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3-[3-Fluoro-5-(5-pyridin-2-yl-2*H*-tetrazol-2-yl)phenyl]-4-methylpyridine: a highly potent and orally bioavailable metabotropic glutamate subtype 5 (mGlu5) receptor antagonist

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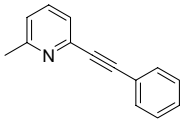
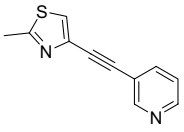
Abstract—Structure–activity relationship studies performed around 3-fluoro-5-(5-pyridin-2-yl-2*H*-tetrazol-2-yl)benzonitrile for the purpose of developing novel mGlu5 receptor antagonists are described. Synthesis of a series of four-ring tetrazoles led to the discovery of 3-[3-fluoro-5-(5-pyridin-2-yl-2*H*-tetrazol-2-yl)phenyl]-4-methylpyridine, a highly potent, brain penetrant, azole-based mGlu5 receptor antagonist.

Glutamate is the principal excitatory neurotransmitter in the central nervous system.[1] As such, it performs a critical function in activating modulatory pathways through G protein-coupled metabotropic glutamate (mGlu) receptors. Group I mGlu receptors, which include the mGlu1 and mGlu5 subtypes, are coupled to the stimulation of phospholipase C resulting in the hydrolysis of inositoltriphosphate and the elevation of intracellular Ca^{2+} levels.[2,3] Excessive activation of mGlu5 receptors has been implicated in several neurological diseases and disorders.[4] A growing body of literature suggests that selective mGlu5 receptor antagonists may be of therapeutic benefit for the treatment of various pain states,[5] psychiatric disorders such as anxiety and depression,[6–8] and other neurological impairments such as drug dependence and mental retardation.[9,10]

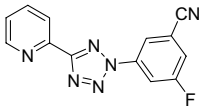
The discovery of the subtype selective mGlu5 receptor antagonists **1** and **2** has led to a better understanding of the role of mGlu5 receptors in the brain and nervous system. These compounds are useful tools for in vitro and in vivo studies and have reinforced the viability of mGlu5 receptors as a molecular therapeutic target.[11,12] In addition to the biaryl alkyne class of antagonists, we have recently reported the discovery of N-linked azole derivatives as novel mGlu5 receptor antagonists.[13] In particular, the tetrazole derivative **3** was shown to have good mGlu5 receptor potency, selectivity,[14] rat pharmacokinetics, and receptor occupancy in rat brain.



1 MPEP



2 MTEP



3

With the goal of further improving the potency and rat pharmacokinetic properties of this novel class of compounds, we herein describe the structure–activity relationship studies (SAR) around the phenyl ring of **3**. Specifically, we sought to replace the nitrile moiety with different aromatic functionalities.

Keywords: Metabotropic glutamate; Antagonist; Tetrazole.
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Synthesis of the tetrazole derivatives described below began with the preparation of tri-substituted anilines, which were synthesized using transition metal-catalyzed couplings starting with 1,3,5-dibromofluoroaniline (Scheme 1). The amine functionality was installed with a Cu-catalyzed amidation using acetamide followed by saponification with NaOH, yielding the aniline **4** in an economical fashion. The remaining bromide in **4** was further derivatized via Pd-catalyzed coupling with $Zn(CN)_2$ or bis(pinacolato)diboron to afford the nitrile **5** and boronate ester **6**, respectively.[15,16]

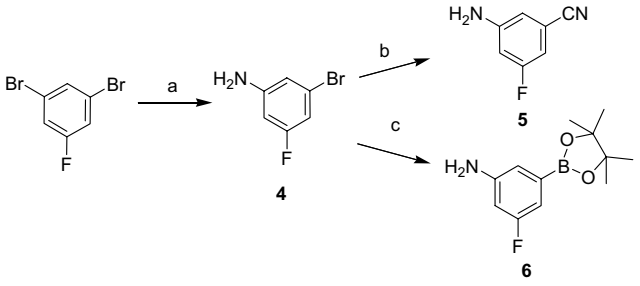
The 1,3-disubstituted tetrazole core was synthesized in a combined three step process from the starting aniline (Scheme 2). The 2-pyridylcarboxaldehyde was condensed with tosyl hydrazide to afford the hydrazone **7**, and each of the anilines was converted to the corresponding diazonium salt under acidic conditions. The two intermediates were combined under aqueous basic conditions to yield the respective tetrazole product in 50–75% yield.

Tetrazole derivatives in which the nitrile moiety of **3** is replaced with aryl rings were prepared by a Suzuki coupling reaction. With compounds **11** and **12**, both aryl boronic acids and aryl halides could be attached to the appropriate tetrazole coupling partner to give the final compounds (Scheme 3). By making use of the basicity of the products, these were readily purified with the use of an acidic ion-exchange resin.[17] The yields were reasonable (50–70%) and the purity excellent (>90% by HPLC).

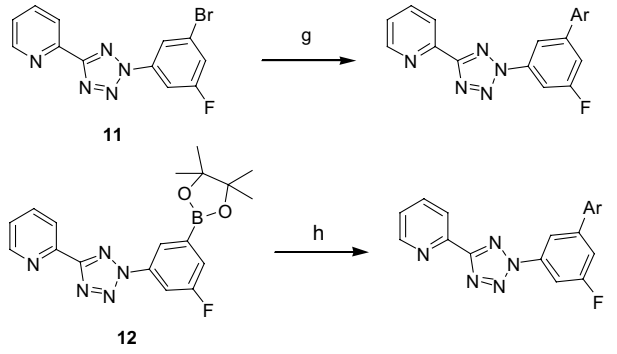
The in vitro functional activity of potential mGlu5 receptor antagonists was determined using a cell-based, high throughput assay that measured the changes in cytosolic Ca^{2+} concentrations by fluorescence detection.[18] Compounds were also tested in a binding assay, which measured the displacement of [H^3]-3-methoxy-5-(pyridin-2-ylethynyl)pyridine from rat cortical membranes.[19] The mGlu5 receptor potency data for the initial set of tetrazole derivatives synthesized is displayed in Table 1.

Phenyl derivative **13** was determined to have moderate in vitro activity (IC_{50} = 247 nM, K_i = 53 nM). Subsequent structure–activity relationship studies on substituted phenyl analogues showed that compounds containing an *ortho*-substituted phenyl ring (**14**, **17**, **19**) were particularly potent in both the functional and binding assays. Furthermore, the 2-pyridyl (**20**) and the 3-pyridyl (**21**) derivatives also maintained mGlu5 receptor potency. Notably, the 2-benzonitrile and the 2-aniline derivatives, compounds **17** (IC_{50} = 6 nM, K_i = 4 nM) and **19** (IC_{50} = 8 nM, K_i = 7 nM), were comparable in potency with compound **3** (IC_{50} = 4 nM, K_i = 12 nM) in both the functional and binding assays.

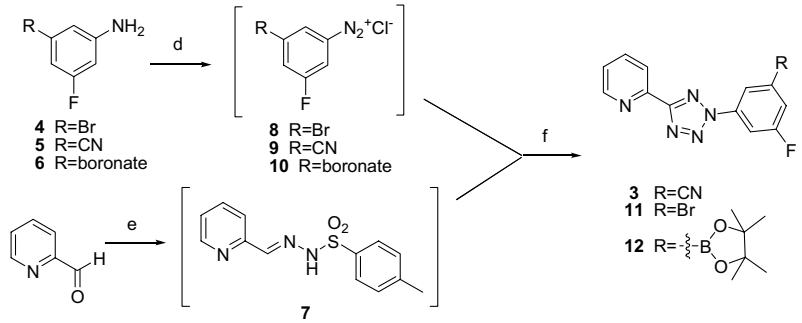
In an attempt to further enhance potency, a second iteration of compounds was synthesized to test whether the



Scheme 1. 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%); (c) bis(pinacolato)diboron, Pd(dppf), KOAc, DMSO, 85 °C, 2 h (82%).

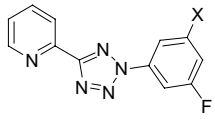


Scheme 3. Reagents and conditions: (g) $ArB(OH)_2$, $Pd(PPh_3)_4$, K_2CO_3, 5:1 DME/H_2O, 90 °C; (h) ArBr, $Pd(PPh_3)_4$, K_2CO_3, 5:1 DME/H_2O, 90 °C.



Scheme 2. Reagents and conditions: (d) $NaNO_2$, HCl, 1:1 H_2O EtOH, 0 °C; (e) tosyl hydrazide, EtOH; (f) NaOH, 0 °C.

Table 1. In vitro data of 4-ring tetrazoles



Compound	X	mGlu5 Ca^{2+} flux IC_{50} (nM)[a]	mGlu5 K_i (nM)[b]
3	CN	4	14
13	Ph	247	53
14	*o*-Tolyl	120	23
15	*m*-Tolyl	1467	NT[c]
16	*p*-Tolyl	>3000	NT[c]
17	2-Benzonitrile	6	4
18	3-Benzonitrile	253	12
19	2-Aniline	8	7
20	2-Pyridyl	39	95
21	3-Pyridyl	12	37
22	4-Pyridyl	956	575

[a] Ca^{2+} flux assay using glutamate (10 μM) as agonist ($n = 2$–4, SD ≈ ±25%).[20]

[b] Displacement by test compounds of [^{3}H]-3-methoxy-5-(pyridin-2-ylethynyl)pyridine from rat cortical membranes ($n = 2$–4, SD ≈ ±25%).[17]

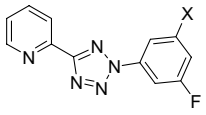
[c] Not tested.

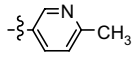
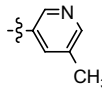
potency enhancing effects of the aryl substitution and the pyridyl moiety were additive. Thus, a series of substituted halopyridines were coupled to **12** using a microwave assisted Suzuki method, affording good yields of the biaryl tetrazole products (75–85%).[21] The substituted pyridyl analogues are listed in Table 2.

As shown in Table 2, when the methyl group was fixed at the *ortho* position and the nitrogen of the pyridyl ring was systematically moved around the ring (**23**, **24**, **25**, **26**), reasonable levels of mGlu5 receptor potency was maintained in each case. In particular, compound **26**, which combines the *ortho*-tolyl of **14** with the 3-pyridyl of compound **21**, had significant potency in the Ca^{2+} flux assay ($IC_{50} = 5$ nM) and in the binding assay ($K_i = 2$ nM). In turn, when the 3-pyridyl functionality was fixed and the methyl substituent moved around the ring as in **26**–**28**, the potency levels decreased, respectively. Interestingly, the 2-aminopyridine derivative **29**, showed diminished potencies relative to the corresponding methyl derivative **26**, and the original aniline derivative **19**. As **26** was the most potent compound synthesized in this series, it was selected for in vivo profiling.

A comparison of the rat receptor occupancy and pharmacokinetics for **3** and **26** are presented in Table 3. As with **3**, **26** possessed significant occupancy levels at 95% in the rat receptor occupancy assay when dosed at 10 mg/kg intraperitoneally (measuring at 1 h). At this time point, the hippocampal and plasma levels were determined to be 1.0 μM and 0.6 μM, respectively, indicating good rat brain penetration. In comparing the pharmacokinetic parameters, both **3** and **26** are bioavailable, however, **3** has a longer half-life ($t_{1/2} = 2.9$ h vs 1.6 h) and a lower clearance than **26** (Cl = 15 mL/min vs 35 mL/min).

Table 2. In vitro data of 4-ring tetrazoles



Cpd	X	mGlu5 Ca^{2+} flux IC_{50} (nM)[a]	mGlu5 K_i (nM)[b]
23		31	24
24		63	33
25		41	45
26		5	2
27		57	36
28		499	272
29		108	20
30		19	31

[a] Ca^{2+} flux assay using glutamate (10 μM) as agonist ($n = 2$–4, SD ≈ ±25%).[15]

[b] Displacement by test compounds of [^{3}H]-3-methoxy-5-(pyridin-2-ylethynyl)pyridine from rat cortical membranes ($n = 2$–4, SD ≈ ±25%).[17]

Table 3. Rat pharmacokinetic data comparison

PK parameter	**3**	**26**
Occ ED_{50}[a]	97%	95%
%F[b]	26	12
C_{max} (μM)[b]	2.5	0.9
$t_{1/2}$ (h)[b]	2.9	1.6
Cl_p (mL/min/kg)[b]	17	39

[a] Following 10 mg/kg dose of 50% PEG 400/H_20 (ip) at 1 h.

[b] iv dosing at 2 mg/kg of 50% PEG 400/H_20, po dosing at 10 mg/kg.

In conclusion, SAR studies on the phenyl ring of tetrazole **3** have shown that the nitrile moiety maybe replaced with a variety of *ortho*-substituted aryl groups. In particular, **26** was highly potent and selective against the mGlu5 receptor. It also had excellent rat brain receptor occupancy, brain penetration, and acceptable pharmacokinetic characteristics. Compound **26** should prove to be a valuable tool for future in vitro and in vivo studies.

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