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5-[(2-Methyl-1,3-thiazol-4-yl)ethynyl]-2,3′-bipyridine: a highly potent, orally active metabotropic glutamate subtype 5 (mGlu5) receptor antagonist with anxiolytic activity

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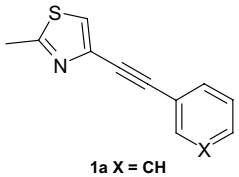
Abstract—Structure–activity relationship studies leading to the discovery of a new, orally active mGlu5 receptor antagonist are described. The title compound, 5-[(2-methyl-1,3-thiazol-4-yl)ethynyl]-2,3′-bipyridine, is highly potent in vitro, has good in vivo receptor occupancy, and is efficacious in the rat fear-potentiated startle model of anxiety following oral dosing.

Metabotropic glutamate (mGlu) receptors are a family of G-protein coupled receptors in the mammalian nervous system that are activated by L-glutamate.[1,2] Eight mGlu receptor subtypes have been identified to date and these are organized into three groups (Group I, II, and III) based on sequence homology. Group I mGlu receptors (mGlu1 and mGlu5) are primarily localized postsynaptically and are widely distributed in many brain regions, including the hippocampus, thalamic nuclei, and spinal cord. Stimulation of mGlu1 and mGlu5 leads to phosphoinositide (PI) hydrolysis and elevation of intracellular Ca^{2+} levels ($[Ca^{2+}]_i$) via G-protein coupling to phospholipase C.[3,4] Excessive activation of mGlu5 receptors has been linked to a number of CNS disorders including pain,[5] anxiety and depression,[6–11,20,21] drug dependence[12] and mental retardation.[13] The development of potent and selective mGlu5 receptor antagonists as potential therapeutic agents has therefore been the focus of significant research in these laboratories.

Recently we reported the discovery of the potent and highly selective mGlu5 receptor antagonists **1a** and **1b** (3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine, MTEP), the latter of which was found to be active in a rodent model of anxiety.[14]



1a X = CH
1b X = N

In this communication structure–activity relationship (SAR) studies around the aryl ring appended to the

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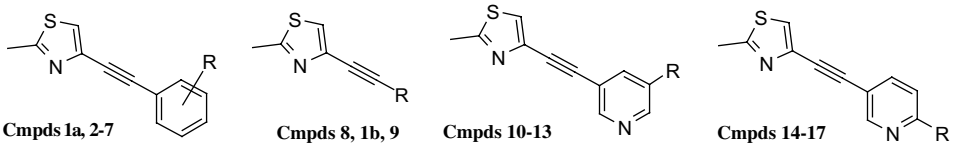
Table 1. In vitro potency data for mGlu5 receptor antagonists[a]



Compound	R	hmGlu5 Ca^{2+} flux IC_{50} (nM)[b]	mGlu5 K_i (nM)[c]	log *D*[d]
1a	H	4 (0.04; 2)	9 (0.18; 3)	3.3
2	2-Me	15 (0.19; 5)	51 (0.02; 2)	3.8
3	3-Me	2 (0.04; 3)	10 (0.12; 2)	3.9
4	4-Me	845 (0.22; 3)	638 (0.08; 2)	3.9
5	2-Ph	423 (0.20; 6)	145 (0.09; 3)	—
6	3-Ph	41 (0.15; 3)	16 (0.05; 3)	4.1
7	4-Ph	22 (0.25; 11)	43 (0.13; 4)	5.0
8	2-Py	53 (0.04; 4)	54 (0.13; 3)	1.9
1b	3-Py	5 (0.4; 3)	12 (0.10; 2)	2.1
9	4-Py	127 (0.14; 4)	218 (0.07; 3)	2.1
10	Ph	2 (0.09; 2)	40 (0.15; 2)	4.1
11	2-Py	22 (0.39; 12)	28 (0.13; 3)	2.8
12	3-Py	3 (0.16; 12)	1 (0.36; 4)	2.4
13	4-Py	18 (0.09; 4)	15 (0.18; 4)	2.6
14	Ph	0.5 (0.33; 4)	2 (0.38; 2)	3.9
15	2-Py	4 (0.24; 5)	16 (0.17; 4)	3.7
16	3-Py	2 (0.05; 3)	2 (0.18; 3)	3.1
17	4-Py	29 (0.26; 3)	21 (0.17; 2)	2.9

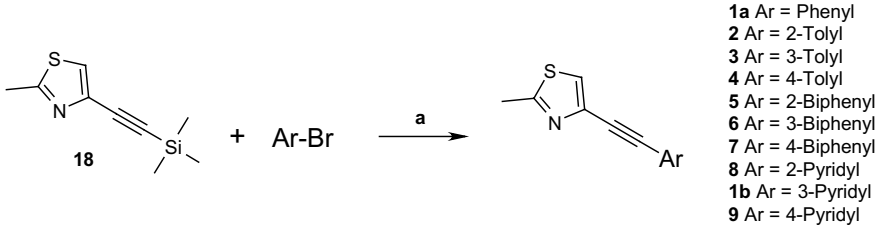
[a] Data are presented as the geometric mean followed in parentheses by the standard deviation and the number of replicates.
[b] Ca^{2+} flux assay using glutamate (10 μM) as agonist.[15–17]
[c] Displacement by test compounds of [^{3}H]-3-methoxy-5-(pyridin-2-ylethynyl)pyridine from rat cortical membranes.[18]
[d] See Ref. 14.

thiazole alkyne motif in **1a** and pyridyl group in **1b** will be described. These efforts have led to the discovery of new derivatives of **1b** that display improved in vitro potency, good rodent pharmacokinetics, and potent anxiolytic activity in vivo. The functional potencies of these compounds in vitro was assessed using an automated assay employing Ltk cells that stably express 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.[15–17] Selectivity over the mGlu1receptor was also determined using the Ca^{2+} flux assay, testing compounds as agonists and antagonists. In all instances, the compounds in Table 1 were inactive at mGlu1 receptors up to 10 μM test concentration. 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 from rat cortical membranes.[18]

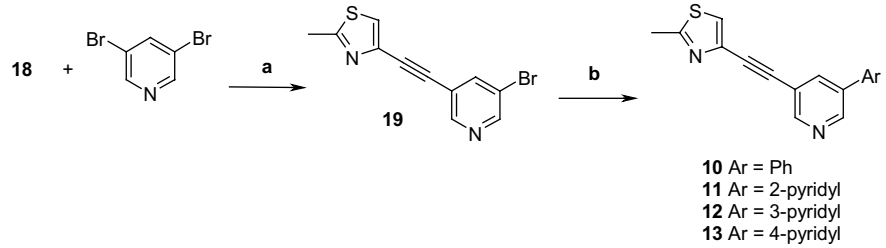
The general scheme for the synthesis of the alkyne derivatives in Table 1 has been described previously.[14,18] Briefly, utilizing Sonogashira cross-coupling methodology, compounds **1a**,**b** and **2**–**9** could be readily prepared from 4-(trimethylsilylethynyl)-2-methyl-1,3-thiazole **18** and the appropriate commercially available pyridyl, aryl or biaryl halide (Scheme 1).

Compounds **10**–**13** were prepared in two steps beginning with the Sonogashira cross-coupling of **18** with 3,5-dibromopyridine (Scheme 2). The resulting common intermediate **19** was used as a Suzuki cross-coupling partner with the appropriate aryl or pyridylboronic acid to obtain **10**, **12**, and **13**. Compound **11** was obtained via Negishi cross-coupling of **19** with 2-pyridylzinc bromide.

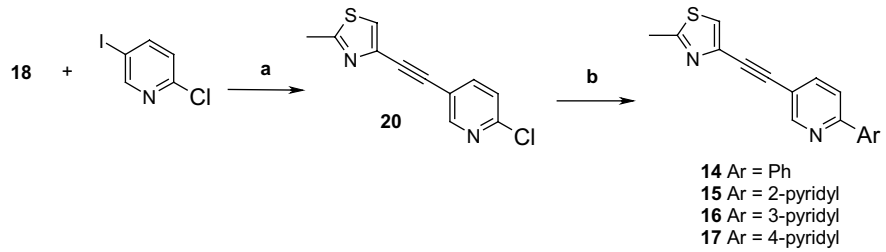
Similarly, compounds **14**–**17** were prepared via Sonogashira cross-coupling of **18** with 2-chloro-5-iodopyr-



Scheme 1. Reagents and conditions: (a) $Pd(PPh_3)_4$, CuI, TBAF, Et_3N, DMF, 70 °C, 4–18 h (41–87%).



Scheme 2. Reagents and conditions: (a) $Pd(PPh_3)_4$, CuI, TBAF, TEA, DMF, 60 °C, 2.5 h (88%); (b) for Ar = Ph; phenylboronic acid, $PdCl_2(PPh_3)_2$, K_2CO_3, DME/H_2O, 80 °C, 5 h (87%); for Ar = 2-pyridyl; 2-pyridylzinc bromide, $Pd(PPh_3)_4$, THF, 65 °C, 18 h (38%); for Ar = 3-pyridyl; 3-pyridylboronic acid, $PdCl_2(PPh_3)_2$, K_2CO_3, DME/H_2O, 80 °C, 4 h (69%); for Ar = 4-pyridyl; 4-pyridylboronic acid, $PdCl_2(PPh_3)_2$, K_2CO_3, DME/H_2O, 80 °C, 18 h (47%).



Scheme 3. Reagents and conditions: (a) $Pd(PPh_3)_4$, CuI, TBAF, TEA, DMF, 70 °C, 5 h (72%); (b) for Ar = Ph; phenylboronic acid, $PdCl_2(PPh_3)_2$, K_2CO_3, DME/H_2O, 80 °C, 4 h (84%); for Ar = 2-pyridyl; 2-pyridylzinc bromide, $Pd(PPh_3)_4$, THF, 65 °C, 30 h (47%); for Ar = 3-pyridyl; 3-pyridylboronic acid, $PdCl_2(PPh_3)_2$, K_2CO_3, DME/H_2O, 80 °C, 6 h (61%); for Ar = 4-pyridyl; 4-pyridylboronic acid, $PdCl_2(PPh_3)_2$, K_2CO_3, DME/H_2O, 80 °C, 18 h (42%).

idine to provide intermediate **20** (Scheme 3). Compound **20** was then subjected to Suzuki cross-coupling conditions with the appropriate aryl or pyridylboronic acid to obtain **14**, **16**, and **17**. Finally, Negishi cross-coupling of intermediate **20** with 2-pyridylzinc bromide gave **15**.

In an effort to probe receptor space around the phenyl ring in **1a**, the effect of methyl and aryl substitution at the *ortho*, *meta*, and *para* positions was determined. It was found that *ortho* (**2**) and *meta* (**3**) substituted methyl derivatives were preferred, while *para*-substitution as in **4** led to a greater than 200-fold reduction in potency. Phenyl substitution, on the other hand, revealed a preference for the *meta* (**6**) and *para* (**7**) biphenyl derivatives over the *ortho* (**5**) analog. Previously[14] we had shown that the 3-pyridyl moiety (as in **1b**) could be used to replace the phenyl group in **1a**, resulting in a lower log *D* while still maintaining high potency in vitro. With this in mind, a 3-pyridyl ring was incorporated into the most potent biphenyl isomers **6** and **7**, providing **10** and the highly potent **14** ($IC_{50} = 0.5$ nM in the Ca^{2+} assay, $K_i = 2$ nM). In an effort to further decrease the log *D* and thereby improve aqueous solubility, a second basic nitrogen was introduced into the phenyl ring of **10** and **14**. This led to compounds **11**–**13** (*meta*-substituted) and **15**–**17** (*para*-substituted), and of these bipyridyl derivatives, **12** and **16** were revealed as the most potent compounds in the series and were therefore selected for further profiling in vivo.

Prior to behavioral evaluation, the rat pharmacokinetic profiles of **12** and **16** were assessed. MTEP (**1b**) is also included for reference (Table 2).

Because of its superior oral bioavailability and high plasma levels, the hydrochloride salt of compound **16** was taken forward to determine in vivo receptor occupancy. Thus at time zero rats were dosed between 0 and 31.25 mg/kg orally and at 59 min [^{3}H]-3-methoxy-5-(pyridin-2-ylethynyl)pyridine was administered via tail vein injection.[19] One minute later, the animals were sacrificed and brain binding was measured. With this paradigm, a dose–response relationship was determined for binding of **16** to mGlu5 receptors in vivo, resulting in an oral Occ_{50} value of 0.52 mg/kg (Figure 1).

The potential use of mGlu5 receptor antagonists for the treatment of mood disorders such as anxiety and depression has been documented recently.[6–11,20,21] Of

Table 2. Selected rat pharmacokinetic data for **1b**, **12**, and **16**

PK parameter	Compound **1b**[a]	Compound **12**[b]	Compound **16**[a]
%*F*	16	26	100
C_{max} (po, μM)	2.0	0.8	6.5
$t_{1/2}$ (iv, h)	8.3	1.6	0.8
Clp (mL/min/kg)	28	29	15

[a] iv dosing at 2 mg/kg, po dosing at 10 mg/kg.
[b] iv dosing at 1 mg/kg, po dosing at 2 mg/kg.

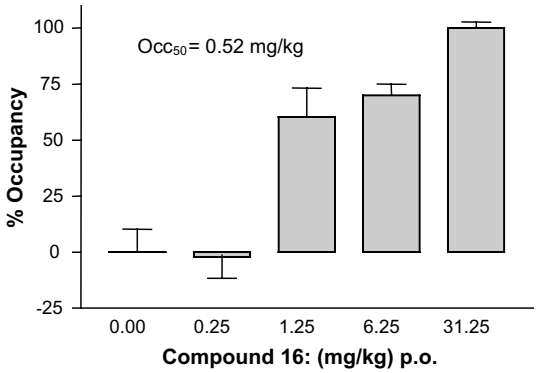


Figure 1. In vivo receptor occupancy dose–response for compound **16**.

particular interest to our research is the ability of these compounds to block fear-conditioning in rats as determined by the fear-potentiated startle (FPS) model of anxiety. Previously **1b** (MTEP) was reported to be five times more potent than the classical mGlu5 receptor antagonist 2-methyl-6-(phenylethynyl)pyridine (MPEP) when dosed ip in the rat FPS model of anxiety ($ED_{50} = 1$ mg/kg ip vs 5 mg/kg ip for MPEP).[14] However, following oral dosing in this model, MTEP showed reduced potency ($ED_{50} = 7$ mg/kg po). On the other hand, as a result of superior oral bioavailability in rats (Table 2), compound **16** at 1 h post-dose exhibited an ED_{50} of 1 mg/kg (po) in the FPS model. Based on this result in conjunction with its in vivo receptor occupancy profile, compound **16** was determined to be the most orally active mGlu5 antagonist described to date.

In conclusion, a detailed exploration of receptor space around the phenyl ring of **1a** led to the discovery of the highly potent mGlu5 antagonist **16**. As a result of excellent rodent oral pharmacokinetics, **16** was profiled, following po dosing, in both an in vivo receptor occupancy assay and the fear-potentiated startle model of anxiety. As compared to the prototypical mGlu5 antagonists MPEP and MTEP,[14] **16** should be considered a significant improvement due to an excellent oral pharmacokinetic profile. Further details of the SAR and pharmacological profile of **16** and related analogues will be reported in due course.

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