ± 6]	7.80 ± 0.10	0.429 [103]	27.3
11k	Cl		0.023 [92 ± 8]	7.65 ± 0.13	0.513 [102]	22.8
11m	F		0.020 [90 ± 3]	7.71 ± 0.09	0.809 [83]	41.1

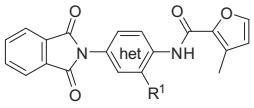
[a] Calcium mobilization $mGlu_1$ assays, values are average of three ($n = 3$) independent experiments performed in triplicate.
[b] Glu Max is expressed as % of PHCCC response.

PAMs. Replacement of the phenyl core with a naphthyl bicyclic ring system, for example, **11i–m**, afforded potent $mGlu_1$ PAMs (EC_{50}s 16 nM to 23 nM), irrespective of the phthalimide substituent, with modest selectivity versus $mGlu_4$ (17.4- to 41-fold). Thus, from these two efforts, while $mGlu_1$ potency and efficacy were attractive, the overall profile and selectivity versus $mGlu_4$ were not appropriate for an in vivo proof of concept tool compound. However, the overall SAR developed with analog libraries **10** and **11** was insightful and overcame the steep SAR previously encountered for this series.

Based on these data, we elected to survey the impact of regioisomers of **10** and **11**, a region of chemical space not previously explored, wherein the substituents were in the 3-position with respect to the phthalimide, for example, analogs **12**. Analogs **12** were accessed as shown in Scheme 1, and these analogs afforded the greatest selectivity versus $mGlu_4$ to date (>793 fold), despite possessing an unsubstituted phthalimide moiety. Here, the fluoro analog, **12a**, was a potent $mGlu_1$ PAM (EC_{50} = 12.6 nM, 84% Glu Max) with >793-fold selectivity versus $mGlu_4$ (EC_{50} >10 μM), and **12b**, the chloro congener, was similarly potent ($mGlu_1$ PAM EC_{50} = 29.1 nM, 68% Glu Max) with >344-fold selectivity versus $mGlu_4$ (EC_{50} >10 μM). Also, these compounds act like pure PAMs and did not show any observable agonistic effect in $mGlu_1$ as well as no activity at $mGlu_5$. Interestingly, the CF_3 analog **12d** was inactive, as were the regioisomeric pyridine and pyrimidine analogs **12f** and **12g**, further highlighting the non-obviousness and challenges in allosteric modulator SAR (Table 3).

From these efforts, **12a** and **12b** emerged as $mGlu_1$ PAMs with the requisite potency and selectivity versus $mGlu_4$ to serve as in vivo proof-of-concept tools; thus, they were profiled in a battery of in vitro and in vivo Drug metabolism and pharmacokinetic (DMPK) assays[9] to further assess their potential. In terms of in vitro predicted hepatic clearance measures, both **12a** and **12b** display moderate hepatic clearance in both human (CL_{HEP} = 9.65 mL/min/kg and 9.25 mL/min/kg, respectively) and rat (CL_{HEP} = 48.5 mL/min/kg and 48.7 mL/min/kg, respectively) hepatic microsomes. Unfortunately, both compounds suffered from high plasma protein binding (F_u <1% in human and rat) as well as high rat brain homogenate binding (F_u <1%). CNS penetration varied significantly between the two PAMs. In our standard rat plasma:brain level (PBL) cassette study (0.2 mg/kg, 10%EtOH:40% PEG 400:50% DMSO, 15 min time point), **12a** displayed a K_p of 1.57, while **12b**, the Cl-congener, showed very poor CNS penetration (K_p = 0.12). While CNS exposure of **12a** was attractive, the low free fraction precluded it from further consideration or advancement as an in vivo $mGlu_1$ PAM tool compound.

Table 3
Structures and activities for analogs **12**



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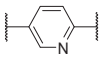
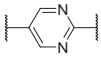
Compd	R^1 or Het/	$hmGlu_1$ EC_{50} (μM)[a] [% Glu Max ± SEM]	$mGlu_1$ pEC_{50} (±SEM)	$hmGlu_4$ EC_{50} (μM) [% Glu Max[b]]	Fold Selective versus $mGlu_4$
12a	F	0.013 [84 ± 2]	7.90 ± 0.08	>10 [—]	>793
12b	Cl	0.029 [68 ± 5]	7.54 ± 0.09	>10 [—]	>344
12c	Me	0.015 [90 ± 2]	7.82 ± 0.09	0.609 [52]	9.8
12d	CF_3	>10 [—]	>5.00	>10 [—]	—
12e	OMe	0.330 [80 ± 6]	6.48 ± 0.19	>10 [—]	>30.3
12f		>10 [—]	>5.00	>10 [—]	—
12g		>10 [—]	>5.00	>10 [—]	—

[a] Calcium mobilization $mGlu_1$ assays, values are average of three ($n = 3$) independent experiments performed in triplicate.
[b] Glu Max is expressed as % of PHCCC response.

In conclusion, our first exploration of central core SAR of the VU0486321 series of $mGlu_1$ positive allosteric modulators (PAMs) provided a combination of both steep and tractable SAR, unexpected findings and new avenues for optimization. Interestingly, analogs where the halogen on the central phenyl core was located adjacent to the 3-furyl amide eliminated activity at $mGlu_4$, providing analogs such as **12a** (>793-fold selective vs $mGlu_4$) and **12b** (>344-fold selective vs $mGlu_4$). DMPK properties hindered these analogs' utility as in vivo probes, but lessons learned will be applied to other scaffolds, and these results will be reported in due course.

Acknowledgments

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