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3-[Substituted]-5-(5-pyridin-2-yl-2*H*-tetrazol-2-yl)benzonitriles: Identification of highly potent and selective metabotropic glutamate subtype 5 receptor antagonists

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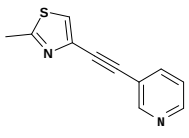
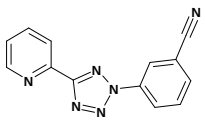
Abstract—Structure–activity relationship studies on the phenyl ring of 3-(5-pyridin-2-yl-2*H*-tetrazol-2-yl)benzonitrile **2** led to the discovery that small, non-hydrogen bond donor substituents at the 3-position led to a substantial increase in in vitro potency. In particular, 3-fluoro-5-(5-pyridin-2-yl-2*H*-tetrazol-2-yl)benzonitrile (**7**) is a highly potent and selective mGlu5 receptor antagonist with good rat pharmacokinetics, brain penetration, and in vivo receptor occupancy.

The excitatory neurotransmitter glutamate activates both ionotropic receptors and G protein-coupled metabotropic glutamate (mGlu) receptors. To date, eight mGlu receptors have been identified and they are categorized as follows: Group I includes mGlu1 and mGlu5 receptors, Group II comprises mGlu2 and mGlu3 receptors, and Group III encompasses the mGlu4 and mGlu6–8 subtypes.[1] The Group I receptors activate phospholipase C, which results in the mobilization of intracellular calcium.[2] A number of reports have indicated that selective antagonism of mGlu5 receptors may improve disease states, such as anxiety and depression,[3–8] pain,[9] drug dependence,[10] and mental retardation.[11]



1-MTEP



2

Keywords: Metabotropic glutamate receptor 5; Antagonist; Tetrazole; Occupancy.
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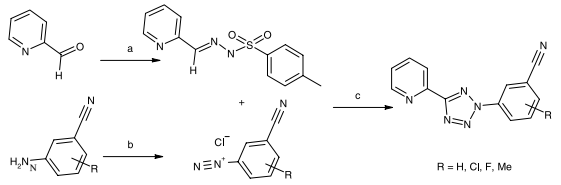
Recent publications from this laboratory have described the discovery of MTEP (**1**), a potent and selective mGlu5 receptor antagonist.[12] In a continuing search for alternative structural series to diaryl-alkynes derivatives, such as MTEP, we developed a series of heteroaromatic azoles.[13] Of the 16 N-linked azoles examined, tetrazole **2** was found to be the most promising in terms of potency, selectivity,[14] brain penetration, and rat pharmacokinetics. However, tetrazole **2** showed only moderate in vitro potency and binding affinity. With the goal of improving the potency of this novel class of compounds further, we herein describe the structure–activity relationship studies (SAR) around the phenyl ring of **2**.

The tetrazole derivatives described herein were constructed, as outlined in Scheme 1. Thus, a 1,3-dipolar cycloaddition was employed between a diazonium salt (derived from the corresponding 3-aminobenzonitrile) and a tosyl hydrazone (derived from condensation of 2-pyridylaldehyde with tosyl hydrazide—Scheme 1).[15]

Those 3-aminobenzonitriles that were not commercially available were synthesized by installing the nitrile on the



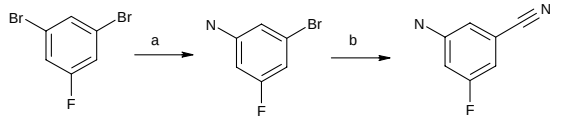
Scheme 1. Reagents and conditions: (a) Tosyl hydrazide, EtOH, rt. (b) $NaNO_2$, HCl, H_2O, EtOH, 0 °C. (c) NaOH, 0 °C.

corresponding bromide using palladium catalyzed cyanation with $Zn(CN)_2$. An example is shown in Scheme 2 for the preparation of the key intermediate 3-amino-5-fluorobenzonitrile.

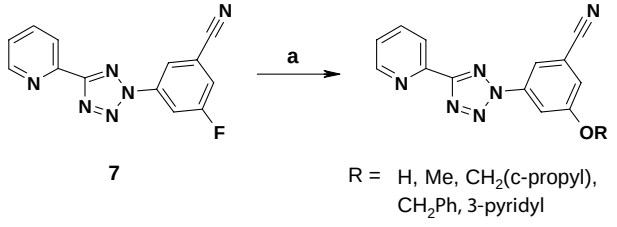
Those derivatives with an alkoxy substituent were prepared by nucleophilic displacement of the fluoro substituent of **7** with an alcohol in the presence of cesium carbonate at an elevated temperature (Scheme 3).

The in vitro functional activity of mGlu5 receptor antagonists was determined using a cell-based, high-throughput assay that measured the changes in cytosolic Ca^{2+} concentrations by fluorescence detection.[16] Compounds were also tested in a binding assay that measured the displacement of [H^3]3-methoxy-5-(pyridin-2-ylethynyl)pyridine from rat cortical membranes.[17] The mGlu5 receptor potency data for the initial set of tetrazole derivatives synthesized are shown in Table 1.

We initially examined the effect of chloride substitution at different positions around the phenyl ring of **2**, while maintaining the 5-nitrile substituent. Thus, derivatives **3**, **5**, and **6** all lost potency, relative to the parent **2**; however, **4** with a 3-chloro-5-nitrile orientation showed a substantial improvement in potency (mGlu5 K_i = 26 nM). Encouraged by this, we decided to focus on the 3,5-orientation and to investigate the effect of other substituents at the 3-position, while fixing the nitrile group at the 5-position.



Scheme 2. Reagents and conditions: (a)—(i) acetamide, CuI, *trans*-1,2-diaminocyclohexane, K_2CO_3, toluene, 130 °C, 18 h (76%); (ii) NaOH (99%). (b) $Zn(CN)_2$, Pd_2dba_3, dppf, DMF, 90 °C, 18 h (85%).



Scheme 3. Reagents and conditions: (a) ROH, Cs_2CO_3, DMF, 140 °C, 16 h.

Table 1. In vitro data for mGlu5 receptor antagonists

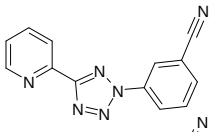
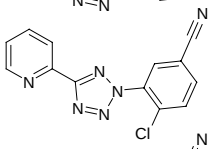
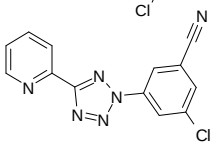
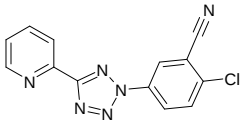
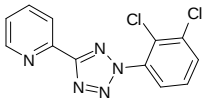
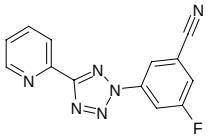
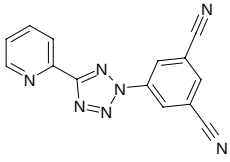
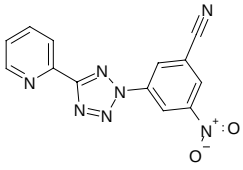
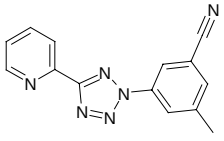
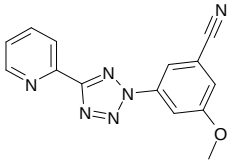
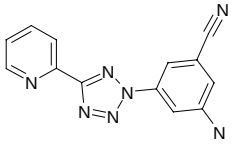
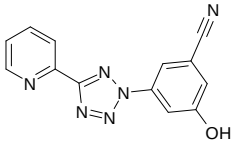
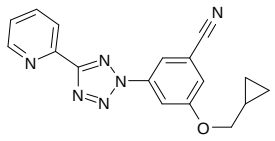
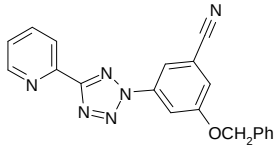
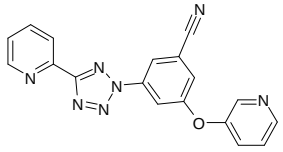
Compound	Structure	mGlu5 Ca^{2+} flux IC_{50} (nM)[a]	mGlu5 K_i (nM)[b]
2		73	190
3		334	>600
4		7	26
5		722	>600
6		1270	>600
7		4	14
8		34	18
9		42	42
10		6	7
11		10	6
12		308	95
13		562	318

Table 1 (*continued*)

Compound	Structure	mGlu5 Ca^{2+} flux IC_{50} (nM)[a]	mGlu5 K_i (nM)[b]
14		975	271
15		>1000	>600
16		15	16

[a] Using glutamate (10 μM) as agonist.
[b] Displacement by test compounds of [^{3}H]3-methoxy-5-(pyridin-2-ylethynyl)pyridine from rat cortical membranes.

Thus, it was found that small, non-hydrogen bond donor groups at the 3-position, such as fluorine (**7**), nitrile (**8**), methyl (**10**), and methoxy (**11**), all had good levels of mGlu5 receptor antagonist activity (mGlu5 K_i = 6–18 nM). Further, electronic nature of the substituent was not important, as an electron-donating methoxy substituent **11** (K_i = 6 nM) and an electron-withdrawing nitrile **8** (K_i = 18 nM) are both potent. However, introduction of a hydrogen bond donor group at the 3-substituent led to a loss of potency against the mGlu5 receptor. Thus, aniline **12** (K_i = 95 nM) or phenol **13** (K_i = 318 nM) both lost significant potency compared to **4**. Similarly, increasing the steric bulk of the alkoxy substituent of **11** (K_i = 6 nM) to methylenecyclopropyl **14** (K_i = 271 nM) or benzyl **15** (K_i = >600 nM) both resulted in a loss of potency. One interesting exception to the loss of potency with increasing steric bulk was the 3-pyridyloxy derivative **16** (K_i = 16 nM). The unexpected activity of this compound probably reflects the fact that the 3-pyridyloxy group is a potent nitrile replacement in this series of mGlu5 receptor antagonists, as disclosed previously.[18]

Having shown that a significant increase in in vitro potency against the mGlu5 receptor may be achieved with derivatives, such as **7** and **10**, we next sought to profile these compounds in terms of rat pharmacokinetics (Table 2).

Table 2. Rat pharmacokinetic data for **2**, **7**, and **10**

	Cl_p (mL/min/kg)[a]	V_d (L/kg)[a]	$t_{1/2}$ (h)[a]	F%[b]	C_{max} (μM)[b]
2	33	5.0	6.9	100	5.8
7	17	1.1	2.9	26	2.5
10	50	1.4	0.4	15	0.8

[a] 2 mg/kg dosed iv (n = 2 Sprague–Dawley rats/group).
[b] 10 mg/kg dosed po (n = 3 Sprague–Dawley rats/group).

Table 3. Rat occupancy and brain penetration for **2** and **7**

	Plasma levels (μM)	Brain levels (μM)	Brain/ plasma (%)	Occ ED_{50} (mg/kg ip)	Occ ED_{50} (mg/kg po)
2	10[a]	15[a]	150	3.0[b]	3.0[c]
7	2.7[d]	2.4[d]	90	1.3[b]	3.6[e]

[a] Measured at 1 h following 10 mg/kg dose ip.
[b] Measured 1 h post-administration (n = 5–6 Sprague–Dawley rats/group).
[c] Measured 30 min post-administration (n = 6–7 Sprague–Dawley rats/group).
[d] Measured at 1 h following 3 mg/kg dose ip.
[e] Measured 2 h post-administration (n = 5–7 Sprague–Dawley rats/group).

Although not as impressive as parent tetrazole **2**, fluoro-derivative **7** still exhibits promising pharmacokinetics with good bioavailability and half-life in rats (F = 26%; $t_{1/2}$ = 2.9 h). Methyl derivative **10** is also bioavailable in rats (F = 15%); however, it suffers from high clearance and a short half-life.

With its excellent in vitro potency against the mGlu5 receptor (Ca^{2+} flux = 4 nM; K_i = 14 nM) and encouraging rat pharmacokinetics, we next profiled **7** in terms of rat brain penetration and in vivo receptor occupancy (Table 3).[19,20]

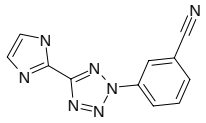
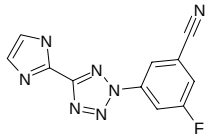
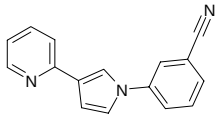
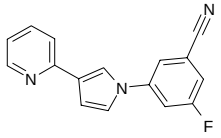
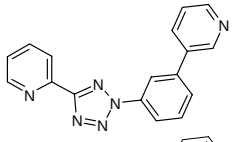
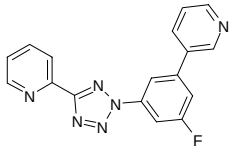
Similar to the parent tetrazole **2**, fluoro-derivative **7** has good rat brain penetration (90% for **7**) with measured drug levels in the brain of 2.4 μM, following a 3 mg/kg ip dose. Together with the high in vitro potency against the mGlu5 receptor, this leads to an excellent occupancy ED_{50} of 1.3 mg/kg ip for **7** (for **2**, ED_{50} = 3.0 mg/kg ip). When examining the po dosing route, reflecting its oral bioavailability of 26%, **7** has an ED_{50} of 3.6 mg/kg po, while the parent tetrazole **2** with 100% bioavailability has an ED_{50} of 3.0 mg/kg po.

Having shown the beneficial effect of substituting **2** with a 3-fluoro substituent on in vitro potency, we next attempted to apply this SAR to related series of mGlu5 receptor antagonists (Table 4).

Thus, substitution of imidazole **17** (K_i = 34 nM),[21] pyrrole **19** (K_i = >600 nM),[13] and bi-aryl **21** (K_i = 69 nM),[22] with a 3-fluoro substituent on the phenyl ring to give **18** (K_i = 9.3 nM), **20** (K_i = 5 nM), and **22** (K_i = 37 nM), respectively, led in each case to an increase in mGlu5 receptor potency.

In conclusion, SAR studies on the phenyl ring of **2** have shown that small, non-hydrogen bond donor groups at the 3-position increase in vitro potency against the mGlu5 receptor. Specifically, the 3-fluoro derivative **7** shows excellent in vitro potency, good rat pharmacokinetics, and excellent in vivo rat receptor occupancy and brain penetration. Subsequent studies have shown that installation of a 3-fluoro substituent in other scaffolds also leads to an improvement in in vitro potency.

Table 4. In vitro data for mGlu5 receptor antagonists

Compound	Structure	mGlu5 Ca^{2+} flux IC_{50} (nM)[a]	mGlu5 K_i (nM)[b]
17		77	34
18		47	9.3
19		190	>600
20		3	5
21		63	69
22		12	37

[a] Using glutamate (10 μM) as agonist.

[b] Displacement by test compounds of [^{3}H]3-methoxy-5-(pyridin-2-yl ethynyl)pyridine from rat cortical membranes.

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