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Re-exploration of the mGlu₁ PAM Ro 07-11401 scaffold: Discovery of analogs with improved CNS penetration despite steep SAR

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Abstract

This letter describes the re-exploration of the mGlu₁ PAM Ro 07-11401 scaffold through a multi-dimensional, iterative parallel synthesis approach. Unlike recent series of mGlu₁ PAMs with robust SAR, the SAR around the Ro 07-11401 structure was incredibly steep (only ~6 of 200 analogs displayed mGlu₁ PAM activity), and reminiscent of the CPPHA mGlu₅ PAM scaffold. Despite the steep SAR, two new thiazole derivatives were discovered with improved *in vitro* DMPK profiles and ~3- to 4-fold improvement in CNS exposure (K_p s 1.01 to 1.19); albeit, with a ~3-fold diminution in mGlu₁ PAM potency, yet comparable efficacy (~5-fold leftward shift of the glutamate concentration-response curve at 10 μ M). Thus, this effort has provided additional CNS penetrant mGlu₁ PAM tools in a different chemotype than the VU0486321 scaffold. These compounds will permit a better understanding of the pharmacology and therapeutic potential of selective mGlu₁ activation, while highlighting the steep SAR challenges that can often be encountered in GPCR allosteric modulator discovery.

Graphical Abstract

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Keywords

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

Efforts towards the development of positive allosteric modulators (PAMs) of the metabotropic glutamate receptor subtype 1 (mGlu₁) were pioneered by Knoflach and co-workers at Roche, resulting in **1-4** (Figure 1).¹⁻³ These small molecule PAMs, coupled with data generated with negative allosteric modulators (NAMs) of mGlu₁,^{4,5} highlighted issues with species differences due to a single amino acid in rat versus human mGlu₁,^{6,7} and thus **4**, a PAM active on both human and rat mGlu₁, emerged as a valuable tool compound, despite modest CNS penetration (K_p = 0.29 and high protein binding (f_u < 0.01). For over a decade, **4** was the only *in vivo* tool compound to study selective mGlu₁ activation.⁸⁻¹¹ Based on recent genetic data implicating *GRM1* in schizophrenia,¹²⁻¹⁴ coupled with data showing that the adverse effect liabilities of group I metabotropic glutamate receptors (mGluRs) are mediated by mGlu₅ and not mGlu₁,¹⁵ our lab has launched a program to develop the next generation of mGlu₁ PAMs.¹⁴⁻¹⁸ In the past year, we have reported on the discovery and optimization of novel mGlu₁ PAMs **5-7** with improved potency (EC₅₀s < 20 nM), DMPK profiles (f_us > 2.0% unbound) and CNS penetration (K_ps > 1) to afford new avenues for target validation and to assess the therapeutic potential of selective mGlu₁ activation.¹⁴⁻¹⁸

Previously, revisiting the mGlu₄ PAM (-)-PHCCC scaffold led to the discovery of improved tool compounds.¹⁹ Therefore, over a decade after its discovery, we felt it was prudent to revisit the Ro 07-11401 scaffold in an effort to develop an *in vivo* tool compound within this series with improved disposition to account for any chemotype or ligand-biased pharmacology and expand the repertoire of validation tools for mGlu₁.

In our functional assays, Ro 07-11401 (**4**) was an equipotent mGlu₁ PAM on both rat (EC₅₀ = 276.5 nM, pEC₅₀ = 6.56 ± 0.08, 109 ± 3% Glu Max) and human (EC₅₀ = 246.0 nM, pEC₅₀ = 6.61 ± 0.08, 96 ± 3% Glu Max), but with a modest disposition profile (*vide infra*).¹⁴⁻¹⁸ Thus, we pursued a multidimensional optimization plan (Figure 2) to explore SAR around **4** in an attempt to improve disposition by identifying replacements for the weakly basic oxadiazole (effectively neutral with the pendant CF₃ moiety) with analogs **9** and the lipophilic 9H-xanthene moiety in analogs **8**. Once identified, optimal moieties would then be combined.

The synthesis was straightforward (Scheme 1). A variety of commercially available 2-amino-4-substituted oxazoles **10** were coupled under HATU conditions with a diverse array of carboxylic acids to provide analogs **8** in yields ranging from 18–54%. Similarly, the 9H-xanthene-9-carboxylic acid **11** was coupled under HATU conditions to a variety of 5- and 6-

membered heterocyclic amines to deliver analogs **9** in 11–94% yields. For both series, many of the amines and acids coupled poorly due to a combination of steric and stereoelectronic effects, as well as a range of poor solubility.

In all, over 200 analogs of **8** and **9** were synthesized and triaged via a 10 μ M single point screen on human mGlu₁, using an EC₂₀ concentration of glutamate, prior to full concentration-response curves (CRCs) on both human and rat mGlu₁. Surprisingly, all analogs **8** (Figure 3) were uniformly inactive mGlu₁ PAMs (no potentiation of an EC₂₀ of glutamate at a concentration of 10 μ M), indicating that the 9*H*-xanthene was a critical pharmacophore. The SAR was remarkably steep, and reminiscent of the steep SAR encountered with the non-MPEP, mGlu₅ PAM CPPHA,²⁰ wherein virtually any modification led to a complete loss of PAM activity. Obviously, these data cast doubt on the success of the campaign with analogs **9**, wherein the 9*H*-xanthene amide was held constant. While SAR once again was steep, active analogs did result (Table 1); however, functionalized pyrazoles, oxazoles, oxadiazoles, thiophenes, piperidines, azetidines, cycloalkyl, a structurally diverse array of tertiary amides and functionalized aryl/benzyl derivatives were also devoid of mGlu₁ PAM activity (inactive up to 10 μ M). Only a sub-set of thiazole amides proved to be active as mGlu₁ PAMs. Here, the 2-thiazole regioisomer **9c** (human EC₅₀ = 1.4 μ M, pEC₅₀ = 5.85 $\pm$ 0.07, 75 $\pm$ 3% Glu Max and rat EC₅₀ = 3.6 μ M, pEC₅₀ = 5.43 $\pm$ 0.17, 111 $\pm$ 7% Glu Max) proved active on both human and rat mGlu₁, while the regioisomeric 5-thiazole congener **9e** was a weak PAM (EC₅₀>10 μ M). Derivatives of **9c** with substitution in the 4-position, such as the 4-CN **9a** and the 4-*i*Pr **9b** congeners, displayed submicromolar activity on human (mGlu₁ EC₅₀ = 0.711 μ M, pEC₅₀ = 6.1 $\pm$ 0.07, 110 $\pm$ 6% Glu Max and mGlu₁ EC₅₀ = 0.90 μ M, pEC₅₀ = 6.04 $\pm$ 0.07, 89 $\pm$ 3% Glu Max, for **9a** and **9b**, respectively) and low micromolar potency on rat (mGlu₁ EC₅₀ = 1.8 μ M, pEC₅₀ = 5.73 $\pm$ 0.13, 124 $\pm$ 8% Glu Max and mGlu₁ EC₅₀ = 2.4 μ M, pEC₅₀ = 5.62 $\pm$ 0.11, 111 $\pm$ 7% Glu Max, for **9a** and **9b**, respectively). Replacement of the thiazole **9e** for the benzothiazole **9f** caused almost a 3-fold increase in potency, whereas a 4,5-diMe thiazole **9g** displayed weak PAM activity. Finally, a 2-pyridyl analog (**9h**) showed moderate activity as an mGlu₁ PAM. In total, we had synthesized and assayed ~200 analogs based on **8** and **9**, and only eight compounds (4% hit rate) displayed significant mGlu₁ PAM activity, highlighting why **4** was advanced early on as an *in vivo* tool in this series. Moreover, no ‘molecular switches’ were noted,²¹ as compounds were either PAMs or inactive.

Having identified **9a** and **9b**, more basic thiazole analogs of **4**, we decided to further profile them, despite the diminution in PAM potency. We assessed their ability to leftward shift a glutamate CRC (an assessment of efficacy), to gauge their ability to render glutamate a more potent agonist (Figure 4). All three compounds induced a comparable, robust leftward shift (4.6 to 6.9-fold) of the glutamate CRC at a 10 μ M concentration.

Then, we assessed the *in vitro* DMPK profiles of **4**, **9a** and **9b**, as well as determined CNS penetration (Table 2).¹⁵ The prototypical PAM in this series, **4**, displayed high intrinsic clearance (at hepatic blood flow) in both rat and human microsomes, very high protein and brain homogenate binding, a mixed CYP profile (with potent, 600 nM inhibition of 2C9) and modest CNS penetration (K_p = 0.29). PAM **9b** looked identical across all of the assays, except for CNS penetration, where the K_p was 1.01 (~3-fold improvement over **4**). In

contrast, PAM **9a** displayed an improved overall profile, except for CYPs, where as **9b** showed an improved CYP profile. In general, inhibition of CYP 2C9 appears to be an issue with this chemotype. Human microsomal intrinsic clearance was reduced by ~50%, predicting a moderate hepatic clearance ($CL_{\text{hep}} = 9.85 \text{ mL/min/kg}$), and there was measureable free drug in human plasma ($F_u = 0.027$) and rat brain homogenate ($F_u = 0.017$). Importantly, CNS penetration was also improved for **9a**, affording a K_p of 1.19 (an ~4-fold increase compared to **4**). While rat intrinsic clearance did not improve beyond that of **4**, the enhanced free drug in brain (>10-fold), coupled with diminished peripheral exposure, provide advantages for this chemical class of mGlu₁ PAMs as CNS tool compounds. Thus, **9a** emerged as an improved mGlu₁ PAM tool compound within the Ro 07-11401 (**4**) series; however, the ideal *in vivo* tool compound from this scaffold remains elusive due to steep SAR.

In conclusion, we revisited the Ro 07-11401 (**4**) series of mGlu₁ PAMs in an effort to develop an improved *in vivo* tool compound within this scaffold. Unlike the recent VU0486321 series of mGlu₁ PAMs which exhibited robust SAR, the SAR of the Ro 07-11401 series was incredibly steep (only ~8 of 200 analogs displayed mGlu₁ PAM activity), and reminiscent of the CPPHA mGlu₅ PAM scaffold. Despite the steep SAR, two new thiazole derivatives (**9a**, VU6000799, and **9b**, VU6000790) were discovered with improved *in vitro* DMPK profiles and ~3- to 4-fold improvement in CNS exposure (K_p s 1.01 to 1.19), albeit an ~3-fold diminution in mGlu₁ PAM potency, yet comparable efficacy (~5 left fold shift of a glutamate CRC at 10 μM). A modeling effort is underway to provide further insight to direct future chemical optimization of this scaffold. Studies are ongoing and will be reported in due course.

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Figure 1.
Structures of representative mGlu₁ PAMs 1-7.

Figure 2. Chemical optimization plan to identify replacements for the lipophilic oxadiazole with analogs **9**, and the lipophilic 9*H*-xanthene with analogs **8**.

Figure 3.
Representative 9*H*-xanthene amide replacement analogs **8** that are inactive mGlu₁ PAMs.

Figure 4.

Efficacy (fold-shift) study of a glutamate CRC in the absence (grey) and presence (blue) of a 10 μ M addition of an mGlu₁ PAM. A) **9a** induces an ~ 5.4 -fold shift in the glutamate CRC with an increase in Glu Max; B) **9b** induces an ~ 4.6 -fold shift in the glutamate CRC with an increase in Glu Max; and C) **4** induces an ~ 6.9 -fold shift in the glutamate CRC with a slight increase in Glu Max.

Scheme 1.

Reagents and conditions: (a) R³CO₂H, DIEA, DMF, 60 °C, 18–54%, (b) H₂NR¹, HATU, DIEA, DCE, rt, 11–94%.

Table 1Structures and activities for analogs **9**.

9			
Cpd	R ¹	hmGlu ₁ EC ₅₀ (μM) ^a [% Glu Max ± SEM]	mGlu ₁ pEC ₅₀ (±SEM)
9a		0.71 [110±6]	6.14±0.12
9b		0.90 [89±3]	6.04±0.07
9c		1.4 [75±3]	5.85±0.07
9d		2.7 [101±7]	5.57±0.11
9e		>10 [54±7]	<5
9f		4.8 [96±11]	5.32±0.20
9g		>10 [68±8]	<5

Cpd	R ¹	hmGlu ₁ EC ₅₀ (μM) ^a [% Glu Max ± SEM]	mGlu ₁ pEC ₅₀ (±SEM)
9h		1.7 [58±3]	5.77±0.11

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

Table 2DMPK characterization of mGlu₁ PAMs **4**, **9a** and **9b**.

Parameter	4	9a	9b
Hum CL _{hep} (mL/min/kg)	19.4	9.85	15.5
Rat CL _{hep} (mL/min/kg)	63.3	62.2	64
Hum <i>F_u</i> plasma	0.014	0.027	0.001
Rat <i>F_u</i> plasma	0.003	0.005	0.002
Rat <i>F_u</i> brain	0.001	0.017	0.001
CYP ₄₅₀ IC ₅₀ (μM)			
1A2 2C9	13 0.6	2.9 2.9	19 2.1
2D6 3A4	>30 >30	10.3 >30	26.5 >30
Rat IV PBL (0.25 mg/kg)			
C _n plasma (ng/mL)	276	80.2	77.8
C _n brain (ng/g)	81	83.6	78.7
K _p (at 0.25 h)	0.29	1.19	1.01