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Lead optimization of the VU0486321 series of mGlu₁ PAMs. Part 1. SAR of modifications to the central aryl core

Pedro M. Garcia-Barrantes^a, Hyekyung P. Cho^{a,b}, Anna L. Blobaum^a, Colleen M. Niswender^{a,b}, P. Jeffrey Conn^{a,b}, and Craig W. Lindsley^{a,b,c,*}

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

^bDepartment of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN 37232, USA

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

Abstract

This letter describes the lead optimization of the VU0486321 series of mGlu₁ positive allosteric modulators (PAMs). While first generation PAMs from Roche were reported in the late 1990s, little effort has focused on the development of mGlu₁ PAMs since. New genetic data linking loss-of-function mutant mGlu₁ receptors to schizophrenia, bipolar disorder and other neuropsychiatric disorders has rekindled interest in the target, but the ideal *in vivo* probe, e.g. 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 mGlu₄.

Graphical abstract

Keywords

mGlu₁; Metabotropic glutamate receptor; Positive allosteric modulator (PAM); Schizophrenia; Structure-Activity Relationship (SAR)

*To whom correspondence should be addressed: craig.lindsley@vanderbilt.edu.

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Recently, deleterious non-synonymous single nucleotide polymorphisms (nsSNPS) in the *GRM1* gene, which encodes metabotropic glutamate receptor subtype 1 (mGlu₁), 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 mGlu₁ PAMs;³ however, neither the first generation Roche mGlu₁ PAM (**1**, Ro 07-11401)⁴ nor our chemically distinct series (**2**, VU0483605),³ derived from an mGlu₄ 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 mGlu₁ PAM with moderate rat PK (CL_p = 13.3. mL/min/kg, t_{1/2} = 54 min), good free fraction (human fu = 0.05, rat fu = 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 mGlu₄ (**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 mGlu₁ and mGlu₄ 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 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 mGlu₁ PAMs with EC₅₀s 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 mGlu₄ varied greatly with respect to mGlu₄ PAM potency (EC₅₀s from 260 nM to >10 µM) and efficacy (% Glu Max from 35 to 122). While lack of activity at mGlu₄ is key for an mGlu₁ PAM *in vivo* probe, a dual mGlu₁/mGlu₄ PAM is intriguing in an antipsychotic agent, as mGlu₄ 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 (mGlu₁ EC₅₀ = 48 nM), but also >208-fold selective versus mGlu₄ (mGlu₄ EC₅₀ >10 µM). All other modifications to either the phenyl core or the phthalimide moiety engendered more significant activity at mGlu₄, but mGlu₁ was still

preferred. Relative to **10a**, the addition of substituents at either R¹ or R² provided potent mGlu₁ 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-CF₃ on the phenyl core was less effective, affording a diminution in mGlu₁ PAM potency relative to **10a**, providing analogs such as **10o**, with an mGlu₁ EC₅₀ 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 mGlu₁ PAM potency (EC₅₀s 35 nM to 387 nM) and a range of selectivity versus mGlu₄ (~18- to 52-fold). Installation of a 2-pyrimidinyl core, analogs **11e–h**, lost significant activity at mGlu₁ (EC₅₀s 200 nM to > 5 μM), and selectivity versus mGlu₄ was eroded. Indeed, both **11e** and **11h**, were equipotent mGlu₁ and mGlu₄ PAMs. Replacement of the phenyl core with a naphthyl bicyclic ring system, e.g., **11i–m**, afforded potent mGlu₁ PAMs (EC₅₀s 16 nM to 23 nM), irrespective of the phthalimide substituent, with modest selectivity versus mGlu₄ (17.4- to 41-fold). Thus, from these two efforts, while mGlu₁ potency and efficacy were attractive, the overall profile and selectivity versus mGlu₄ 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, e.g., analogs **12**. Analogs **12** were accessed as shown in Scheme 1, and these analogs afforded the greatest selectivity versus mGlu₄ to date (>793 fold), despite possessing an unsubstituted phthalimide moiety. Here, the fluoro analog, **12a**, was a potent mGlu₁ PAM (EC₅₀ = 12.6 nM, 84% Glu Max) with >793-fold selectivity versus mGlu₄ (EC₅₀ > 10 μM), and **12b**, the chloro congener, was similarly potent (mGlu₁ PAM EC₅₀ = 29.1 nM, 68% Glu Max) with >344-fold selectivity versus mGlu₄ (EC₅₀ > 10 μM). Also, these compounds act like pure PAMs and did not show any observable agonistic effect in mGlu₁ as well as no activity at mGlu₅. Interestingly, the CF₃ 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.

From these efforts, **12a** and **12b** emerged as mGlu₁ PAMs with the requisite potency and selectivity versus mGlu₄ 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) assays⁹ 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* mGlu₁ PAM tool compound.

In conclusion, our first exploration of central core SAR of the VU0486321 series of mGlu₁ 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-furly amide eliminated activity at mGlu₄, providing analogs such as **12a** (>793-fold selective versus mGlu₄) and **12b** (>344-fold selective versus mGlu₄). 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.

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Figure 1. Structures of reported mGlu₁ 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, r.t., 59–97%; (b) H_2 , Pd/C, EtOH, rt, 94–99%; (c) 3-methylfuran-2-carbonyl chloride, DIEA, DCM, r.t., 39–98%; (d) i. TFA, DCM, r.t. ii. phthalic anhydrides, AcOH, reflux, 53–94%.

Table 1

Structures and activities for analogs **10**.

<div> </div> 10						
Cpd	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

^dCalcium mobilization mGlu₁ assays, values are average of three (n=3), independent experiments performed in triplicate.

^b Glu Max is expressed as % of PHCC response, which is used as a normalization control as the % max values vary on a daily basis for the mGlu4 assay.

Table 2

Structures and activities for analogs **11**.

Cpd	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

11						
Cpd	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
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 mGlu₁ assays, values are average of three (n=3) independent experiments performed in triplicate.

^b Glu Max is expressed as % of PHCCC response.

Table 3

Structures and activities for analogs **12**.

12					
Cpd	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 mGlu₁ assays, values are average of three (n=3) independent experiments performed in triplicate.^b Glu Max is expressed as % of PHCCC response.