3-[(2-Methyl-1,3-thiazol-4-yl)ethynyl]-pyridine: A Potent and Highly Selective Metabotropic Glutamate Subtype 5 Receptor Antagonist with Anxiolytic Activity

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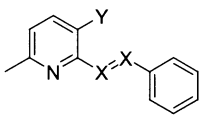
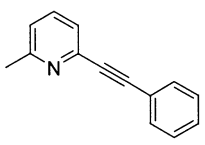
Abstract: 2-Methyl-6-(phenylethynyl)pyridine (**3**), a potent noncompetitive mGlu5 receptor antagonist widely used to characterize the pharmacology of mGlu5 receptors, suffers from a number of shortcomings as a therapeutic agent, including off-target activity and poor aqueous solubility. Seeking to improve the properties of **3** led to the synthesis of compound **9**, a highly selective mGlu5 receptor antagonist that is 5-fold more potent than **3** in the rat fear-potentiated startle model of anxiety.

Introduction. Glutamate is the principal excitatory transmitter in the central nervous system acting through ionotropic glutamate receptors; however, it also plays a major role in activating modulatory pathways through G-protein-coupled metabotropic glutamate (mGlu) receptors.[1,2] Group I mGlu receptors include the mGlu1 and mGlu5 subtypes, which are coupled to stimulation of phospholipase C resulting in phosphoinositide hydrolysis and elevation of intracellular Ca^{2+} levels ($[Ca^{2+}]_i$).[3,4] Excessive activation of mGlu5 receptors has been implicated in several diseases, and selective mGlu5 receptor antagonists may be of therapeutic benefit in the treatment of various pain states,[5] psychiatric disorders such as anxiety and depression,[6−11] and other neurological impairments such as drug addiction and drug withdrawal.[12]

The discovery in these laboratories of the first subtype selective, noncompetitive mGlu5 receptor antagonists, **1** and **2**,[13] led to the subsequent identification of 2-methyl-6-(phenylethynyl)pyridine (MPEP, **3**), a structurally related compound with similar selectivity but improved in vitro potency at mGlu5 receptors.[14] These



1 X=N, Y=OH
2 X=CH, Y=H



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pharmacological tools have led to a plethora of research to understand the role of mGlu5 receptors in the brain and nervous system and hence the viability of mGlu5 receptors as a molecular therapeutic target.[15]

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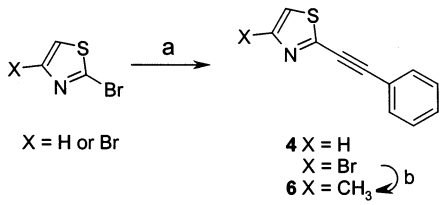
Scheme 1[a]

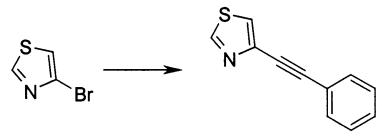


X = H or Br

4 X = H
X = Br
6 X = CH_3

[a] Reagents and conditions: (a) for X = H; phenylacetylene, $PdCl_2(PPh_3)_2$, CuI, NEt_3, DME, 80 °C, 16 h, (60%); for X = Br; phenylacetylene, $Pd(PPh_3)_4$, CuI, NEt_3, DME, 80 °C, 18 h, (86%); (b) Me_4Sn, $Pd_2(dba)_3$, $P(tBu)_3$, CsF, 1,4-dioxane, 100 °C, 5 h, (17%).

Scheme 2[a]



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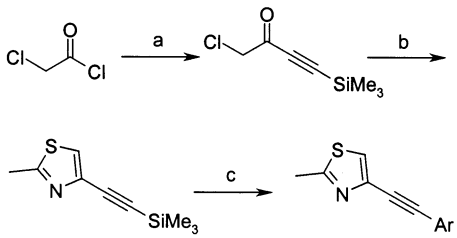
[a] Reagents and conditions: phenylacetylene, $Pd(PPh_3)_4$, CuI, NEt_3, DME, 80 °C, 5 h, (77%).

Scheme 3[a]



7 Ar = Ph
8 Ar = 2-pyridyl
9 Ar = 3-pyridyl
10 Ar = 4-pyridyl

[a] Reagents and conditions: (a) BTMSA, $AlCl_3$, CH_2Cl_2, 0 °C, 2 h and then 25 °C, 1 h, (54%); (b) thioacetamide, DMF, 25 °C, 16 h, (72%); (c) for Ar = Ph; iodobenzene, $PdCl_2(PPh_3)_2$, PPh_3, CuI, NEt_3, TBAF, Bu_4NI, DMF, 70 °C, 36 h, (73%); for Ar = 2-py; 2-bromopyridine, $PdCl_2(PPh_3)_2$, CuI, NEt_3, TBAF, DMF, 70 °C, 30 min, (73%); for Ar = 3-py; 3-bromopyridine, Pd $(PPh_3)_4$, CuI, NEt_3, TBAF, DME, 70 °C, 26 h, (65%); for Ar = 4-py; 4-bromopyridine hydrochloride, $Pd(PPh_3)_4$, CuI, NEt_3, TBAF, DME, 70 °C, 32 h, (75%).

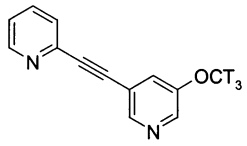


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Figure 1. [^{3}H]-3-methoxy-5-(pyridin-2-ylethynyl)pyridine.

While compound **3** has proven to be a useful tool for both in vitro and in vivo studies, it has a number of drawbacks as a drug. First, compound **3** is not completely selective for mGlu5 receptors. For example, it has been shown to block *N*-methyl-D-aspartate (NMDA) receptors,[16,17] albeit at high concentrations, and our own studies suggest the presence of other off-target activities of **3**. Second, the compound is poorly water soluble, as indicated by a log *D* value of 3.5, which would limit solubility in CSF and very likely reduce in vivo efficacy.

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Table 1. In Vitro Potency Data for mGlu5 Receptor Antagonists[a]

	A	B	hmGlu5 Ca^{2+} Flux IC_{50} (nM)[b]	mGlu5 K_i (nM)[c]	logD[d]
3		Ph	2 (0.25; 5)	12 (0.40; 9)	3.5
4		Ph	81 (0.22; 12)	153 (0.21; 3)	3.2
5		Ph	97 (0.36; 3)	142 (0.14; 3)	2.6
6		Ph	13 (0.05; 3)	32 (0.11; 3)	3.6
7		Ph	6 (0.74; 5)	6 (0.45; 3)	3.3
8		2-Py	53 (0.05; 4)	48 (0.12; 3)	1.9
9		3-Py	5 (0.40; 3)	16 (0.20; 4)	2.1
10		4-Py	122 (0.16; 4)	214 (0.08; 3)	2.1

[a] Data are presented as the geometric mean followed in parentheses by the standard deviation and the number of replicates. [b] Ca^{2+} flux assay using glutamate as agonist.[13,19,20] [c] Displacement by test compounds of [^{3}H]-3-methoxy-5-(pyridin-2-ylethynyl)pyridine (**11**) bound to rat cortical membranes.[21] [d] See ref 18.

Table 2. In Vitro Selectivity Data for mGlu5 Receptor Antagonists **3** and **9**

	mGlu1[a] IC_{50} (μM)	NR2B[b] IC_{50} (μM)	MAO_A[c] IC_{50} (μM)
3	>100	18	8 (6, 13)
9	>100	>300	30 (17, 40)

[a] Ca^{2+} flux assay, tested as agonist and antagonist. [b] Ca^{2+} flux assay; data generated from a six-point CRC, n = 8−22 cells/concentration tested. [c] Data are presented as the geometric mean IC_{50} followed in parentheses by the upper and lower SE (n = 3).

We therefore sought mGlu5 receptor antagonists with improved selectivity, a lower log *D* value, and good efficacy in vivo. With this goal in mind, we set out to explore the SAR of **3** in order to identify a compound possessing all of these desirable properties.

Medicinal Chemistry and SAR. The Sonogoshira cross-coupling methodology employed for the synthesis of the alkyne derivatives described herein is summarized in Schemes 1−3 (yields are not optimized). Two in vitro assays were employed in the initial research phase in addition to the determination of log *D* for compounds using an HPLC method.[18] The functional potency of compounds was assessed using an automated assay employing Ltk cells stably expressing human recombinant mGlu5 receptors. 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.[13,19,20] Binding to native mGlu5 receptors in vitro was determined by measuring the displacement by test compounds of [^{3}H]-3-methoxy-5-(pyridin-2-ylethynyl)pyridine (**11**, Figure 1) from rat cortical membranes.[21]

The 1,3-thiazole ring is a classical isostere of pyridine, and therefore, a logical first step was to prepare analogues in which the pyridyl ring (A) in **3** was replaced with a thiazole moiety. Replacement of the 6-methylpyridine moiety in **3** with 2- or 4-thiazolyl units, as in **4** and **5**, gave compounds with reduced in vitro potency at the mGlu5 receptor (Table 1). By analogy with **3**, introduction of a methyl group adjacent to the thiazole nitrogen in **4** and **5** gave compounds **6** and **7** with greatly enhanced in vitro potency at the mGlu5 receptor. In particular, 2-methyl-4-(phenylethynyl)-1,3-thiazole (**7**) inhibited mGlu5 recepor mediated Ca^{2+} flux with IC_{50} = 6 nM and K_i = 6 nM in the binding assay, which is in the same potency range as compound **3** in these assays. However, log *D* values (Table 1) indicated that both **3** (log *D* = 3.5) and **7** (log *D* = 3.3) are lipophilic molecules. It was hypothesized that introduction of a second basic nitrogen into the molecular framework of **7** would reduce the log *D* and increase aqueous solubility. A nitrogen scan in the phenyl ring (B) of compound **7** demonstrated that, while the 2-and 4-pyridyl isomers (**8** and **10**) were less potent than **7**, the 3-pyridyl isomer (**9**) was potent in vitro (IC_{50} = 5 nM in the Ca^{2+} flux assay, K_i = 16 nM). Furthermore, 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine (MTEP, **9**) has a log *D* value of 2.1 compared with 3.5 for **3** and 3.3 for **7** (Table 1). On the basis of these data, **9** was selected for further in vitro and in vivo evaluation.

In Vitro Profiles of Compounds 3 and 9. To identify any off-target activities, **3** and **9** were profiled extensively against a battery of in vitro assays. Experiments were performed initially at a drug concentration of 10 μM. For assays in which inhibition was detected at the single dose, concentration−response curves were generated. Like **3**, compound **9** is highly selective for the mGlu5 receptor over the mGlu1 receptor (Table 2). Additionally **9** has no effect when tested against other mGlu receptor subtypes (e.g., mGlu1, mGlu2, mGlu7) or against ionotropic glutamate receptors, including AMPA and kainate subtypes (e.g., Glu1, Glu3, Glu5, Glu6). It should be noted that **3** and **9** were not tested against glutamate transporters or against enzymes for which glutamate is a substrate. Since it had been reported that **3** is an antagonist of NMDA NR2B-containing receptors in vitro,[16,17] **3** and **9** were tested for their ability to block NMDA/glycine-evoked increases in intracellular calcium in a cell line stably expressing recombinant human NMDA receptors. In this assay, **3** inhibited NR1A/2B receptors (IC_{50} = 18 μM, Table 2) while **9** produced only 19% inhibition at a concentration of 300 μM. Further profiling revealed that **3** displaced [^{125}I]methyl (1*R*,2*S*,3*S*,5*S*)-3-(4-iodophenyl)-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylate ([^{125}I]RTI-55) from MAO_A with IC_{50} = 8 μM compared with IC_{50} = 30 μM

Table 3. In Vivo Data for mGlu5 Receptor Antagonists **3** and **9**[a]

	Occ ED_{50} [b,c] (mg/kg ip)	plasma levels[d,e] (μM)	brain levels[d,e] (μM)	CSF levels[d,f] (μM)	FPS ED_{50} [b,g] (mg/kg ip)
3	2.1 (1.1, 3.4)	0.39 ± 0.02 (7)	0.83 ± 0.05 (7)	0.21 ± 0.3 (5)	5 (3.7, 6.66)
9	1 (0.6, 1.2)	1.2 ± 0.2 (6)	1.4 ± 0.2 (6)	1.0 ± 0.27 (5)	1 (0.65, 2.0)

[a] All in vivo measurements taken at 1 h after administration. [b] Data presented as mean followed in parentheses by ED_{50} values from individual experiments (95% confidence interval). [c] n = 4−6 rats/group. [d] Data presented as mean ± SEM (n), where n = number of rats/group. [e] Dose = 3 mg/kg ip. [f] Dose = 30 mg/kg po. [g] n = 8 rats/group.

for **9**. Together, these results indicate that **9** exhibits fewer off-target effects than **3** and thus greater specificity for the mGlu5 receptor.

In Vivo Profiles of Compounds 3 and 9. To evaluate the brain penetration of **3** and **9** and to correlate affinity at the mGlu5 receptor with in vivo efficacy, an in vivo receptor occupancy assay was employed.[22] Briefly, at time zero rats were dosed with the test compound intraperitoneally and at 59 min. [^{3}H]-3-methoxy-5-(pyridin-2-ylethynyl)pyridine (**11**)[21] was administered via tail vein injection. One minute later, the animals were sacrificed and brain binding was measured. With this paradigm, dose−response relationships were determined for binding to the mGlu5 receptor in vivo and it was found that **9** is twice as potent as **3** in this assay (Table 3). In other experiments, plasma, brain (hippocampus), and CSF levels for **3** and **9** were measured following dosing in rats. Interestingly, while both **3** and **9** exhibited similar drug levels in the hippocampus (Table 3), the concentration of **9** in CSF (1 μM) was approximately 5-fold higher than the concentration of **3** (0.21 μM). This may be a consequence of the lower log *D* value for **9** and therefore greater aqueous solubility compared with **3** (Table 1).

There is growing evidence of a role for mGlu5 receptors in the modulation of mood disorders including depression and anxiety.[6−11] For example, **3** is reported to be active in the rodent Geller−Seifter model,[6,7] ultrasonic vocalization,[6] elevated plus maze,[7,9] social exploration test,[7] marble burying test,[7] conflict drinking test,[9] and four-plate test.[9] Compound **3** also reduces stress-induced hyperthermia in mice.[8] Recently **3** was also shown to block fear-conditioning in rats as determined in the fear-potentiated startle (FPS) model of anxiety.[6,11] The FPS model was therefore selected to assess the relative potencies of **3** and **9** in a rodent model of anxiety. Compounds **3** and **9** were administered intraperitoneally to rats, and both compounds were found to block the expression of fear in this paradigm. However, the ED_{50} for **9** was calculated to be 1 mg/kg compared with 5 mg/kg for **3** (Table 3), indicating that **9** is 5-fold more potent than **3** in this animal model.

Conclusion. Exploration of the SAR around **3** resulted in the discovery of compound **9**, a potent and selective mGlu5 receptor antagonist with fewer off-target effects than **3**. Furthermore, **9** is more potent than **3** in vivo (rats) in both a receptor occupancy assay and in the fear-potentiated startle model of anxiety. Further details of the SAR and pharmacological profile of **9** and analogues will be reported in due course.

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Supporting Information Available: Experimental details for the preparation of compounds **4**−**10** and log *D* determination using HPLC. This material is available free of charge via the Internet at http://pubs.acs.org.

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