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[^{3}H]-M-MPEP, a Potent, Subtype-Selective Radioligand for the Metabotropic Glutamate Receptor Subtype 5

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Abstract—The synthesis of a new potent, subtype-selective radioligand [^{3}H]-M-MPEP (2-methyl-6-((3-methoxyphenyl)ethynyl)-pyridine) and its in vitro pharmacological characteristics are described.

Introduction

L-Glutamate is the main excitatory neurotransmitter in the mammalian brain and acts through a heterogeneous family of two major types of receptors: ionotropic (iGluRs) and metabotropic glutamate receptors (mGluRs). Ionotropic glutamate receptors are ligand gated channels, whereas metabotropic receptors are G-protein coupled receptors (GPCRs) linked to second messenger pathways. To date eight metabotropic receptor subtypes have been identified and classified into three groups according to their amino acid identities, second messenger coupling and pharmacology.[1] Group I mGluRs (1 and 5) are coupled to the phosphoinositide/Ca^{2+} cascade. Group II mGluRs (2 and 3) and Group III mGluRs (4–8) are negatively coupled to adenylate cyclase. mGlu receptors are members of the superfamily 3 of GPCRs and possess a unique structure with a large N-terminus involved in the glutamate recognition site.[2–4]

mGluR Ligands

The vast majority of known mGluR ligands are amino acid derivatives and therefore interact with the conserved glutamate binding site.[5] Until recently the lack of potent and selective ligands has hampered the development of radioligands to devise useful receptor binding assays. The identification of potent and Group II selective ligands such as DCG-IV, LY354740 and LY341495 opened up the possibility for the radiolabeling and recent reports described the characterization of [^{3}H]-LY354740 (**1**), [^{3}H]-LY341495 (**2**), [^{3}H]-DCG-IV (**3**) and [^{3}H]-quisqualatic acid (**4**), respectively (Fig. 1).[6–9] Recent work by Mutel et al.[10] demonstrated that a non-selective agonist, [^{3}H]-Quisqualate (**4**) (Fig. 1), could be a useful tool to label Group I mGlu receptors (1 and 5). However, this radioligand does not allow us to selectively label each Group I subtype and therefore there is still a lack of subtype-selective ligands.

Chemistry

Recently, we reported the identification and the characterization of a series of non-competitive mGluR5 selective antagonists illustrated by SIB-1757 [6-methyl-2-(phenazo)-pyridin-3-ol] (**5**), SIB-1893 [(*E*)-2-methyl-6-(phenylethenyl)-pyridine] (**6**) and MPEP [2-methyl-6-(phenylethynyl)-pyridine] (**7**) (Fig. 2).[11,12] In the course of our optimization program we aimed to identify a potent and suitable derivative for radiolabeling in view of the development of a radioligand based binding assay. Such a radioligand binding technique could be a useful tool to investigate the receptor distribution and pharmacology.

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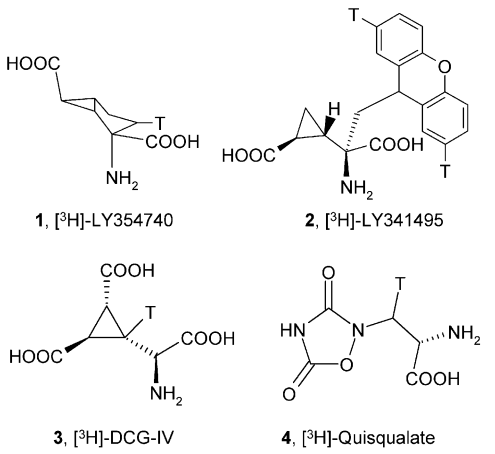


Figure 1. Structures of currently known tritiated radioligands for metabotropic glutamate receptors and the corresponding radiolabeling position.

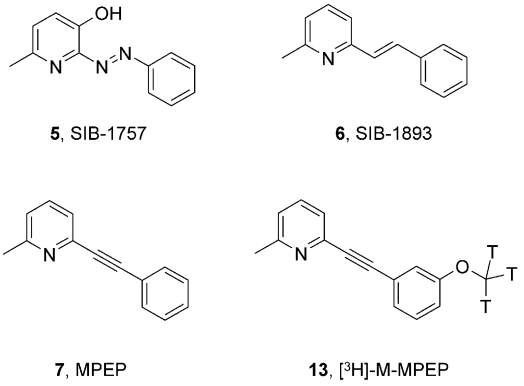


Figure 2. Structures of non-competitve antagonists.

The lead optimization process of this series started from the original leads **5** (SIB-1757) and **6** (SIB-1893) (Fig. 2).[11] Several lines of chemical modifications were investigated such as modification of the nature and the length of the spacer between the two aromatic moieties. These modifications led to the identification of MPEP [6-methyl-2-(phenylethynyl)pyridine], the first potent and selective $mGlu_5$ receptor antagonist.[12] Further investigations of the substituents around the phenyl ring allowed the identification of the methoxy substituted derivative 2-methyl-6-((3-methoxyphenyl)ethynyl)-pyridine (M-MPEP, **13**), a more potent derivative ($IC_{50} = 10$ nM, PI turnover assay; Fig. 3; Table 1) which was considered for radiolabeling at the methoxy position.

The synthesis of the radioligand is shown in Scheme 1 and uses 2,6-dimethyl-pyridine **8** and 3-hydroxy-benzaldehyde **9** as starting materials. **8** and **9** are condensed into the styrene derivative **10** in acetic anhydride. This intermediate was dibrominated and the mixture of dibromo derivatives **11** eliminated using potassium tert-butylate to deliver **12**, which contains the necessary phenolic function for the methylation. Introduction of the methyl can be performed either with dimethylsulfate or with methyliodide as alkylating agents. The latter is more suitable for the radiosynthesis since [^{3}H]-methyl-iodide is commercially available.

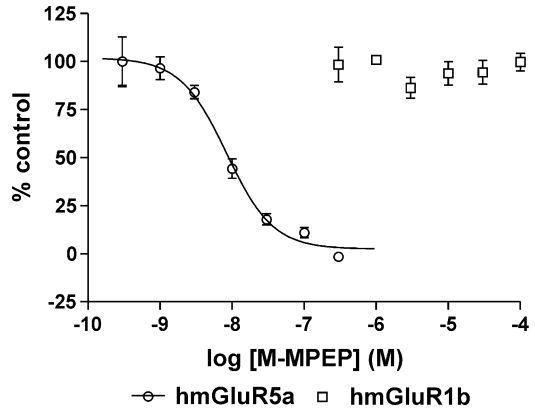


Figure 3. Effects of M-MPEP on PI turnover at the human mGluR5a stably expressed in L(tk−) cells (○) and the human mGluR1b stably expressed in CHO-K1 cells (□). Results are expressed as percentage (mean ± SEM) of the stimulation obtained with the agonists at the respective EC_{80} (quisqualate at 0.3 and 20 μM for human mGluR5a and mGluR1b, respectively). Percentages were pooled from $N = 3$ independent experiments performed in triplicate.

Table 1. In vitro functional activity and binding affinity for the recombinant human mGlu5 receptor

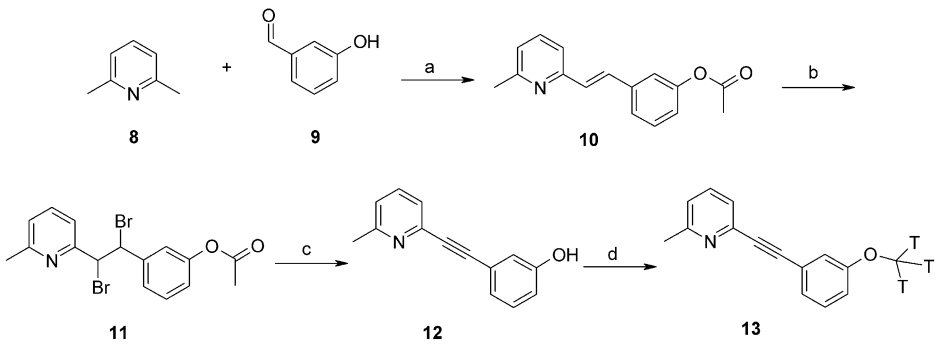
	IC_{50} (nM)	
	PI-hydrolysis[a]	[^{3}H]-M-MPEP displacement[b]
13, M-MPEP	10	3.6
7, MPEP	36	20
6, SIB-1893	3000	1100

[a]Data taken from refs 11,12.
[b]Displacement of [^{3}H]-M-MPEP from membrane of L(tk−) cells expressing the human mGluR5.

In Vitro Pharmacology

M-MPEP (2-(3-methoxy-phenylethynyl)-6-methyl-pyridine) has been functionally characterized at the human mGlu receptor subtypes 1, 2, 4, 7 and at the recombinantly expressed ionotropic glutamate hNMDA 1A/2A, 1A/2B, hGlu3 (AMPA) and hGlu6 (kainate) receptors. At these receptor subtypes M-MPEP did not show activity up to 10 μM (data not shown). Furthermore, using standard receptor binding techniques M-MPEP was tested for affinity at a panel of twenty-four G-protein coupled receptors and did not show any significant affinity up to 10 μM (data not shown). At the human mGlu5 receptor expressed in L-(tk−) cells, M-MPEP inhibits quisqualate induced PI hydrolysis with an IC_{50} of 10 nM (Fig. 3).

The in vitro binding characteristics of [^{3}H]-M-MPEP were studied in membranes of recombinant human mGluR5 expressing L(tk−) cells (150–200 μg tissue/protein) and using the filtration technique to separate the bound from the free ligand. Time course association/dissociation studies were determined and showed that binding was >90% of total binding at a concentration of 2 nM of [^{3}H]-M-MPEP, fully reversible and saturable. Quantification showed a B_{max} 879 ± 32 fmol/mg protein and a $K_d = 2.0 \pm 0.3$ nM. Displacement studies



Scheme 1. Reagents and conditions: (a) Ac_2O, 165 °C, 3.5 h (53%); (b) Br_2, CCl_4, RT (dark), 1.5 h (80%); (c) KOH, KOtBu, crown ether 18C6, THF, 50 °C, 1.5 h (59%); (d) (cold) KOH, dimethyl sulfate, dioxane, 40 °C, 7 h (78%); (d) (radioactive): [^{3}H]-MeI (0.5 equivalent), K_2CO_3, DMF, 100 °C/180 min, purification on reversed-phase chromatography. Radiochemical purity 98%, specific activity 86 Ci/mmol.

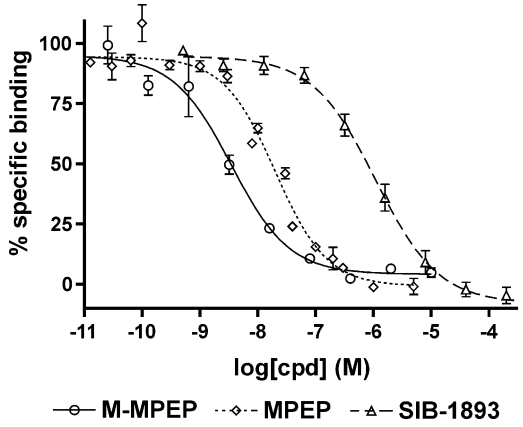


Figure 4. Displacement of [^{3}H]-M-MPEP by M-MPEP (○), MPEP (◇) and SIB-1893 (△). Values are presented as percent of specific binding in the absence of compound tested and represent mean ± SEM of $N = 3$ independent experiments performed in triplicate.

using [^{3}H]-M-MPEP and membranes of hmGluR5 expressing cells showed the following rank order of affinity: M-MPEP > MPEP ≫ SIB-1893 (Fig. 4), which is in full accordance with the rank order of potency determined using functional assays such as the PI hydrolysis (Table 1).[11,12]

Discussion

Our results show that the introduction of a methoxy moiety in the 3-position of the phenyl ring of MPEP allowed the identification of a more potent derivative with the same functional efficacy and subtype selectivity for the metabotropic glutamate receptor 5. Furthermore, this new derivative was radiolabeled at the methoxy position using a phenolic precursor (**12**) and tritiated methyliodide. The labeling can be achieved at the last step of the synthesis and leads to a radioligand with high specific activity and radiochemical purity.

The characterization of this novel radioligand using membranes of cells recombinantly expressing the human mGlu5 receptor showed that the ligand has a high affinity ($K_d = 2$ nM) and a high percentage of specific binding > 90% over the 1–10 nM concentration range.

Displacement studies of [^{3}H]-M-MPEP with the non-labeled M-MPEP and two known antagonists MPEP and SIB-1893 led to IC_{50} values of the same order of magnitude as in the functional assay and the same rank order of potency.

In summary, the characterization, in vitro, of [^{3}H]-M-MPEP showed that this new radioligand is a very useful tool to label the mGlu5 receptor subtype.

References and Notes

1. Conn, P. J.; Pin, J. P. *Annu. Rev. Pharmacol. Toxicol.* **1997**, *37*, 205.
2. Takahishi, K.; Tsuchida, K.; Tanabe, Y.; Masayuki, M.; Nakanishi, S. *J. Biol. Chem.* **1993**, *268*, 19341.
3. O'Hara, L. J.; Sheppard, P. O.; Thogersen, H.; Venezia, D.; Haldema, B. A.; McGrane, V.; Houamed, K. M.; Thomsen, C.; Gilbert, T. L.; Mulvihill, E. R. *Neuron* **1993**, *11*, 41.
4. Pin, J. P.; De Colle, C.; Bessis, A. S.; Acher, F. *Eur. J. Pharmacol.* **1999**, *375*, 277.
5. Schoepp, D. D.; Jane, D. E.; Monn, J. A. *Neuropharmacology* **1999**, *38*, 1431.
6. Schaffhauser, H.; Grayson Richards, J.; Cartmell, J.; Chaboz, S.; Kemp, J. A.; Klingelschmidt, A.; Messer, J.; Stadler, H.; Woltering, T.; Mutel, V. *Mol. Pharmacol.* **1998**, *53*, 228.
7. Johnson, B. G.; Wright, R. A.; Arnold, M. V.; Wheeler, W. J.; Ornstein, P. L.; Schoepp, D. D. *Neuropharmacology* **1999**, *38*, 1519.
8. Ornstein, P. L.; Arnold, M. B.; Bleisch, T. J.; Wright, R. A.; Wheeler, W. J.; Schoepp, D. D. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1919.
9. Cartmell, J.; Adam, G.; Chaboz, S.; Henningsen, R.; Kemp, J. A.; Klingelschmidt, A.; Metzler, V.; Monsma, F.; Schaffauser, H.; Wichmann, J.; Mutel, V. *Br. J. Pharmacol.* **1998**, *123*, 497.
10. Mutel, V.; Ellis, G. J.; Adam, G.; Chaboz, S.; Nilly, A.; Messer, J.; Bleuel, Z.; Metzler, V.; Malherbe, P.; Schlaeger, H.-J.; Roughley, B. S.; Faull, R. L. M.; Grayson Richards, J. *J. Neurochem.* **2000**, *75*, 2590.
11. Varney, M. A.; Cosford, N. D. P.; Jachec, C.; Rao, S. P.; Sacaan, A.; Lin, F.-F.; Bleicher, L.; Santori, E. M.; Flor, P. J.; Allgeier, H.; Gasparini, F.; Kuhn, R.; Hess, S. D.; Velicelebi, G.; Johnson, E. C. *J. Pharmacol. Exp. Ther.* **1999**, *290*, 170.
12. Gasparini, F.; Lingenhöhl, K.; Stoehr, N.; Flor, P. J.; Heinrich, M.; Vranesic, I.; Biollaz, M.; Allgeier, H.; Heckendorn, R. J.; Urwyler, S.; Varney, M. A.; Johnson, E. C.; Hess, S. D.; Rao, S. P.; Sacaan, A. I.; Santori, E. M.; Veliçelebi, G.; Kuhn, R. *Neuropharmacology* **1999**, *38*, 1493.