Bioorganic & Medicinal Chemistry Letters 19 (2009) 3275–3278

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Discovery of a potent and brain penetrant mGluR5 positive allosteric modulator

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ARTICLE INFO

Article history:
Received 24 February 2009
Revised 19 April 2009
Accepted 20 April 2009
Available online 24 April 2009

Keywords:
mGluR5
Glutamate
Allosteric
Positive allosteric modulator
Rat
Mouse

ABSTRACT

This Letter describes the discovery of a novel series of mGluR5 positive allosteric modulators (PAMs). The lead compound, **11c**, exhibits excellent potency (EC_{50} = 30 nM) in vitro, and reaches high brain levels in both rats and mice after oral administration.

Glutamate is the principal excitatory neurotransmitter in the mammalian central nervous system, and it mediates its effects through interaction with ionotropic and metabotropic receptors. The metabotropic glutamate receptors (mGluRs) are class C GPCRs and eight subtypes are known. They are divided into three groups based on their preferred G protein coupling and pharmacological responses to various ligands: Group I consisting of mGluR1 and mGluR5, group II consisting of mGluR2 and mGluR3, and group III consisting of mGluR4, mGluR6, mGluR7, and mGluR8.[1] These receptors contain two distinct domains; a large extracellular domain which binds glutamate at the orthosteric binding site, and a heptahelical transmembrane domain, which has been found to bind a variety of ligands at one or more allosteric binding sites.[2] The effects of glutamate binding on receptor activation can be modulated either positively or negatively by the binding of ligands to an allosteric site.

Activation of mGluR5 has been postulated to ameliorate both positive symptoms and cognitive deficits in schizophrenia.[3–9] It has proven difficult to devise selective agonists at this receptor because of its close homology with the mGluR1 receptor, particularly at the orthosteric binding site.[10] Recently, positive allosteric modulators (PAMs) binding to sites in the transmembrane domain have been reported.[11–13] Unlike orthosteric agonists, PAMs can be highly selective for the mGluR subtype of interest because they bind to sites with lower levels of homology than the L-Glu binding site. We now wish to report a new series of mGluR5 PAMs.[14] The lead compound of this series exhibits excellent potency and metabolic stability, sufficient aqueous solubility to allow in vivo dosing in DMSO-free vehicles, and high brain exposure after oral administration to rodents.

A screening campaign of a collection of approximately 50,000 compounds using a functional Ca^{2+} flux assay yielded a moderately potent hit **1** (Fig. 1). The assay was set up using transiently transfected BHK cells to detect PAMs by adding the test compound together with a low concentration of L-Glu (0.30 μM roughly corresponding to an EC_{10} of this agonist).[15] Potentiation of this low response indicates PAM activity of the test compound. In this assay, screening hit **1** had an EC_{50} for potentiation of 0.97 μM and potentiated the L-Glu signal to 73% of its maximum value at saturating L-Glu concentration.

The structure of **1** shares the (2-phenylethynyl)aryl motif with the previously reported mGluR5 PAM **2**,[16] and also with the negative allosteric modulator (NAM) MPEP (**4**),[10,17] while the structure of the PAM CDPPB (**3**)[9,18] is unrelated. We reasoned that one of the aminal hydrogen bond acceptor nitrogen atoms of **1** might overlap with the carbonyl hydrogen bond acceptor of **2** and that both of these compounds would bind similarly to the receptor. Based on this hypothesis, transformation of the aminal-bearing benzene ring to a pyridine ring and exploration of alternative hydrogen bond acceptors to replace the aminal moiety were investigated.

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doi:10.1016/j.bmcl.2009.04.095

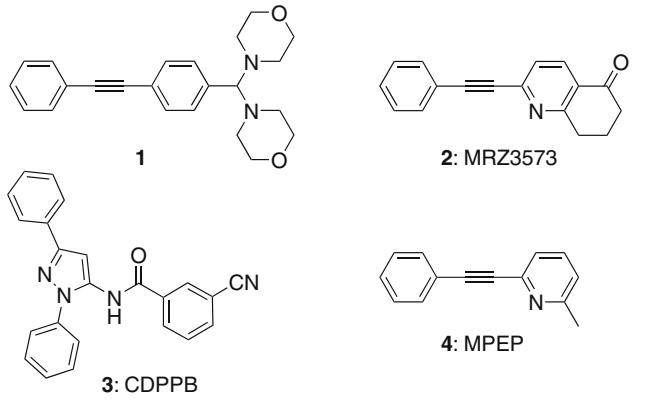


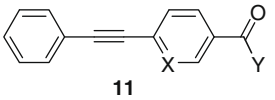
Figure 1. Original screening hit **1**, previously known PAMs **2** and **3**, and NAM **4**.

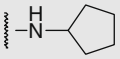
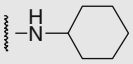
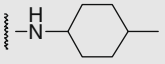
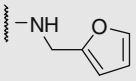
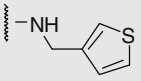
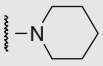
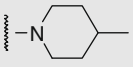
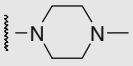
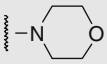
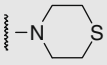
Carboxylic acid esters and amides were selected as suitable hydrogen bond acceptors, and a flexible synthesis of the target compounds **7** and **11** was developed (Scheme 1).[19] Iodonicotinic acid **5**[20] was esterified and coupled with phenylacetylene using a microwave-assisted Sonogashira reaction to afford ethyl ester **7**. This compound could be transformed into the primary amide **11a** (Table 1) using NaCN and methanolic ammonia, or could be hydrolyzed to the acid **8**. This acid, or the corresponding benzoic acid **10** (obtained from oxidation of commercially available aldehyde **9**) was conveniently coupled with a variety of primary and secondary amines using solid-supported carbodiimide as a coupling reagent.

By this protocol, 16 compounds were prepared, and their potencies as mGluR5 PAMs were evaluated (Table 1). Small substituents on the carbonyl yielded very weakly active or inactive compounds, for example, **7** and **11a**, but larger substituents afforded compounds with a range of activities. Both secondary and tertiary amides were active, and the optimum potency was achieved with five or six heavy atoms in a five- or six-membered lipophilic ring. Three pairs of compounds, pair-wise identical except for the aromatic CH to N transformation in the amide-bearing ring, where included to investigate the effect of this substitution. In all three cases, the pyridine derivative was more potent than the benzene one.

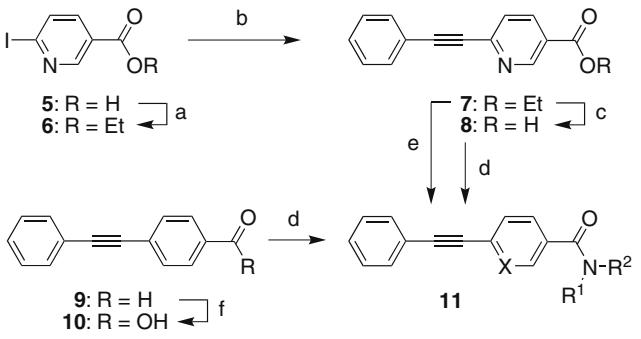
The most potent compound, **11c**, was selected for further investigation. First, its allosteric mode of action was confirmed by its inability to displace the orthosteric agonist [^{3}H]-quisqualic acid in a radioligand binding assay. At concentrations above 1 μM, **11c** actually enhanced binding of [^{3}H]-quisqualic acid slightly

Table 1
Structures and potencies of compounds **7** and **11**



Compound	X	Y	EC_{50}[a] (μM)
11a	N	NH_2	6.6
7	N	OEt	[b]
11b	N	$HNCH_2CH_2CH(CH_3)_2$	0.51
11c	N		0.030
11d	N		0.24
11e	N		1.2
11f	N		0.18
11g	CH		1.2
11h	N		0.14
11i	N		0.28
11j	CH		0.72
11k	N		0.13
11l	CH		3.1
11m	N		1.6
11n	N		0.99
11o	N		0.046

[a] EC_{50} of the test compound for the potentiation of the response to a low concentration of L-Glu (0.30 μM roughly corresponding to an EC_{10} of this agonist).
[b] No effect at 10 μM.



Scheme 1. Reagents and conditions: (a) EtOH, H_2SO_4, 3 h, reflux, 63% yield; (b) PhC≡CH, $PdCl_2(PPh_3)_2$, CuI, Et_3N, 140 °C, 10 min, μw, 96% yield; (c) LiOH, THF, water, rt, 2.5 h, 73% yield; (d) HNR^1R^2, PS-Carbodiimide, 1,2-dichloroethane, 50 °C, 18 h; (e) NaCN, NH_3, MeOH, 50 °C, 16 h; (f) $NaClO_2$, KH_2PO_4, 2-methyl-2-butene, *t*-BuOH, water, rt, 1.5 h, 99% yield.

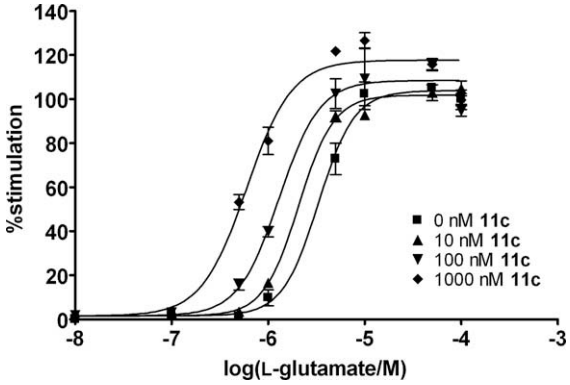


Figure 2. Concentration–response curves of L-glutamate alone and together with three fixed concentrations of **11c** at the human mGlu5 receptor. The EC_{50} for L-glutamate was 3.3 μM in the absence of **11c** and shifted to 2.1, 1.3, and 0.6 μM in the presence of **11c** at 10, 100, and 1000 nM, respectively.

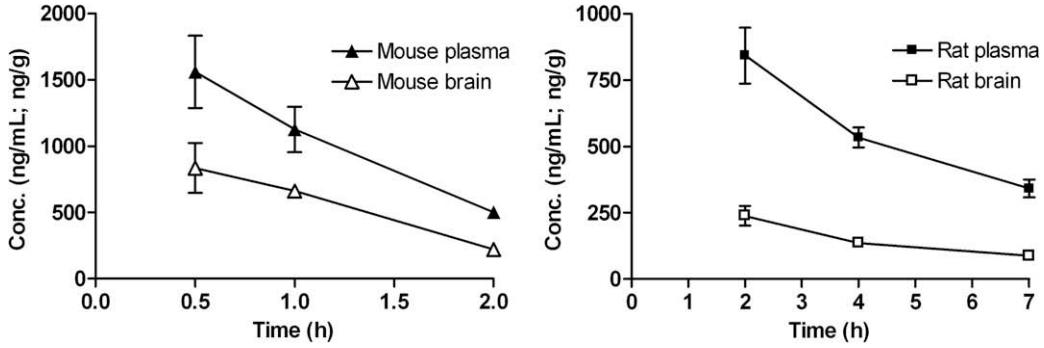


Figure 3. Plasma (ng/mL) and brain (ng/g) concentration–time courses of **11c** following oral administration of 5 mg/kg (C57/6J mice) and 2.5 mg/kg (Lister Hooded rats). Each data point represents an average of $n = 3 \pm$ SEM.

(ca. 30%, see Supplementary data), clearly demonstrating that **11c** does not bind to the orthosteric site. Because of its structural similarity with MPEP (**4**), it was expected that **11c** would displace [^{3}H]-MPEP or the structurally similar [^{3}H]-methoxy-PEPy[2,21] in a radioligand binding assay. It has been shown that some PAMs, for example, CDPPB (**3**) do displace [^{3}H]-methoxy-PEPy,[9] while at least CPPHA does not.[22] Interestingly, for CDPPB (**3**) binding affinity versus [^{3}H]-methoxy-PEPy was two orders of magnitude lower than functional efficacy. Indeed **11c** was found to displace [^{3}H]-MPEP with $K_i = 1.8$ μM, suggesting that this compound binds to the same site as MPEP (**4**) and CDPPB (**3**). Next, concentration–response curves (CRCs) of L-Glu were recorded in the presence of three fixed concentrations of **11c**. As expected, a concentration-dependent leftward shift of the L-Glu CRC was observed, but at the highest tested concentration of **11c**, 0.60 μM, a clear depression of the maximum response of the mGluR5 receptor to L-Glu was detected. At this concentration, an eightfold shift of the CRC was found. Because other mGluR5 PAMs, for example, CDPPB (**3**),[9,18] DFB[23] and CPPHA[22] were not reported to exhibit this depression of the maximum response, we suspected it to be an artifact of our assay.[24] To test this hypothesis, a new assay was developed using stably transfected BHK cells. In this new assay, L-Glu EC_{10} was 1.0 μM and the EC_{50} of **11c** for the potentiation of the response to this L-Glu concentration was 0.90 μM. CRCs of L-Glu were again recorded in the presence of three fixed concentrations of **11c** (Fig. 2). As with the old assay, a leftward shift of the L-Glu CRC was observed, but no depression of the maximum response was seen. Also, under these assay conditions, no depression of the maximum response was observed for the PAM reference compound CPPHA (see Supplementary data). At 1.0 μM, the highest tested concentration of **11c**, a 5.5-fold shift of the CRC was found, as the L-Glu EC_{50} shifted from 3.3 to 0.6 μM.

The selectivity of compound **11c** was evaluated against a panel of 63 receptors and seven enzymes using binding assays. At 10 μM, **11c** caused less than 50% inhibition of radioligand binding in all cases. In a second evaluation against a panel of 34 GPCRs using functional assays designed to detect both agonism and antagonism, **11c** caused less than 30% activation or inhibition at 10 μM in all cases. A full panel of mGlu receptors was not available to us, but at mGluR1, **11c** showed no agonism, antagonism or allosteric modulation at 10 μM.

Low aqueous solubility has hampered in vivo experiments with previous mGluR5 PAMs,[18] or in the case of CDPPB (**3**), necessitated the use of rather harsh vehicles containing significant fractions of DMSO.[6,9] The solubility of compound **11c** in aqueous pH 7.4 buffer was found to be modest, approximately 0.5 μg/mL corresponding to 1.7 μM. Because this is >50-fold above the EC_{50} value, and significantly above the highest concentration tested in the L-Glu CRC shift described above (Fig. 2), solubility should not limit the usefulness of **11c** as an in vivo tool compound. Exposure studies with compound **11c** showed it to be orally absorbed with high plasma concentrations following administration of 5 mg/kg in mice and 2.5 mg/kg in rats (Fig. 3). A vehicle consisting of PEG400/Cremophor (80/20 v/v) dosed in a volume of 5 mL/kg was used for all experiments.[15] The ability of compound **11c** to permeate the blood-brain barrier was verified from analysis of brain homogenate samples. Brain-plasma distribution ratios of 0.5 and 0.3 were found in mice and rats, respectively, over the entire duration of the concentration–time courses. The elimination half-lives were approximately 1 and 4 h in mice and rats with similar half-lives in plasma and brain within the species. These pharmacokinetic properties make compound **11c** useful as tool compound for in vivo pharmacological investigations in rodents.

In conclusion, a potent mGluR5 PAM, **11c**, has been discovered, and the adequate physicochemical properties and good metabolic stability of this lead should make it useful as an in vivo pharmacological tool for investigating the mGluR5 receptor.

Acknowledgment

The authors thank Mr. Jude Alfred for technical assistance.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.04.095.

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24. Measured EC_{50} values for these compounds using this assay were in accordance with those reported in the literature.