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Discovery of highly potent, selective, orally bioavailable, metabotropic glutamate subtype 5 (mGlu5) receptor antagonists devoid of cytochrome P450 1A2 inhibitory activity

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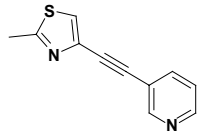
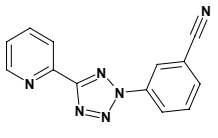
Abstract—Structure–activity relationship studies focused on bio-isosteric replacements of 2-pyridyl resulted in mGlu5 receptor antagonists with reduced inhibition of cytochrome P450 1A2. This led to highly potent, selective and orally bioavailable 2-imidazolyl tetrazoles such as (**10**) that are devoid of cytochrome P450 inhibitory activity.

Glutamate is the major excitory neurotransmitter in the nervous system exerting it's effect through both ionotropic receptors and the G-protein coupled metabotropic glutamate (mGlu) receptors. The eight mGlu receptor subtypes identified to date are classified into three groups based on sequence homology.[1] In group I, mGlu1 and mGlu5 are primarily localized postsynaptically and coupled via phospholipase C; activation leads to phosphoinositide hydrolysis and elevation of intracellular Ca^{2+} levels.[2] Selective antagonists of the mGlu5 receptor may be useful to treat several disease states including anxiety and depression,[3–8] pain,[9] drug dependence[10] and mental retardation.



1: MTEP



2

We have recently described the discovery of MTEP (**1**), a potent and selective mGlu5 receptor antagonist with anxiolytic properties.[11] In a continuing search for alternative structural series to diaryl-alkynes derivatives such as MTEP, we developed a series of heteroaromatic azoles exemplified by tetrazole **2**.[12] Compound **2** was found to be a potent and selective[13] mGlu5 receptor antagonist (mGlu5 Ca^{2+} flux = 73 nM) with good rat brain receptor occupancy (Table 1). In vitro profiling of **2** against cytochrome P450 (CYP) isoforms revealed that although **2** did not significantly inhibit CYP 3A4, 2D6, 2C9 or 2C19 (IC_{50} > 14 μM), it was a potent inhibitor of CYP 1A2 (IC_{50} = 3.8 μM). Indeed, this CYP 1A2 inhibition was observed for the tetrazole mGlu5 receptor antagonists as a class—vide supra. At 90% receptor occupancy, the concentration of **2** in plasma (9.7 μM) is higher than the CYP 1A2 IC_{50} and this constituted a potential liability for this series. To eliminate the possibility of drug–drug interactions, we sought to develop

Keywords: Metabotropic glutamate; Antagonist; Tetrazole; Cytochrome P450 1A2.
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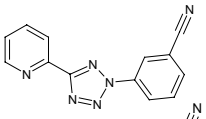
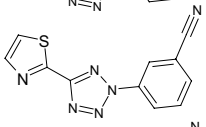
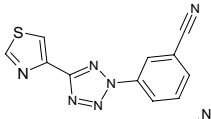
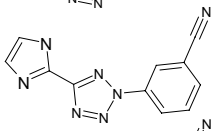
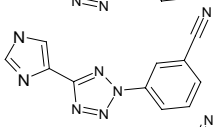
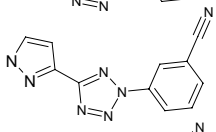
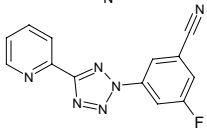
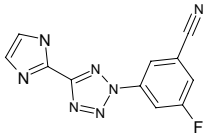
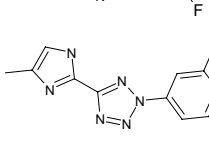
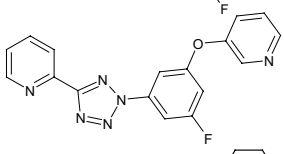
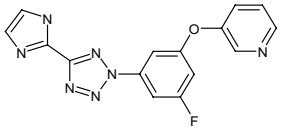
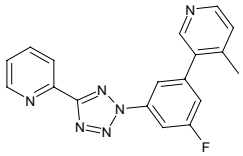
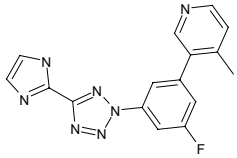
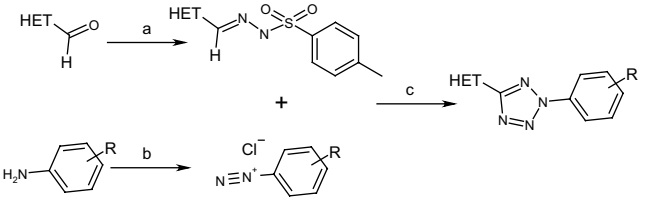
Table 1. In vitro data for mGlu5 receptor antagonists

Compound	Structure	mGlu5 Ca^{2+} flux (nM)[a]	mGlu5 K_i (nM)[b]	CYP 1A2 (μM)[c]	% Occ[d]
2		73	102	3.8	90
3		142	183	1.0	—[e]
4		148	157	1.7	—[e]
5		77	34	>14	—[e]
6		>2000	>2000	—[e]	—[e]
7		>2000	>2000	—[e]	—[e]
8		3.9	14	2.3	97
9		47	9.3	>14	95
10		77	19	>14	97
11		6.7	12	0.7	90
12		9.7	3.2	5.1	69
13		5.2	1.8	0.5	95
14		20	3.5	3.5	29

[a] Using glutamate (10 μM) as agonist ($n = 2–4$, SD < ±25%).[17]
[b] Displacement by test compounds of [^{3}H]-3-methoxy-5-(pyridin-2-ylethynyl)pyridine from rat cortical membranes ($n = 2–4$, SD < ±25%).[18]
[c] Recombinant cytochrome P450 protocols.[19]
[d] Measured 1 h post-administration of 10 mg/kg compound ip.[20]
[e] Not tested.



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

tetrazole derivatives that did not inhibit the major CYP isoforms and in particular CYP 1A2. Our efforts towards this goal are described herein.

Briefly, the N-linked tetrazoles derivatives were prepared by reaction of a tosyl hydrazone (derived from the condensation of a heterocyclic aldehyde with tosyl hydrazide) with a diazonium salt under basic conditions (Scheme 1).[12,14] In turn the diazonium salt was derived from an appropriately substituted aniline.

Recent disclosures from this laboratory have described structure–activity relationship (SAR) studies around the phenyl ring of tetrazole **2** and demonstrated that significant increases in potency at the mGlu5 receptor maybe achieved by employing the appropriate substitution pattern.[12,15,16] However, in each case these improvements in potency also led to an increase in CYP 1A2 inhibition (Table 1, compounds **2**, **8**, **11**, **13**). Concerned that the pyridyl moiety in **2** might be responsible for CYP 1A2 inhibition, we carried out a survey of pyridyl ring replacements while keeping constant the 3-cyanophenyl ring. Selected examples from these SAR studies are shown in Table 1.

Compared with **2**, 2-thiazolyl derivative **3** (Ca^{2+} flux = 142 nM) and 5-thiazolyl derivative **4** (Ca^{2+} flux = 148 nM) maintained potency against the mGlu5 receptor but in both cases showed an undesired increase in CYP 1A2 inhibition. On the other hand, 2-imidazolyl derivative **5** also maintained mGlu5 receptor potency in the functional (Ca^{2+} flux = 77 nM) and binding (K_i = 34 nM) assays but more importantly CYP 1A2 inhibition was greatly reduced (IC_{50} > 14 μM: compared to 3.8 μM for **2**). In turn, moving the nitrogen around the ring as in 3-imidazolyl derivative **6** and 2-pyrazolyl derivative **7** led to a dramatic loss of mGlu5 receptor potency (Ca^{2+} flux >2000 nM).

Having demonstrated the improvement in CYP 1A2 inhibition upon replacing the 2-pyridyl moiety of **2** with a 2-imidazolyl moiety (as in **3**), we applied this finding to the lead compounds derived from SAR studies on the phenyl ring of tetrazole **2**.[12,15,16] Thus tetrazole **8** is highly potent against the mGlu5 receptor (Ca^{2+} flux = 3.9 nM, K_i = 14 nM) and showed high rat receptor occupancy (97% at 10 mg/kg ip) but inhibited CYP 1A2 with an IC_{50} of 2.3 μM. Gratifyingly, replacement of the 2-pyridyl ring in **8** with a 2-imidazolyl ring (**9**) greatly reduced CYP 1A2 inhibition (IC_{50} > 14 μM) while mGlu5 receptor binding potency (K_i = 9.3 nM) and rat receptor occupancy (95%) were maintained. Similarly, 4-methyl-2-imidazolyl derivative **10** maintained potency in the binding assay (K_i = 19 nM) and was a poor inhibitor of CYP 1A2 (IC_{50} > 14 μM). Interestingly, both **9** (Ca^{2+} flux = 47 nM) and **10** (Ca^{2+} flux = 77 nM) were somewhat less active in the Ca^{2+} flux functional assay compared to **8** (Ca^{2+} flux = 3.9 nM). However when tested in a phosphatidylinositol hydrolysis assay,[18] functional potency for **10** was maintained (IC_{50} = 23 nM) compared to **8** (IC_{50} = 41 nM).

The trend of reducing CYP 1A2 inhibition while maintaining mGlu5 receptor potency and selectivity was observed with other lead compounds also. Thus in comparing aryl ethers **11** and **12**, 2-imidazolyl derivative **12** has reduced CYP 1A2 inhibition while maintaining mGlu5 receptor potency in both the functional (Ca^{2+} flux = 9.7 nM) and binding (K_i = 3.2 nM) assays. Similarly, in comparing biaryls **13** and **14**, mGlu5 receptor potency is maintained while CYP 1A2 inhibition is reduced. However, in both **12** and **14** there is a reduction of mGlu5 receptor occupancy in vivo.

A full profile of CYP inhibition and rat pharmacokinetics comparing **8** and **10** is shown in Tables 2 and 3.

Both **8** (K_i = 14 nM) and **10** (K_i = 19 nM) are potent and selective mGlu5 receptor antagonists. When screened

Table 2. CYP P450[a] profile of **8** and **10**

Compound	mGlu5 K_i (nM)	1A2 (μM)	3A4 (μM)	2D6 (μM)	2C9 (μM)	2C19 (μM)
8	14	2.3	>14	>14	>14	>14
10	19	70	>14	>14	>14	>14

[a] Recombinant cytochrome P450 Gentest based protocols.[19]

Table 3. Rat occupancy[a] and PK[b] profile of **8** and **10**

Compound	Rec Occ[b] (%)	Brain (μM)[c]	%F	$t_{1/2}$ (h)	Vd (L/kg)	Cl (mL/min/kg)	AUC (μM h)
8	97	9.7	24	2.9	1.1	15	9.6
10	97	8.1	22	0.3	0.6	40	3.4

[a] Rat brain receptor occupancy measured 1 h post-administration of 10 mg/kg ip (PEG 400).[20]
[b] 2 mg/kg dosed iv (solution in PEG 400/water (1/1)), 10 mg/kg dosed po (Sprague–Dawley rats).
[c] Measured at 1 h post-administration of 10 mg/kg ip (PEG 400).

for inhibition against a panel of the major cytochrome P450 isoforms, imidazole **10** did not inhibit any CYP isoforms at the test concentration (IC_{50}'s > 14 μM)—specifically the CYP 1A2 IC_{50} = 70 μM. In contrast, pyridine **8**, although not inhibitory against 3A4, 2D6, 2C9, 2C19, inhibits CYP 1A2 with IC_{50} = 2.3 μM.

Both compounds showed high rat receptor occupancy (97%) when dosed at 10 mg/kg intraperitoneally (measuring at 1 h). Furthermore at this time point the brain levels of **8** and **10** were 9.7 and 8.1 μM, respectively, indicating both compounds were highly brain penetrant. In comparing rat pharmacokinetics, both compounds are bioavailable (%F = 22–24%), however, imidazole **10** suffers from a short high-life ($t_{1/2}$ = 0.3 h) and high clearance (Cl = 40 mL/min/kg) when compared to **8** ($t_{1/2}$ = 2.9 h, Cl = 15 mL/min/kg). Due to the short half-life in rat, these 2-imidazolyl tetrazole compounds were not developed further.

In conclusion, SAR studies have shown that replacing the 2-pyridyl ring of the tetrazole class of mGlu5 receptor antagonist with a 2-imidazolyl ring leads to compounds that do not inhibit CYP 1A2 (nor any of the other major CYP isoforms) while maintaining high potency, selectivity and in vivo receptor occupancy. However, a short half-life in rat precluded these compounds from further development.

Acknowledgements

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