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# [$^3$H]-Methoxymethyl-MTEP and [$^3$H]-Methoxy-PEPy: Potent and Selective Radioligands for the Metabotropic Glutamate Subtype 5 (mGlu5) Receptor

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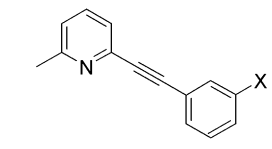
**Abstract**—The design, synthesis, and characterization of two potent, non-competitive radioligands, [$^3$H]-methoxymethyl-MTEP and [$^3$H]-methoxy-PEPy, that are selective for the mGlu5 receptor are described.

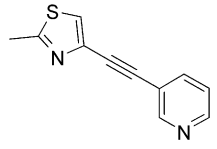

## Introduction

Metabotropic glutamate (mGlu) receptors are a family of G-protein coupled receptors in the mammalian nervous system that are activated by L-glutamate.[1] Group I mGlu receptors (mGlu1 and mGlu5) are primarily localized at the periphery of the postsynaptic membranes of synapses in many brain regions, including the hippocampus, thalamic nuclei, cerebellar cortex, and spinal cord. Stimulation of mGlu1 and mGlu5 leads to phosphoinositide hydrolysis and elevation of intracellular $Ca^{2+}$ levels ($[Ca^{2+}]_i$) via G-protein coupling to phospholipase C. Excessive activation of mGlu5 receptors has been implicated in several disease states including pain,[2] anxiety and depression,[3–8] and drug addiction or withdrawal.[9] Research in these laboratories has therefore focused on the discovery of potent and selective mGlu5 receptor antagonists as potential therapeutic agents.

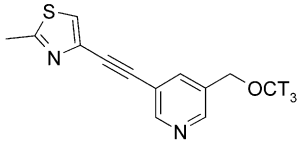
Recently we reported that exploration of SAR around the prototypical non-competitive mGlu5 receptor antagonist MPEP (**1**) led to the discovery of 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine (MTEP; **3**) a potent and highly selective mGlu5 receptor antagonist active in animal models of anxiety.[10] To accomplish the goal of discovering compounds such as MTEP the design and synthesis of highly potent and selective radiolabeled mGlu5 receptor antagonists was required, which allowed the development of robust in vitro and in vivo binding assays.[11,12] The recent disclosure by researchers at Novartis of a selective mGlu5 receptor radioligand [$^3$H]-2-[(3-methoxyphenyl)ethynyl]-6-methylpyridine ([$^3$H]-M-MPEP; **2**),[13] a derivative of MPEP (**1**), prompted



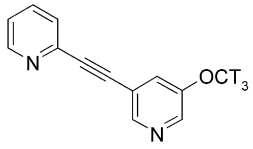
**1** X = H; MPEP
**2** X = $OCT_3$; [$^3$H]-M-MPEP



**3** MTEP



**4** [$^3$H]-Methoxymethyl-MTEP



**5** [$^3$H]-Methoxy-PEPy

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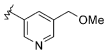
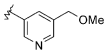
**Table 1.** In vitro data for mGlu5 receptor antagonists

A—≡—B

| Compd | A | B | mGlu5 $Ca^{2+}$ Flux $IC_{50}$ (nM)[a] | LogD[b] |
|---|---|---|---|---|
| **1** |  | Ph | 2 | 3.5 |
| **2** |  | 3-MeOPh | 7 | 3.0 |
| **3** |  | 3-Py | 5 | 2.1 |
| **7** |  |  | 3 | 2.5 |
| **4** |  |  | 7 | 2.4 |
| **6** |  |  | 19 | 2.0 |
| **5** |  |  | 1 | 2.25 |

[a]$Ca^{2+}$ flux assay using glutamate (10 μM) as agonist. Concentration–response curves were performed using 12 concentrations, performed in duplicate wells in two or more separate experiments.[14–16]
[b]See ref 10.

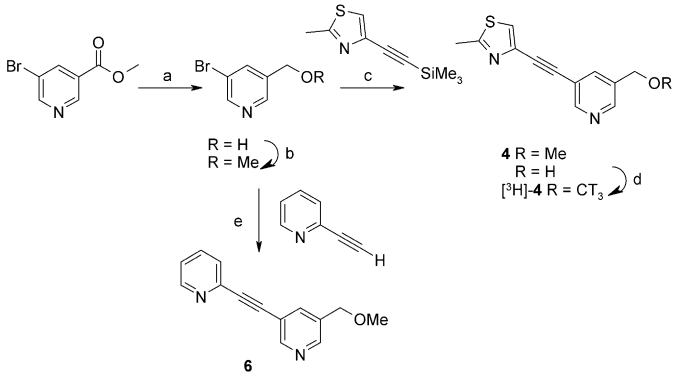
the present communication reporting our parallel efforts to develop potent and highly selective tritiated mGlu5 receptor antagonists. This work resulted in the discovery of [$^3$H]-3-(methoxymethyl)-5-[(2-methyl-1,3-thiazol-4-yl)-ethynyl]pyridine ([$^3$H]-methoxymethyl-MTEP; **4**), and [$^3$H]-3-methoxy-5-(pyridin-2-ylethynyl)pyridine ([$^3$H]-methoxy-PEPy; **5**). The synthesis and in vitro characterization of these pharmacological tools and described herein.

## Chemistry

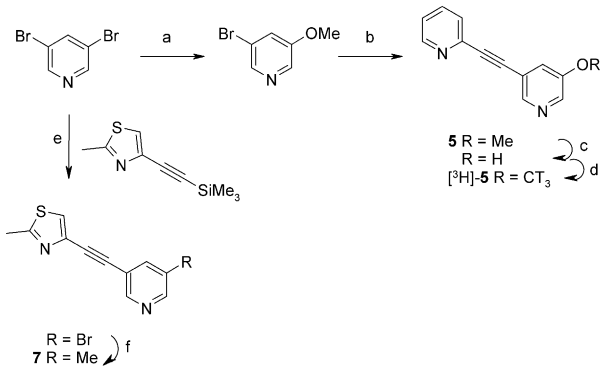
The development of a viable radioligand typically requires the optimization of two parameters: (a) high specific binding (i.e., high affinity for the receptor in question), and (b) low non-specific binding to other endogenous binding sites. Non-specific binding is generally more pronounced for highly lipophilic molecules. We therefore sought to identify selective mGlu5 receptor antagonists with excellent in vitro potency at the mGlu5 receptor and low LogD (low lipophilicity). Thus, in addition to the determination of LogD for compounds using an HPLC method,[10] the functional potency of compounds in vitro was assessed using an automated assay employing Ltk-cells stably expressing human recombinant mGlu5 receptors (Table 1). This cell-based assay measures changes in cytosolic $Ca^{2+}$ concentrations ($[Ca^{2+}]_i$) by fluorescence detection using the $Ca^{2+}$-sensitive dye fura-2.[14–16]

Comparison of MPEP (**1**) and MTEP (**3**) indicated that while both compounds show similar potency at the mGlu5 receptor in vitro (Table 1), MTEP (**3**) is much less lipophilic, with LogD = 2.1 versus 3.5 for **1**. MTEP (**3**) appeared, therefore, to be a promising initial scaffold and SAR studies revealed that methyl substitution was tolerated at the 5-pyridyl position, as in compound **7**, giving a slight enhancement of potency ($IC_{50}$ = 3 nM). Further investigation of the SAR demonstrated that a methoxy substituent on the pyridyl methyl group of **7** was also tolerated as in compound **4** (methoxymethyl-MTEP) with $IC_{50}$ = 7 nM. Interestingly **4**, selected as a candidate for tritium labeling, exhibits similar in vitro functional potency to **2** (M-MPEP) in the $Ca^{2+}$ flux assay (Table 1) however **4** (LogD = 2.4) is less lipophilic than **2** (LogD = 3.0), suggesting the potential for lower non-specific binding for **4**. Efforts to reduce lipophilicity even further led to replacement of the thiazole ring in **4** with a 2-pyridyl moiety to give **6** (LogD = 2.0) although this compound displayed a significant loss of in vitro potency ($IC_{50}$ = 19 nM). Truncation of the methoxymethylene unit in **6** to a methoxy moiety gave **5** (methoxy-PEPy), a compound with excellent functional potency ($IC_{50}$ = 1 nM) and low lipophilicity (LogD = 2.25). On the basis of these results **4** and **5** were selected for tritium labeling and evaluation in binding experiments.

The synthesis of compounds **4** to **7** is summarized in Schemes 1 and 2. Compounds **4** and **6** were each prepared in three steps from methyl 5-bromonicotinate via Sonogoshira cross-coupling of the appropriate alkyne precursor with 3-bromo-5-methoxymethylpyridine (Scheme 1). Compound **5** was prepared by Sonogoshira cross-coupling of 2-ethynylpyridine with 3-bromo-5-methoxypyridine, obtained by reaction of sodium




**Scheme 1.** Reagents and conditions: (a) $LiAlH_4$, THF, −78 °C (63%); (b) NaH, MeI, THF, 0–25 °C, 60 h, (90%); (c) R = Me: $PdCl_2(PPh_3)_2$, $PPh_3$, CuI, $NEt_3$, $Bu_4NI$, $Bu_4NF$, DMF, 45 °C, 60 h, (58%); R = H: $PdCl_2$, $PPh_3$, CuI, $NEt_3$, $Bu_4NF$, DME, 75 °C, 60 h, (44%); (d) [$^3$H$_3$]CI, NaH, THF, 0–25 °C; radiochemical purity >99%, specific activity 79.8 Ci/mmol; (e) $PdCl_2$, $PPh_3$, CuI, $NEt_3$, DME, 75 °C, 6 d (58%).




**Scheme 2.** Reagents and conditions: (a) NaOMe, DMF, 60 °C, 18 h (70%); (b) $PdCl_2(PPh_3)_2$, CuI, $NEt_3$, DMF, 80 °C, 3 h (21%); (c) $AlBr_3$, $CH_2Br_2$, 0–25 °C, 1 h (76%); (d) [$^3H_3$]CI, NaH, DMF, 0–25 °C; radiochemical purity 99.9%, specific activity 67.6 Ci/mmol; (e) $Pd(PPh_3)_4$, CuI, $NEt_3$, $Bu_4NF$, DMF, 65 °C, 7 h, (57%); (f) $Pd(PPh_3)_4$, $Me_2Zn$, DME, 70 °C, 2 h, (60%).

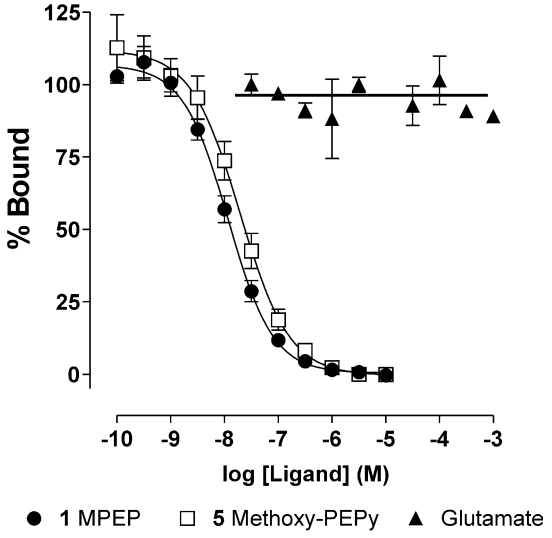



**Figure 1.** [$^3$H]-**5** Binding to rat brain membranes. **1** $IC_{50}$ = 20 (14, 30) nM; **5** $IC_{50}$ = 12 (7, 20) nM.

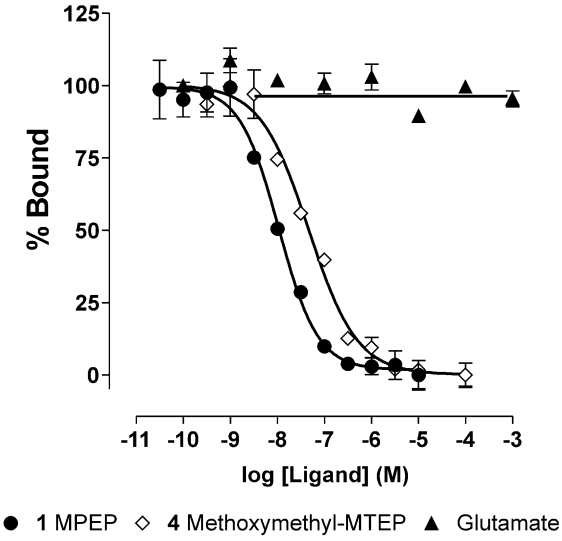



**Figure 2.** [$^3$H]-**4** Binding to human mGlu5 receptors. **1** $IC_{50}$ = 11 nM; **4** $IC_{50}$ = 47 nM.

methoxide with 3,5-dibromopyridine, while **7** was prepared by Sonogoshira cross-coupling of 4-(trimethylsilylethynyl)-2-methyl-1,3-thiazole with 3,5-dibromopyridine followed by Negishi cross-coupling of the monobromo product with dimethylzinc (Scheme 2).

## Pharmacological Characterization

Both [$^3$H]-**4** and [$^3$H]-**5** showed high specific binding to rat brain membranes, as defined with **1** (10 μM) as cold displacer, and the specific binding was greater than 90% of total binding.[11,12] Analysis of rat brain binding of [$^3$H]-**4** or [$^3$H]-**5** revealed a single binding site that was saturable and of high affinity with $K_d$ = 20 nM for [$^3$H]-**4** and $K_d$ = 3.4 nM for [$^3$H]-**5**. Association experiments demonstrated that both [$^3$H]-**4** and [$^3$H]-**5** binding reached equilibrium within 60 min. when incubated at room temperature and that dissociation occurs rapidly in the presence of cold **1** (10 μM). The ability of **1** or **5** to displace [$^3$H]-**5** binding to rat brain membranes was determined and the concentration–response curves are shown in Figure 1. Binding of [$^3$H]-**4** to cells stably expressing recombinant human mGlu5 receptors was also investigated and found to be fully reversible and of high affinity (Fig. 2).

## Conclusion

Two potent and selective tritiated mGlu5 receptor antagonists ([$^3$H]-**4** and [$^3$H]-**5**) were designed, synthesized and the in vitro pharmacology evaluated in binding assays. These radioligands have enabled the development of in vitro binding assays using either rat brain tissue or cells expressing recombinant hmGlu5 receptors. The use of [$^3$H]-**4** and [$^3$H]-**5** in an in vivo receptor occupancy assay in rodents which has been critical to mGlu5 receptor antagonist research in these laboratories are described in detail elsewhere.[11,12]

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