Letters

6,7-Dihydro-2-benzothiophen-4(5*H*)-ones: A Novel Class of GABA-A α5 Receptor Inverse Agonists

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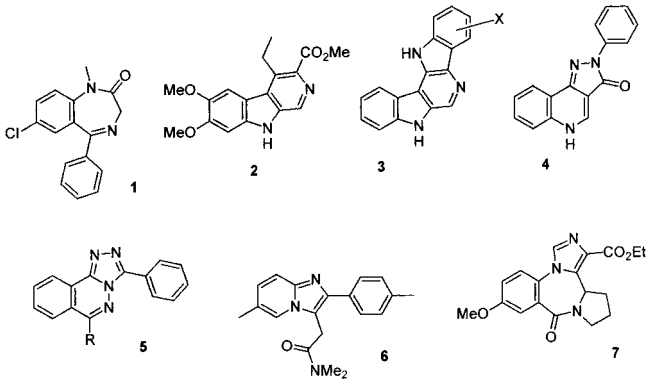
Abstract: Nonselective inverse agonists at the benzodiazepine binding site on the GABA-A chloride ion channel enhance cognitive performance in animals but cannot be used in the treatment of cognitive disorders because of anxiogenic and convulsant side effects. We have identified a novel series of GABA-A α5 receptor ligands during our search for α5 receptor inverse agonists as potential cognition enhancers. In particular, 6,6-dimethyl-3-(2-hydroxyethyl)thio-1-(thiazol-2-yl)-6,7-dihydro-2-benzothiophen-4(5*H*)-one (**26**) has been identified as a functionally selective GABA-A α5 inverse agonist.

Introduction. γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in mammalian brain. The binding of GABA to the receptor is modulated by binding of chemical entitites to allosteric sites on the GABA ion channel complex—one of the most important of which is the benzodiazepine (BZ) site. Ligands that bind to this allosteric site are categorized either as agonists (which increase the affinity of GABA for the ion channel complex), antagonists (which bind to the GABA-A receptor but have no intrinsic efficacy), or inverse agonists (which reduce the affinity of GABA for the GABA-A receptor). Partial agonists and partial inverse agonists span this efficacy range. GABA-A receptors are composed of five heteromeric subunits of which various subfamilies (α, β, γ, δ, ϵ, π, and θ) have been identified using molecular cloning techniques.[1] Of these, the α, β, and γ subunits are the most important since it is recombinant receptors expressing those subunits which most closely mimic the functional and biochemical response of native GABA-A receptors from mammalian brain cells. The major BZ-sensitive GABA-A receptor subtypes in the brain are α1βxγ2, α2βxγ2, α3βxγ2, and α5βxγ2. Nonselective BZ agonists such as diazepam (1), which bind to different GABA-A receptor subtypes with equal affinity, have found therapeutic use as anxiolytics and anticonvulsants.[2] However, they also impair learning and memory processes.[3,4] Conversely, nonselective inverse agonists enhance cognitive performance in animal models[5] but are anxiogenic,[6] convulsant,[7] pro-convulsant,[8] or may alter attentional processing.[9] As a consequence, nonselective BZ inverse agonists cannot be used to treat neurological disorders associated with cognitive impairment in humans. Although α5-containing GABA-A receptors constitute less than 5% of the total GABA-A receptor population in the brain, they are primarily located in the hippocampus, a region of the brain associated with learning and memory, where they represent 20% of all GABA-A receptors.[10,11] Thus, we rationalized that a selective α5 inverse agonist may have therapeutic utility as a cognition-enhancing agent that may lack the unwanted side effects associated with activity at other GABA-A receptor subtypes. In addition to benzodiazepines such as **1** (Chart 1), a variety of different chemical classes have been shown to bind to the BZ site on the GABA-A receptor. These include the β-carbolines such as DMCM (**2**),[12−14] pyridinoindoles (**3**),[15,16] pyrazoloquinolinones (**4**),[17−20] triazolo[3,4-*a*]phthalazines (**5**),[21] imidazo[1,2-*a*]pyridines such as zolpidem (**6**),[22−24] and imidazo[1,5-*a*]benzodiazepines exemplified by **7**.[25−29] Of these the imidazobenzodiazepines[30] and some diazepam analogues[31] exhibit binding selectivity for the α5β3γ2 subunit compared to the other receptor subtypes.

Chart 1. Different Structural Classes of Benzodiazepine Site Ligands



In this Letter, we describe a novel class of GABA-A receptor ligands which have high affinity for the α5 subtype and identify a functionally selective GABA-A α5 inverse agonist.

Results and Discussion. As well as investigating the established chemical classes, we sought a structurally novel series of GABA-A receptor ligands with selective in vitro α5 inverse agonist activity. To achieve this goal, a directed screening approach was adopted using the Merck sample collection. Known BZ site ligands, including the pyridazinone **8** (Figure 1),[32] were used as the starting point for similarity searching.[33] Using this strategy, 6,6-dimethyl-3-methylthio-1-(pyrazol-3-yl)-6,7-dihydro-2-benzothiophen-4(5*H*)-one (**9**, Figure 2)[34] was identified as a high affinity GABA-A α5 receptor ligand (K_i 5.2 nM) that had 4−13-fold binding

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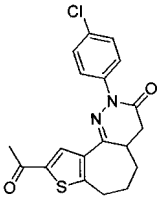

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Figure 1. 9-Acetyl-2-(4-chlorophenyl)-2,4,4a,5,6,7-hexahydro-3*H*-thieno[2′,3′:6,7]-cyclohepta[1,2-c]pyridazin-3-one (**8**)—a high affinity BZ site ligand.

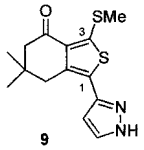

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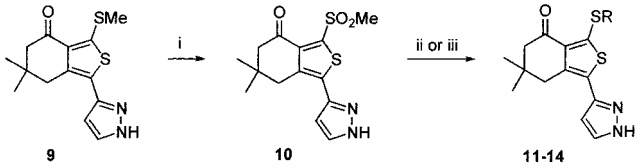
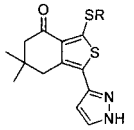
Figure 2. 6,6-Dimethyl-3-methylthio-1-(pyrazol-3-yl)-6,7-dihydro-2-benzothiophen-4(5*H*)-one (**9**)—a novel GABA-A receptor ligand.

Scheme 1[a]



[a] Reagents: (i) DCM, dioxan, *m*-CPMA; (ii) RSH, NaOH, EtOH, 25 °C or 70 °C; (iii) RSNa, THF, 25 °C.

Table 1. Binding Affinity of the C3-Thio-Substituted Thiophenes at the GABA-A Receptor Subtypes



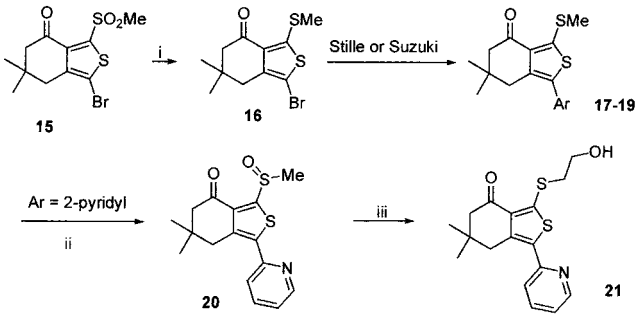
		K_i (nM) GABA-A αxβ3γ2 receptors[a]				
No.	R	α5	α1	α2	α3	Sel.[b]
2	-	2.2 ± 1.0	10 ± 1	13 ± 5	7.5 ± 1.2	3-6
9	Me	5.2 ± 1.5	66 ± 2	56 ± 17	21 ± 9	4-13
11	Et	1.6 ± 0.1	12 ± 5	16 ± 3	16 ± 2	8-10
12	^{i}Pr	2.0 ± 0.3	5 ± 1	12 ± 5	7 ± 1	3-6
13	$(CH_2)_2OH$	2.9 ± 0.5	31 ± 8	21 ± 4	24 ± 4	7-11
14		1.4 ± 0.6	4.3 ± 0.6	19 ± 3	48 ± 2	3-34

[a] Displacement of [^{3}H] Ro15-1788 binding from recombinant human GABA-A receptor subtypes. K_i values are the geometric mean ± SEM of three independent determinations. [b] Binding selectivity for GABA-A α5 receptors over GABA-A α1, α2, and α3 receptors.

selectivity over the GABA-A α1, α2, and α3 receptor subtypes.[35] Pyrazole **9** bears little resemblance to known BZ site ligands and represents a novel class of GABA-A receptor ligands.[36]

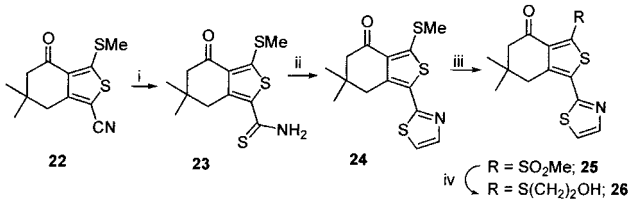
Following a structure−activity study it was established that, in terms of GABA-A α5 affinity, a thioether at the C3 position was optimal. Modification of the thio substituent was carried out according to Scheme 1, in which **9** was converted to the sulfone **10** which was then reacted with the appropriate thiolate to produce the thio-substituted derivatives **11**−**14**. The binding results, shown in Table 1, demonstrate that the C3 position was tolerant of a variety of thio substituents ranging from the more lipophilic isopropyl group (**12**) to substituents bearing polar functionality such as hydroxyethylthio

Scheme 2[a]



[a] Reagents: (i) MeSNa, THF; (ii) DCM, *m*-CPBA (1.0 equiv), −50 °C; (iii) NaOH, $HS(CH_2)_2OH$, EtOH.

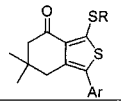
Scheme 3[a]



[a] Reagents: (i) H_2S, Et_3N, pyridine; (ii) chloroacetaldehyde, EtOH, reflux; (iii) DCM, *m*-CPBA (2.0 equiv), 25 °C; (iv) NaOH, $HS(CH_2)_2OH$, EtOH.

(**13**). At the C1 position, a nitrogen-containing heteroaromatic ring was found to be essential for high α5 affinity, and of these, 2-pyridyl and 2-thiazolyl were optimal. The pyridyl substituents were introduced using either Stille or Suzuki chemistry via the bromothiophene **16**, as indicated in Scheme 2. In the case of the thiazoles, the heterocycle was assembled by heating the thioamide **23** with chloroacetaldehyde (Scheme 3). The position of the nitrogen atom in the C1-heterocycle was crucial for optimum α5 binding affinity; it was those isomers in which the nitrogen is adjacent to the point of attachment of the heterocycle that had the highest α5 affinity (Table 2). Irrespective of the C1 or C3 substituent, all the analogues that we examined exhibited higher affinity at the GABA-A α5 receptor compared to the other GABA-A receptor subtypes. The efficacy of the thiophenes at the GABA-A α5 receptor subtype was measured using two-electrode voltage clamp recording from *Xenopus laevis* oocytes, transiently expressing the GABA-A α5β3γ2 receptor subtype.[29,37] As shown in Table 3, the nature of the C1-heterocycle had a significant effect on the functional response elicited by the compound. Whereas the pyrazoles **9** and **14** are antagonists at GABA-A α5 receptors, the thiazolyl and pyridyl analogues are α5 inverse agonists. In particular, the pyridines **17**, **18**, and **21** and the thiazole **26** have high α5 inverse agonism and are comparable to that of the nonselective, full BZ inverse agonist DMCM.[38,39] In addition to having 1 order of magnitude binding selectivity for the GABA-A α5 receptor over the α1, α2, and α3 subtypes, in vitro 6,6-dimethyl-3-(2-hydroxyethyl)-thio-1-(thiazol-2-yl)-6,7-dihydro-2-benzothiophen-4(5*H*)-one (**26**) is also a functionally selective α5 inverse agonist. Concentration−response curves were generated using whole cell patch-clamp recording from mammalian fibroblast (Ltk$^-$) cells[40] stably expressing the GABA-A αxβ3γ2 receptor subtype (x = 1, 2, 3, 5). As shown in Table 4, in the presence of a submaximal dose

Table 2. Binding Affinity of the C1-Heteroaryl-thiophenes at the GABA-A Receptor Subtypes



No.	Ar	R	K_i (nM) GABA-A αxβ3γ2 receptors[a] α5	α1	α2	α3	Sel.[b]
17		Me	1.7 ± 0.3	45 ± 3	26 ± 4	25 ± 4	15-26
18		Me	5.8 ± 1.2	165 ± 34	31 ± 7	33 ± 4	5-28
19		Me	9.1 ± 0.9	172 ± 28	43 ± 4	35 ± 8	4-19
21			0.5 ± 0.1	3.6 ± 1.0	5.2 ± 1.1	10 ± 1	7-20
24		Me	2.2 ± 0.2	61 ± 16	44 ± 10	47 ± 10	20-28
26			1.6 ± 0.4	20 ± 2	16 ± 2	20 ± 1	10-13

[a] Displacement of [^{3}H] Ro15-1788 binding from recombinant human GABA-A receptor subtypes. K_i values are the geometric mean ± SEM of three independent determinations. [b] Binding selectivity for GABA-A α5 receptors over GABA-A α1, α2, and α3 receptors.

Table 3. Efficacy of the Thiophenes at GABA-A α5β3γ2 Receptors

no.	α5β3γ2 efficacy (% ± SEM)[a]
2[b]	−34 ± 5
9	−3 ± 4
14	−7 ± 1
17	−31 ± 2
18	−40 ± 5
19	−20 ± 5
21	−39 ± 3
24	−24 ± 2
26	−38 ± 2

[a] Efficacy of the BZ site ligands is determined as the percentage modulation of the submaximal (EC_{20}) response to GABA. Values given are the arithmetic mean ± SEM of at least three individual cells from the α5β3γ2s receptor subtype transiently expressed in *Xenopus laevis* oocytes. [b] Efficacy is the arithmetic mean ± SEM of 11 individual cells from the α5β3γ2 receptor subtype transiently expressed in *X. laevis* oocytes.

Table 4. Efficacy of **2** and **26** in L(tk$^-$) Cells Expressing Different GABA-A Receptor Subtypes

compd	efficacy at GABA-A αxβ3γ2 receptors (%)[a] α1	α2	α3	α5
2	−71 ± 1.5	−53 ± 2.7	−62 ± 2.0	−57 ± 0.7
26	−21 ± 2.1[b]	−1 ± 1.9[b]	−3 ± 1.2[b]	−51 ± 1.8

[a] Maximum modulation of the current produced by **2** or **26** relative to a submaximal (EC_{20}) GABA response. Values are the mean maximum modulation ± SEM from at least five individually fitted concentration−response curves. [b] Values are the arithmetic mean ± SEM of at least five individual cells, produced using [**26**] = 100 nM from αxβ3γ2 (x = 1, 2, or 3) receptor subtypes stably expressed in mammalian fibroblast L(tk$^-$) cells.

(EC_{20}) of GABA, **26** behaves as a full inverse agonist at the GABA-A α5β3γ2 receptor, a low efficacy inverse agonist at the α1 subtype and an antagonist at α2 and α3. Compounds such as **26** will be valuable tools in delineating the involvement of α5-containing GABA-A receptors in memory processes.[41]

Conclusion. Using a directed screening approach, a series of 6,7-dihydro-2-benzothiophen-4(5*H*)-ones has been identified as a novel class of GABA-A receptor ligands. In particular, these compounds exhibit high affinity for the GABA-A α5 receptor subtype and have some binding selectivity over the GABA-A α1, α2, and α3 receptor subtypes. From this series, several compounds have high inverse agonism at the GABA-A α5 receptor, and 6,6-dimethyl-3-(2-hydroxyethyl)thio-1-(thiazol-2-yl)-6,7-dihydro-2-benzothiophen-4(5*H*)-one (**26**) has been identified as a binding and functionally selective GABA-A α5 receptor inverse agonist.

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Supporting Information Available: Experimental details including microanalysis, high-resolution mass spectra, HPLC data, and melting points for all test compounds as well as a detailed description of the biological test methods used. This material is available free of charge via the Internet at http://pubs.acs.org.

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(41) A paper describing the in vivo properties of **26** and an expanded SAR for the series will be submitted in due course.

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