Selective, Orally Active γ-Aminobutyric Acid$_A$ α5 Receptor Inverse Agonists as Cognition Enhancers

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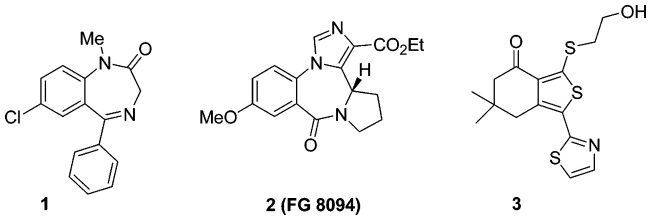
Received October 24, 2003

Abstract: Nonselective inverse agonists at the γ-aminobutyric acid$_A$ (GABA-A) benzodiazepine binding site have cognition-enhancing effects in animals but are anxiogenic and can precipitate convulsions. Herein, we describe novel GABA-A α5 subtype inverse agonists leading to the identification of **16** as an orally active, functionally selective compound that enhances cognition in animals without anxiogenic or convulsant effects. Compounds of this type may be useful in the symptomatic treatment of memory impairment associated with Alzheimer's disease and related dementias.

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in mammalian brain, and γ-aminobutyric acid$_A$ (GABA-A) receptors constitute the largest population of inhibitory neurotransmitter receptors. The GABA-A receptor is a GABA-gated chloride ion channel with multiple allosteric modulatory sites, in addition to the GABA binding site, and the benzodiazepine (BZ) site is one of the most studied of these. Ligands that bind to the BZ site can influence the binding of GABA to the receptor and thereby alter the flux of chloride ions through the ion channel. Ligands at the BZ site are categorized as agonists, inverse agonists, or antagonists. Agonists act by increasing the frequency of channel opening to give a net hyperpolarization of the neuron and a decreased excitability. BZ inverse agonists have the opposite effect and decrease the frequency of channel openings, resulting in a depolarization and an increased neuronal excitability. Between the two efficacy extremes, there is a continuum of partial agonists and partial inverse agonists as well as antagonists that do not alter chloride flow and are functionally silent. These different efficacies are reflected in different behavioral effects of BZ site ligands.

GABA-A receptors are pentameric assemblies of a large range of subunits (α1−6, β1−3, γ1−3, δ, ϵ, π, and θ), of which the α subunit is of particular importance in determining the pharmacology of the BZ binding site.[1] The major BZ-sensitive GABA-A receptor subtypes in the brain are α1βxγ2, α2βxγ2, α3βxγ2, and α5βxγ2, and their distribution in the brain shows distinct regional variations. For example, the α5-subunit-containing receptors represent less than 5% of total brain GABA-A receptors, yet in the hippocampus, a region of the brain associated with learning and memory processes, they constitute 20% of all GABA-A receptors.[2,3]

Nonselective BZ agonists (similar activity at the different GABA-A receptor subtypes) such as diazepam (**1**) are used clinically for the treatment of anxiety and epilepsy.[4] However, they also induce amnesia in humans



and animals.[5,6] Conversely, nonselective BZ receptor inverse agonists have cognition-enhancing effects in animal models[7] but are anxiogenic[8] and convulsant[9] or proconvulsant[10] and, as such, cannot be used to treat cognitive deficits in humans. By use of genetically modified (knock-in) mice, it has been demonstrated that α1-subunit-containing GABA-A receptors mediate sedative/muscle-relaxant effects while α2/α3-subunit-containing receptors mediate anxiolytic and anticonvulsant effects.[11−13] The role of α5-subunit-containing receptors, however, remains largely undefined. Given the abundance of α5-subunit-containing receptors in the hippocampus,[14] it has been hypothesized that this subtype may be involved in cognitive processes.[15] Further, we have proposed that a selective α5 inverse agonist may have therapeutic utility as a cognition-enhancing agent without the unwanted side effects associated with activity at other receptor subtypes. Most drugs currently used in the treatment of cognitive deficiency act through the cholinergic system and have moderate clinical efficacy. GABA-A α5 subtype selective inverse agonists may offer an alternative mechanism for the symptomatic treatment of memory impairment associated with Alzheimer's disease and related dementias.

Of the numerous structural classes that have been shown to bind to the BZ site, relatively few are selective for the α5 subtype. These include imidazobenzodiazepines[16] (e.g., FG 8094, **2**) and some diazepam analogues,[17] both of which exhibit binding selectivity for α5-subunit-containing receptors compared to the other receptor subtypes. In addition, we recently described novel 6,7-dihydro-2-benzothiophen-4(5*H*)-ones (e.g., **3**) that have binding selectivity for the α5 subtype.[18,19] However, typically these compounds had low oral bioavailability in animals, preventing their further development. In this communication, we describe the design, synthesis, and biological evaluation of orally active GABA-A α5 receptor inverse agonists leading to the identification of an *efficacy selective* compound that enhances cognition in animals while being devoid of anxiogenic, convulsant, and proconvulsant activity.

In this study, the efficacies of compounds at the GABA-A receptor subtypes were determined at cloned

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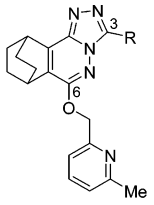
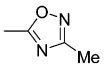
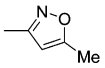
10.1021/jm031076j CCC: $27.50
Published on Web 03/27/2004

Table 1. Binding Affinities and Efficacies of Bicyclo-2.2.2-Triazolopyridazines at Cloned Human GABA-A Receptor Subtypes



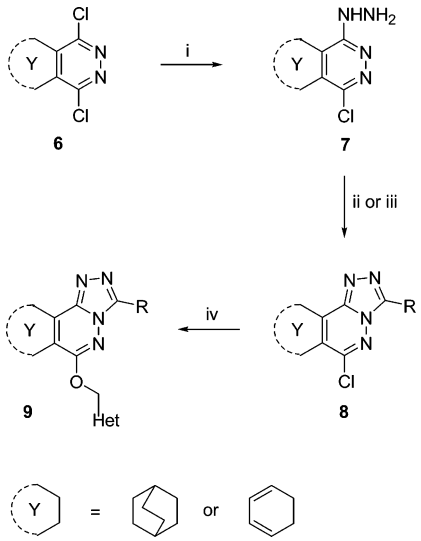
		K_i (nM) GABA-A αxβ3γ2 receptors[a]				Efficacy at GABA-A αxβ3γ2 receptors (%)[b]			
No.	R	α1	α2	α3	α5	α1	α2	α3	α5
DMCM		16 ± 3.4	11 ± 2.7	10 ± 2.7	**2.3 ± 0.58**	-36 ± 5	-34 ± 6	-52 ± 4	**-52 ± 4**
CDZ		770 ± 170	460 ± 71	740 ± 160	**520 ± 140**	+165 ± 6	+101 ± 8	+173 ± 10	**+134 ± 14**
4		170 ± 35	46 ± 5.9	25 ± 2.0	**2.9 ± 0.45**	+53 ± 12	+36 ± 6	+44 ± 9	**+15 ± 3**
5		74 ± 6.8	17 ± 0.85	7.6 ± 1.1	**1.1 ± 0.18**	-15 ± 2	-10 ± 3	-21 ± 5	**-35 ± 8**
10		270 ± 45	110 ± 20	48 ± 1.6	**3.7 ± 0.57**	+17 ± 11	+9 ± 0.3	+38 ± 8	**+7 ± 1**
11		250 ± 35	63 ± 12	40 ± 4.6	**3.1 ± 0.24**	+27 ± 5	-6 ± 3	-4 ± 6	**-24 ± 4**
12		280	86	41	**4.6**	+21 ± 7	+15 ± 2	-1 ± 4	**-29 ± 4**

[a] Displacement of [^{3}H]Ro 15-1788 binding from recombinant human GABA-A receptor subtypes. K_i values are the mean of at least two independent determinations; the standard error of the mean (SEM) is given where at least three independent determinations were performed. [b] Efficacy is determined as the percentage modulation of the submaximal (EC_{20}) response to GABA. Values are the arthimetic mean ± SEM of at least three independent cells from GABA-A receptor subtypes transiently expressed in *Xenopus laevis* oocytes.

human α1-, α2-, α3-, and α5-containing receptors transiently expressed in *Xenopus* oocytes by measurement of the modulatory effect on the GABA EC_{20} ion current using two-electrode voltage clamp electrophysiology.[20] The efficacy scale from full inverse agonist to full agonist is unsymmetrically distributed about the point of zero efficacy (antagonism). Thus, methyl 6,7-dimethoxy-4-ethyl-*β*-carboline-3-carboxylate (DMCM), which is regarded as a nonselective full inverse agonist, has an efficacy of −52% at α5-containing receptors while that of the nonselective full agonist chlordiazepoxide (CDZ) is +134%; a similar pattern is observed at the other receptor subtypes (Table 1).

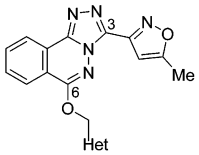
The starting point for our work was the triazolopyridazine **4** prepared in the course of the Merck GABA-A α2/α3 agonist program for the discovery of novel anxiolytics.[21] **4** has high GABA-A α5 receptor affinity combined with 8- to 40-fold binding selectivity over GABA-A α1, α2, and α3 receptor subtypes (Table 1); it is, however, a very low-efficacy partial agonist at GABA-A α5 receptors with somewhat higher efficacies at the other receptor subtypes. Other groups have demonstrated that the conversion of GABA-A receptor inverse agonists and antagonists to partial agonists can be achieved within structural classes by the introduction of appropriate lipophilicity.[22,23] On the basis of this precedent, the C-3 phenyl substituent of **4** was replaced with the more hydrophilic oxadiazole ring to give **5**. This resulted in a dramatic switch in the α5 efficacy profile to give an inverse agonist (−35%) while maintaining excellent α5 affinity (Table 1). **5** displays somewhat weaker inverse agonism at α1, α2, and α3 receptors. A range of different C-3 heterocycles was investigated using the chemistry shown in Scheme 1.[24] Thus, conversion of the 3,6-dichloropyridazines **6** to the monohydrazinopyridazines **7** followed by coupling with heterocyclic carboxylic acids and dehydration gave the 6-chloropyridazines **8**. Reaction of **8** with alkoxides gave derivatives with the general structure **9**. The results shown in Table 1 demonstrate that a range of different heterocycles was tolerated at the C-3 position, the compounds displaying high α5 affinity and a range of efficacies with the level of inverse agonism being dependent on the hydrophilicity of the heterocycle (e.g., **10** and **11**). All analogues showed higher affinity at α5 receptors than at the other subtypes, particularly

Scheme 1[a]



[a] Reagents: (i) $NH_2NH_2 \cdot H_2O$, EtOH, reflux; (ii) (a) RCO_2H, CDI, DMF or RCO_2H, BOPCl, Et_3N, CH_2Cl_2; (b) cat. $Et_3N \cdot HCl$, xylene, reflux; (iii) RCOCl, Et_3N, room temp, then reflux; (iv) $HetCH_2OH$, NaH or LHMDS, DMF.

Table 2. Binding Affinities and Efficacies of Triazolophthalazines at Cloned Human GABA-A Receptor Subtypes



		K_i (nM) GABA-A αxβ3γ2 receptors[a]				Efficacy at GABA-A αxβ3γ2 receptors (%)[b]			
No.	Het	α1	α2	α3	α5	α1	α2	α3	α5
13		0.88 ± 0.05	1.6 ± 0.20	1.3 ± 0.35	**0.79 ± 0.15**	+15 ± 6	+12 ± 3	+3 ± 3	**-15 ± 8**
14		0.93 ± 0.17	1.5 ± 0.38	0.96 ± 0.16	**0.62 ± 0.08**	-2 ± 1	+15 ± 5	-4 ± 3	**-46 ± 2**
15		1.3 ± 0.18	0.64 ± 0.12	0.80 ± 0.17	**0.87 ± 0.15**	-5 ± 3	+9 ± 2	-3 ± 2	**-35 ± 1**
16		0.88 ± 0.19	0.58 ± 0.17	0.61 ± 0.17	**0.66 ± 0.14**	-4 ± 2	+12 ± 3	+4 ± 4	**-29 ± 3**
17		4.2	2.8	5.2	**2.0**	-8 ± 1	-25 ± 4	-6 ± 4	**-33 ± 1**

[a] Displacement of [^{3}H]Ro 15-1788 binding from recombinant human GABA-A receptor subtypes. K_i values are the mean of at least two independent determinations; the standard error of the mean (SEM) is given where at least three independent determinations were performed. [b] Efficacy is determined as the percentage modulation of the submaximal (EC_{20}) response to GABA. Values are the arthimetic mean ± SEM of at least three independent cells from GABA-A receptor subtypes transiently expressed in *Xenopus laevis* oocytes.

compared to α1-containing receptors (40- to 75-fold). As well as displaying excellent binding selectivity, the furan **11** and isoxazole **12** also showed functional selectivity for the α5 subtype, being inverse agonists at α5 and weak partial agonists or antagonists at α1, α2, and α3. Unfortunately, neither of these compounds were orally bioavailable in rats. In particular, both compounds were found to be rapidly and extensively metabolized when incubated with rat liver microsomes, and a detailed study revealed that a major route of metabolism, in vitro and in vivo, for compounds of this class was through hydroxylation of the [2.2.2]-bicyclic ring system. To remove this source of metabolism, the bicyclic ring system was modified to afford triazolophthalazines (Scheme 1, Table 2).

13, the triazolophthalazine analogue of **12**, retained excellent α5 binding affinity and functional selectivity, although there was a modest reduction in the level of α5 inverse agonism. Interestingly, replacement of the [2.2.2] bicycle led to complete loss of α5 binding selectivity. Although **13** showed poor oral bioavailability in rats, deletion of the methyl group from the C-6 pyridyl substituent (**14**) yielded good bioavailability (41%). **14** has subnanomolar α5 affinity and, although it has no binding selectivity, shows enhanced functional selectivity compared to **12**. Although **14** was not convulsant when dosed alone to mice, it was proconvulsant, potentiating pentylenetetrazole (PTZ) induced seizures in mice[25] when given at high doses corresponding to full α5 receptor occupancy.[26]

A variety of heterocycles was explored as replacements for the C-6 pyridine ring of **14** to provide further optimization of in vivo properties. Of particular interest were the isomeric triazoles **15**−**17**, all of which retained excellent α5 affinity, high α5 inverse agonism, and functional selectivity (Table 2). The position of the methyl substituent on the triazole ring proved critical, giving rise to subtle changes in the overall efficacy profile of the isomers that manifested itself in vivo. Thus, **15** and **17** were proconvulsant in the PTZ assay while **16** showed no effects at doses that occupied approximately 95% of α5 receptors. **16** also showed no anxiogenic effects[27] in rats at doses that gave greater than 85% occupancy of BZ receptors[28] and was orally bioavailable in rats, dogs, and rhesus monkeys. In the delayed matching to position test in the water maze,[29] a hippocampal-dependent cognitive assay, **16** significantly enhanced the performance of rats at an oral dose of 0.3 mg/kg, corresponding to approximately 40% of BZ receptor sites being occupied. Thus, **16** enhances cognition in animals without anxiolytic, convulsant, or proconvulsant side effects and, on the basis of its overall in vitro and in vivo profile, was selected for clinical evaluation.

Supporting Information Available: Experimental details for the synthesis of **16** and NMR and mass spectral data, microanalysis results, and melting points for test compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM031076J