Identification of a Novel, Selective $GABA_A$ α5 Receptor Inverse Agonist Which Enhances Cognition

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In pursuit of a $GABA_A$ α5-subtype-selective inverse agonist to enhance cognition, a series of 6,7-dihydro-2-benzothiophen-4(5*H*)-ones has been identified as a novel class of $GABA_A$ receptor ligands. These thiophenes have higher binding affinity for the $GABA_A$ α5 receptor subtype compared to the $GABA_A$ α1, α2, and α3 subtypes, and several analogues exhibit high $GABA_A$ α5 receptor inverse agonism. 6,6-Dimethyl-3-(2-hydroxyethyl)thio-1-(thiazol-2-yl)-6,7-dihydro-2-benzothiophen-4(5*H*)-one (**43**) has been identified as a full inverse agonist at the $GABA_A$ α5 receptor and is functionally selective over the other major $GABA_A$ receptor subtypes. **43** readily penetrates into the CNS to give selective occupancy of $GABA_A$ α5 receptors. In addition, **43** enhances cognitive performance in rats in the delayed 'matching-to-place' Morris water maze test—a hippocampal-dependent memory task—without the convulsant or proconvulsant activity associated with nonselective, $GABA_A$ receptor inverse agonists.

Introduction

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in mammalian brain. There are three classes of GABA receptors: $GABA_A$ and $GABA_C$ receptors are ligand-gated ion channels whereas $GABA_B$ receptors are G-protein-coupled receptors.[1] Of these, it is the $GABA_A$ receptor family which has been the most widely studied since this family is the site of action of a number of clinically important drugs, including benzodiazepines (BZs), barbiturates, and anesthetics.[2] $GABA_A$ receptors are pentameric assemblies of protein derived from a family of genes encoding a number of subunits (α1−6, β1−3, γ1−3, δ, ϵ, π, and θ). The majority of $GABA_A$ receptors in the brain contain α-, β-, and γ-subunits,[3,4] and those containing α1, α2, α3 or α5 in conjunction with a β- and γ2-subunit (around 80% of the total $GABA_A$ receptor population)[3] contain a BZ binding site which allosterically modulates the functional effects of GABA. For example, the binding of the prototypic benzodiazepine diazepam (**1**) to the BZ site on the $GABA_A$ receptor enhances the action of GABA by increasing the frequency of GABA-activated channel openings. Diazepam is a nonselective BZ agonist (or positive allosteric modulator), in that it has equivalent affinity and efficacy for α1-, α2-, α3-, and α5-containing $GABA_A$ receptors; nonselective BZ agonists have found therapeutic use as anxiolytics and anticonvulsants.[5] However, they also impair learning and memory processes.[6,7] In contrast, the β-carboline DMCM (**2**) produces a decrease in the frequency of GABA-activated channel openings, and it is termed an inverse agonist[8,9] (or negative allosteric modulator). The opposing effects of agonists and inverse agonists on $GABA_A$ receptor function are reflected in whole animal pharmacology in that nonselective BZ inverse agonists enhance cognitive performance in animal models[10] but can be anxiogenic,[11] convulsant,[12] and proconvulsant,[13] and they also may alter attentional processing.[14] Finally, a compound which binds to the BZ site but has no effect on the frequency of GABA-induced channel openings is termed a BZ antagonist.

Using genetically modified (knock-in) mice it has been demonstrated that $GABA_A$ receptors containing an α1-subunit mediate the sedative/muscle relaxant effects of benzodiazepines, whereas α2- and/or α3-subunit-containing receptors mediate the anxiolytic and anticonvulsant effects.[15−17] $GABA_A$ α5 receptors have a relatively restricted distribution, being primarily expressed in the hippocampus, a region of the brain associated with learning and memory. Although α5 receptors account for less than 5% of the total $GABA_A$ receptor population in the brain, in the hippocampus they represent 20% of all $GABA_A$ receptors,[3,18,19] thereby implicating this $GABA_A$ receptor subtype in learning and memory processes. Thus, we proposed 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.

Selective $GABA_A$ α5 inverse agonism may be achieved either in terms of binding selectivity (i.e., a high affinity α5 inverse agonist which has very low affinity at the other $GABA_A$ receptor subtypes) or functional selectivity (i.e., an inverse agonist at the $GABA_A$ α5 receptor but low efficacy at the other $GABA_A$ receptor subtypes). Of the many different structural classes of $GABA_A$ receptor ligands, it is only the imidazobenzodiazepines,[20,21] for example L-655,708 (**3**), and some diazepam analogues[22] which are reported to exhibit binding selectivity for α5-containing $GABA_A$ receptors compared to the other receptor subtypes.

In this manuscript, we describe a novel class of $GABA_A$ receptor ligands[23] which have higher affinity for

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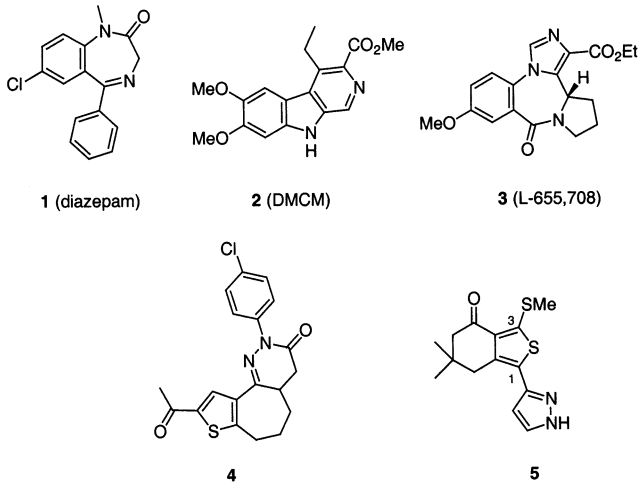
Chart 1. $GABA_A$ Benzodiazepine Site Ligands



the α5-subtype compared to the other $GABA_A$ receptor subtypes. Several examples exhibit high α5 inverse agonism and, in particular, we describe the synthesis and biological evaluation of a functionally selective $GABA_A$ α5-subtype receptor full inverse agonist which enhances cognitive performance in a hippocampal-dependent memory task, without the convulsant or proconvulsant liabilities associated with nonselective $GABA_A$ inverse agonists.

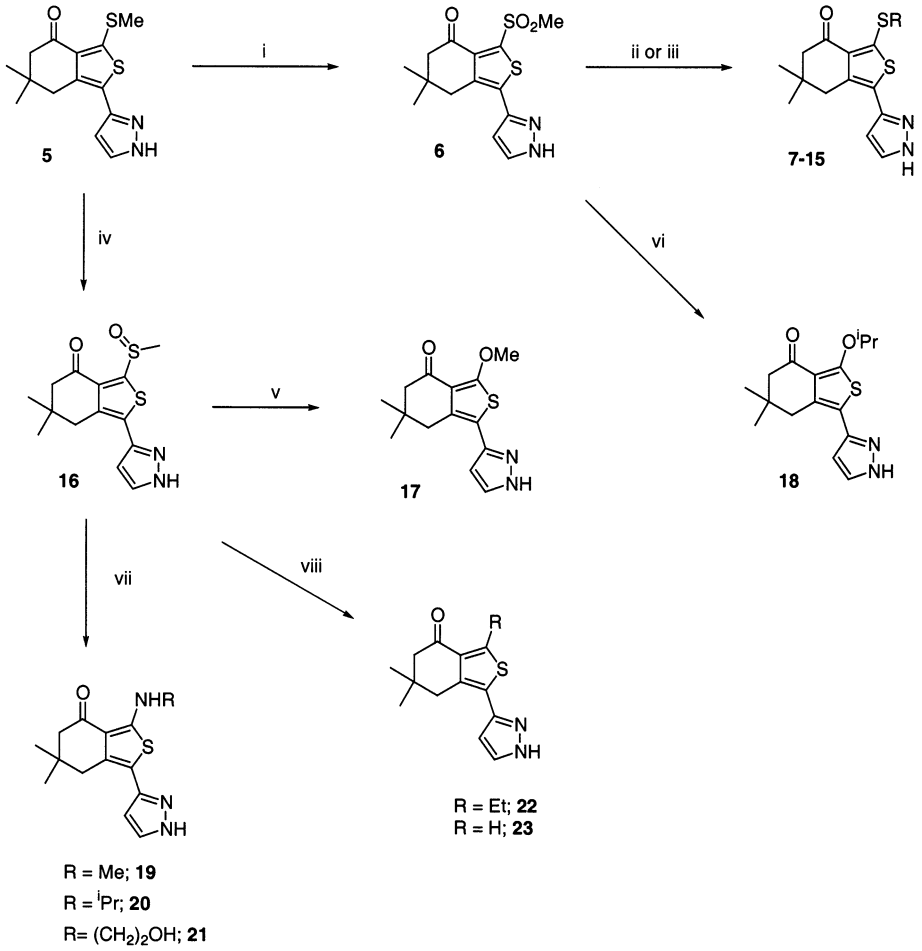
Lead Identification. The approach we followed in order to identify a novel and selective $GABA_A$ α5 inverse agonist was to adopt a similarity searching technique, using two-dimensional atom pair and topological torsion descriptors.[24] By comparing known BZ ligands such as the pyridazinone **4** (K_i Bz binding sites: 4.2 nM)[25] with compounds from the Merck database, this directed screening approach ultimately identified 6,6-dimethyl-3-methylthio-1-(pyrazol-3-yl)-6,7-dihydro-2-benzothiophen-4(5*H*)-one (**5**)[26] as a high affinity $GABA_A$ α5 receptor ligand (K_i 5.2 nM) that has 4−13-fold binding selectivity over the $GABA_A$ α1, α2, and α3 receptor subtypes. The tetrahydrobenzothiophene **5** represents a structurally novel class of $GABA_A$ receptor ligands and was used as the starting point in our structure−activity studies.

Chemistry. Synthesis of the C3-substituted analogues was carried out according to Scheme 1. Modification of the thioalkyl group was achieved by oxidizing **5** to the sulfone **6** followed by reaction with the desired thiolate. Similarly, reaction of **6** with sodium isopropoxide afforded the isopropyl ether **18**. The methoxy (**17**), alkylamino (**19**−**21**), and ethyl (**22**) derivatives were prepared via reaction of the sulfoxide **16** with sodium methoxide, the appropriate alkylamine, and ethylmagnesium bromide, respectively. In the case of the Grignard reaction, the unsubstituted thiophene **23** was also obtained. The introduction of heterocyclic substituents (and phenyl) at the C1 position was accomplished according to Schemes 2−4. As shown in Scheme 2, the majority of C1-substituents were incorporated using either Stille or Suzuki methodology via the bromothiophene **25**. The oxadiazole **36** was synthesized by transforming the commercially available carboxylic acid **35**[26] into the imidazolide which, in turn, was reacted with acetamide oxime (Scheme 3). The thiazole (**39**) and triazole (**41**) analogues were both obtained from the cyanothiophene **37**,[26] via the thioamide (**38**) and imino ether (**40**) derivatives, respectively (Scheme 4).

Results and Discussion

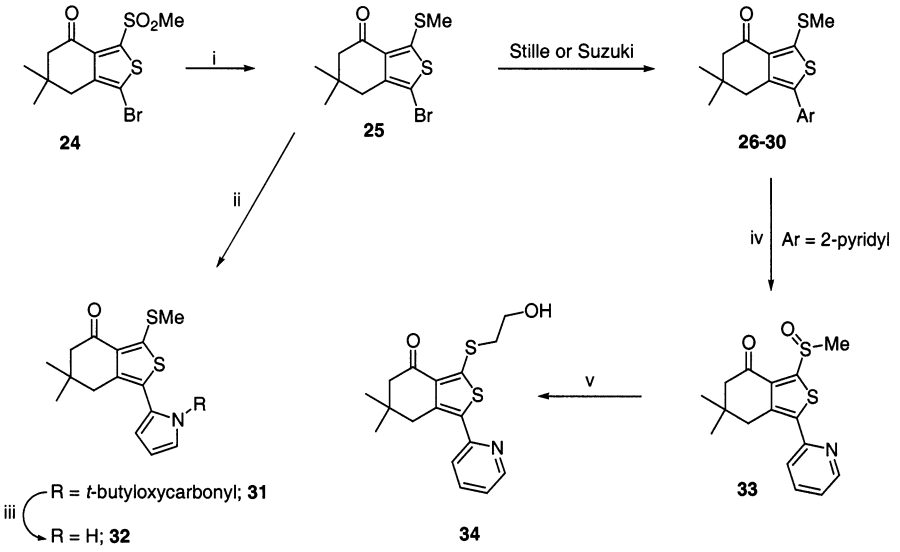
In Vitro Binding Affinity. The in vitro binding affinity for the thiophenes at the human $GABA_A$ receptor subtypes are shown in Tables 1 and 2. In general, it was found that a variety of thioether substituents are well tolerated at the C3 position of the tetrahydrobenzothiophene skeleton. As shown in Table 1, homologation of the methylthio substituent to ethylthio (**7**) or propylthio (**8**) had no detrimental effect on α5 affinity or subtype selectivity. Further extension to butylthio (**9**) did, however, result in an order of magnitude decrease in α5 binding affinity compared to **7**, although selectivity for the $GABA_A$ α5 subtype over the α1, α2, and α3 receptors was retained. The isopropylthio derivative **10** also had high $GABA_A$ α5 affinity but, as indicated by the *tert*-butylthio analogue **11**, there is a limit to the extent of steric crowding that can be tolerated adjacent to sulfur. The introduction of an hydroxyl group onto the thioalkyl substituent had no detrimental effect on either $GABA_A$ α5 affinity or subtype selectivity (cf. **12** with **7** and **8** with **13**). Heteroarylthio substituents also produced high affinity α5 ligands and, in particular, thiazolylthio (**14**) and thienylthio (**15**) were particularly well tolerated. In general, replacing the alkylthio substituent with alkyloxy or alkylamino resulted in a decrease in α5 affinity, although one exception was the isopropylamino derivative **20** which has similar α5 affinity to the isopropylthio analogue **10**. The ethyl derivative **22** has similar α5 affinity compared to **5**, but, as a general rule, replacing alkylthio with alkyl proved detrimental to $GABA_A$ α5 binding (data not shown). The importance of having a substituent at C3 is demonstrated by the lack of activity demonstrated by **23**. The results in Table 2 show the $GABA_A$ receptor subtype binding affinities for the C1-substituted heterocyclic derivatives. At the C1 position a heteroaromatic ring was found to be essential for high α5 affinity as demonstrated by the lack of $GABA_A$ receptor affinity displayed by the phenyl derivative **30**. The position of the nitrogen atom in the 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. For example, comparison of the pyridyl analogues **26**−**28** reveals that it is the 2-pyridyl isomer **26** which has the highest α5 affinity, whereas for the thiazolyl isomers the 2-thiazolyl derivative **39** has 7-fold higher affinity for the α5-subtype compared to the 5-thiazolyl isomer **29**. Indeed, of all the heterocyclic groups we incorporated at C1, in terms of α5 binding affinity, we found that 2-pyridyl or 2-thiazolyl were the best. It should be noted that the existence of nitrogen adjacent to the point of attachment is not, in itself, sufficient to produce high affinity ligands. For example, the pyrrole **32**, which possesses only a H-bond donor group and no H-bond acceptor, and the 3-methyloxadiazolyl derivative **36** have an order of magnitude lower $GABA_A$ α5 affinity compared to the analogous pyrazole **5**. As was observed in the pyrazole series, the C3-(2-hydroxyethylthio) substituent is well tolerated when there is a 2-pyridyl (**34**) or 2-thiazolyl group (**43**) at C1, and, in general, the

Scheme 1[a]



[a] Reagents: (i) DCM, dioxane, *m*-CPBA (2.0 equiv); (ii) RSH, NaOH, EtOH, 25 °C or 70 °C; (iii) RSNa, THF, 25 °C; (iv) DCM, dioxane, *m*-CPBA (1.0 equiv); (v) NaOMe, MeOH, 70 °C; (vi) NaO^{i}Pr, ^{i}PA, 70 °C; (vii) RNH_2, MeOH or EtOH, 100 °C; (viii) EtMgBr, THF, −10 °C.

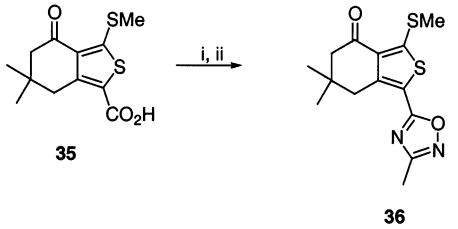
Scheme 2[a]



[a] Reagents: (i) MeSNa, THF; (ii) $Pd(PPh_3)_4$, ethylene glycol dimethyl ether, Na_2CO_3, H_2O, 1-(*tert*-butyloxycarbonyl)pyrrol-2-yl boronic acid; (iii) TFA; (iv) DCM, *m*-CPBA (1.0 equiv), −50 °C; (v) NaOH, $HS(CH_2)_2OH$, EtOH.

2-hydroxyethylthio derivatives had slightly higher affinity at the α1-, α2-, α3-, and α5-subtypes compared to the thiomethyl analogues. Similarly, the homologous C3-(2-hydroxypropylthio) group (e.g., **13** and **44**) has no detrimental effect on α5 affinity. All the analogues that we examined had higher affinity for the α5-subtype than for any of the other $GABA_A$ receptor subtypes. In particular, the C1-(2-thiazolyl) series maintained at least 1 order of magnitude selectivity for the α5 receptor over its next nearest subtype, and in the case of the

Scheme 3[a]



[a] Reagents: (i) Dioxane, 1,1′-carbonyldiimidazole; (ii) acetamide oxime, 100 °C.

thiazolylthio analogue **45** α5-subtype selectivity was 17−28-fold over the other $GABA_A$ receptor subtypes.

In Vitro Efficacy. Of those compounds which demonstrated good α5 binding affinity, a number was selected and the efficacy at the human $GABA_A$ α5 receptor subtype determined using two-electrode voltage clamp recording from *Xenopus laevis* oocytes, which transiently expressed the $GABA_A$ α5β3γ2 receptor subtype.[27,28] The α5 efficacy results obtained for those compounds tested are shown in Tables 1 and 2. In this assay, the control compound **2**, which is regarded as a nonselective, full BZ inverse agonist,[29,30] produced an efficacy reading of −34%. All three pyrazoles (**5**, **14**, **20**) are essentially antagonists at $GABA_A$ α5 receptors whereas the pyridines (**26**−**28**, **34**) are all α5 inverse agonists. A comparison of the methylthio analogues **26**−**28** reveals that the nature of the pyridyl isomer at C1 influences the level of α5 inverse agonism, with efficacy values ranging from −20% for the 4-pyridyl derivative **28** to −40% for the 3-pyridyl isomer **27**. In the thiazole series the C3 substituent has a significant effect on the α5 efficacy. Whereas the methylthio (**39**) and hydroxyethylthio (**43**) analogues are inverse agonists at the $GABA_A$ α5 receptor ($GABA_A$ α5 efficacy: −24% and −38%, respectively), the hydroxypropylthio (**44**) and thiazolylthio (**45**) derivatives are a partial agonist ($GABA_A$ α5 efficacy: +25%) and an antagonist ($GABA_A$ α5 efficacy: −5%), respectively. It is noteworthy that the introduction of a single methylene unit into the hydroxyethylthio substituent of **43** converts a full BZ inverse agonist into a partial agonist, thus demonstrating the difficulty of obtaining a robust structure−efficacy relationship for this series of compounds.

Further In Vitro Characterization of 43. The efficacy profile of **43** was examined in more detail using a whole cell patch clamp technique. This procedure differs from the two-electrode voltage clamp, in which compounds were assayed at a single concentration in transiently infected *Xenopus laevis* oocytes, in that human $GABA_A$ receptors are stably expressed and efficacy is determined over a wider concentration range. As shown in Table 3 and Figure 1, using whole cell patch-clamp recording from mammalian fibroblast L(tk$^-$) cells,[31] stably expressing the human $GABA_A$ αxβ3γ2 receptors (x = 1, 2, 3, 5), in the presence of a submaximal dose (EC_{20}) of GABA, **43** has similar efficacy at the $GABA_A$ α5β3γ2 receptor (−51%) compared to the full nonselective inverse agonist **2** (−57%). The EC_{50} value of **43** at the α5-subtype is 0.7 nM, which complements its binding affinity of 1.6 nM. It is a low efficacy inverse agonist at the α1-subtype (−21%) and an antagonist at α2 (−1%) and α3 (−3%). In addition to being a functionally selective, full α5 inverse agonist with 10−13-fold α5 binding selectivity over the α1-, α2-, and α3-subtypes, **43** had relatively low affinity for the α4β3γ2 receptors (K_i 106 nM) and was essentially inactive (K_i 1800 nM) at the α6β3γ2-subtype. Furthermore, when examined in 96 radioligand binding and enzyme assays,[32] the only off-target activity which was <1 μM was at the rat adenosine A_1 receptor (K_i 29 nM).

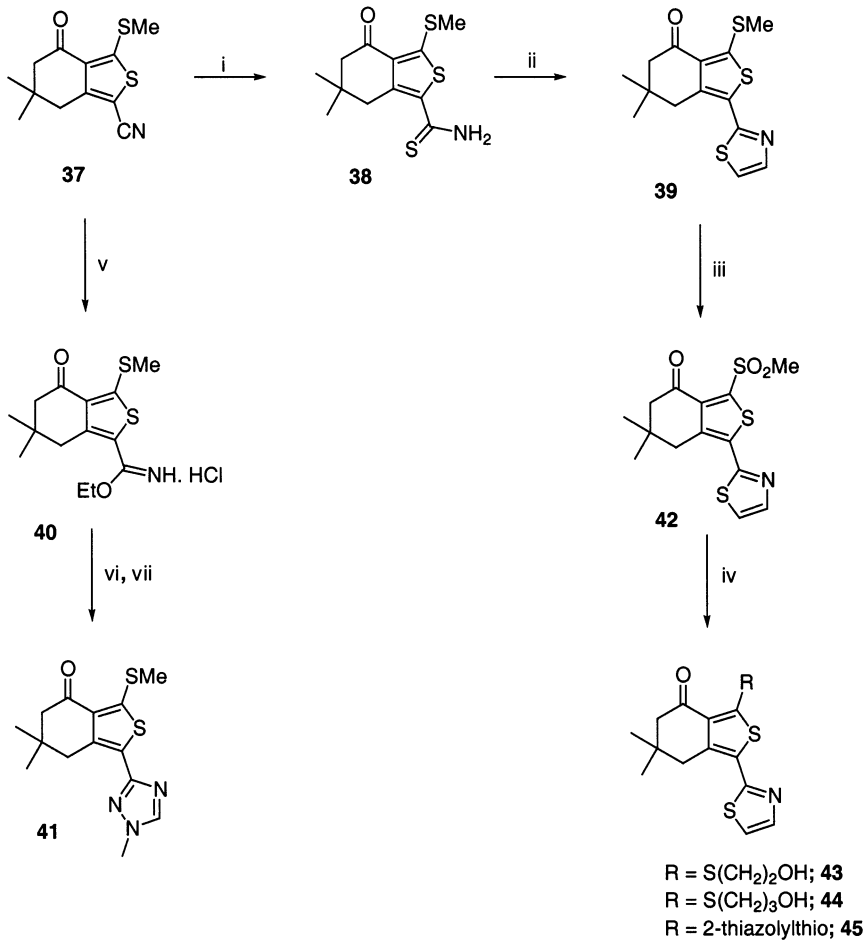
In Vivo Characterization of 43. To assess the occupancy of **43** at the $GABA_A$ receptor subtypes in the rat, an in vivo radioligand binding assay[33] was used. Using [^{3}H]Ro 15−1788 as the radioligand, when administered at 3 mg/kg ip in the rat the occupancy of $GABA_A$ receptors by **43** was 20% (Figure 2a). Ro 15−1788 is a nonselective $GABA_A$ ligand which binds equally to all the diazepam-sensitive $GABA_A$ receptor subtypes, and therefore the relative contribution of the α5-subtype to the total Bz binding site $GABA_A$ receptor population (i.e., combined α1 + α2 + α3 + α5-subtypes) measured using [^{3}H]Ro 15−1788 is relatively small (∼5%).[3] In this experiment, the major contribution to the $GABA_A$ receptor occupancy is from the α1 + α2 + α3-subtypes. When [^{3}H]L-655,708 is used as the radioligand, the occupancy in the rat at 3 mg/kg ip was 80% (Figure 2b). L-655,708 is a $GABA_A$ α5 binding selective ligand[21] and therefore, assuming that the human and rat in vitro binding data are comparable, the in vivo binding assay is essentially measuring occupancy of only α5-containing receptors. These data indicates that not only is thiazole **43** brain penetrant in the rat with high occupancy of α5 binding sites at 3 mg/kg ip, but that the α5 binding selectivity observed in vitro, is confirmed in vivo.

The proconvulsant potential of **43** was determined in mice. At 3 mg/kg ip in the mouse, 91% of $GABA_A$ α5-containing receptors ([^{3}H]L-655,708 binding) were occupied, and, at this dose, **43** had no proconvulsant or convulsant effects (Figure 3).[34] This contrasts with the significant *convulsant* effects reported in mice for the *nonselective* BZ inverse agonist **2**.[12]

Having found no significant off-target activities with **43** and having shown that **43** is a functionally selective $GABA_A$ α5 inverse agonist with good occupancy of α5-containing receptors and relatively poor occupancy of α1, α2 and α3-containing receptors, **43** was selected as an appropriate probe to test the hypothesis that a selective $GABA_A$ α5 receptor inverse agonist may enhance cognition.

To investigate the effect that **43** has on learning and memory, we assessed the performance of rats dosed with **43** in the water maze, a hippocampal-dependent cognitive test.[35] The delayed 'matching-to-place' variant of the water maze was used in which the position of the submerged platform in a 2 m diameter pool varied each day, but the location remained constant for the duration of that day.[36] The rat in a given test was required to find the platform four times each day. On the first trial of each day the rat must search the pool for the hidden platform. If it locates the platform more rapidly on trial 2, this indicates that the rat has remembered the platform position from trial 1. Around the pool are spatial cues to assist the rat in navigating its way to the platform. Thus, the improvement in memory can be quantified by calculating the difference between the time taken to reach the platform on trial 1 compared with subsequent trials. As can be seen in Figure 4, rats

Scheme 4[a]



[a] Reagents: (i) H_2S, Et_3N, pyridine, (ii) chloroacetaldehyde, EtOH, reflux; (iii) DCM, *m*-CPBA (2.0 equiv), 25 °C; (iv) RSH, NaOH, EtOH; (v) EtOH, HCl; (vi) EtOH, $MeNHNH_2$, 50 °C; (vii) HCO_2H, 100 °C.

dosed with **43** at 0.3 mg/kg ip showed a significant improvement in performance between trial 1 and trial 2 compared with vehicle-treated animals ($p < 0.05$). At this dose the occupancy of $GABA_A$ α5 receptors in the rat ([^{3}H]L-655,708 binding) was 40%. Since **43** was without effect on swim speed, these data indicate that the functionally selective $GABA_A$ α5 inverse agonist **43** significantly enhanced performance in this hippocampal-dependent memory task.

These results, therefore, clearly provide pharmacological evidence for the involvement of $GABA_A$ α5 receptors in cognitive processes and complements the recent molecular genetic evidence provided by Collinson et al.[37] and Crestani et al.[38] who used α5 'knock-out' and 'knock-in' mice, respectively, to demonstrate a role for the $GABA_A$ α5-subtype in cognitive processing.

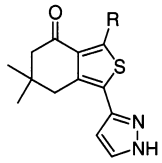
Conclusion

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 which have binding selectivity for the $GABA_A$ α5 receptor subtype over the α1-, α2-, and α3-subtypes. Several examples have high inverse agonism at the $GABA_A$ α5 receptor. In particular, 6,6-dimethyl-3-(2-hydroxyethyl)thio-1-(thiazol-2-yl)-6,7-dihydro-2-benzothiophen-4(5*H*)-one (**43**) has been identified as a high affinity, brain penetrant, functionally selective, full $GABA_A$ α5 receptor inverse agonist which improves cognitive performance in a hippocampal-dependent memory task, without the convulsant and proconvulsant side effects associated with nonselective full $GABA_A$ receptor inverse agonists.

Experimental Section

General. All reactions were carried out under a nitrogen atmosphere, using commercially available anhydrous solvents. Thin-layer chromatography was performed on glass-backed precoated Merck silica gel (60 F_{254}) plates, and flash chromatography was carried out using 40−63 μm silica gel. Proton NMR spectra were measured on a Bruker AC250, Bruker DPX360, or Bruker DPX400 spectrometer in the solvent specified. Chemical shifts are measured in ppm downfield from TMS as an internal standard, and coupling constants are measured in hertz (Hz). Mass spectra were recorded on VG Quattro spectrometer using positive ionization electrospray (ES^+). High-resolution mass spectra (HRMS) were obtained at MSD using a micromass Q-Tof spectrometer using electrospray (ES^+) ionization or by M-Scan Ltd., Sunninghill, Ascot, Berkshire, UK. Combustion analyses were conducted by Butterworth Laboratories, Teddington, Middlesex, U.K. Melting points were determined on a Reichert hot stage apparatus and are uncorrected. 6,6-Dimethyl-3-methylthio-1-(pyrazol-3-yl)-6,7-dihydro-2-benzothiophen-4(5*H*)-one (**5**), 1-bromo-6,6-dimethyl-3-methanesulfonyl-6,7-dihydro-2-benzothiophen-4-(5*H*)-one (**24**), 6,6-dimethyl-3-methylthio-6,7-dihydro-2-benzothiophen-4(5*H*)-one-1-carboxylic acid (**35**) and 6,6-dimethyl-3-methylthio-4-oxo-4,5,6,7-tetrahydro-2-benzothiophene-1-carbonitrile (**37**) were obtained from Maybridge Chemical Co. Ltd., Trevillett, Tintagel, Cornwall, U.K. High performance

Table 1. Binding Affinity and Efficacy of the C3-Thio-substituted Thiophenes



		K_i (nM) human $GABA_A$ αxβ3γ2 receptors[a]					Efficacy[b]
No.	R	α5	α1	α2	α3	Sel.[c]	α5
DMCM (**2**)	-	2.2 ± 1.0	10 ± 1	13 ± 5	7.5 ± 1.2	3-6	-34 ± 5[d]
5	SMe	5.2 ± 1.5	66 ± 2	56 ± 17	21 ± 9	4-13	-3 ± 4
7	SEt	1.6 ± 0.1	12 ± 5	16 ± 3	16 ± 2	8-10	
8	SPr	4.6 ± 1.3	22 ± 3	28 ± 5	41 ± 13	5-9	
9	SBu	18 ± 5	57 ± 13	184 ± 28	182 ± 45	3-10	
10	S^{i}Pr	2.0 ± 0.3	5.0 ± 1.3	12 ± 5	7.0 ± 1.3	3-6	
11	S^{t}Bu	170 ± 10	>300	>300	>300	>2	
12	$S(CH_2)_2OH$	2.9 ± 0.5	31 ± 8	21 ± 4	24 ± 4	7-11	
13	$S(CH_2)_3OH$	4.7 ± 0.6	60 ± 15	46 ± 16	50 ± 6	10-13	
14		1.4 ± 0.6	4.3 ± 0.6	19 ± 3	48 ± 2	3-34	-7 ± 1
15		2.4 ± 0.1	10 ± 2	7.6 ± 2.5	18 ± 1	3-8	
17	OMe	67 ± 8	>300	>300	>300	>5	
18	O^{i}Pr	15 ± 4	119 ± 65	182 ± 53	69 ± 19	8-18	
19	NHMe	132 ± 1	>300	>300	>300	>2	
20	NH^{i}Pr	3.9 ± 0.4	72 ± 10	44 ± 4	87 ± 14	11-22	-2 ± 4
21	$NH(CH_2)_2OH$	78 ± 19	>300	>300	>300	>4	
22	Et	11 ± 2	211 ± 44	162 ± 49	240 ± 63	15-22	
23	H	>300	>300	>300	>300	-	

[a] Displacement of [^{3}H]Ro 15−1788 binding from recombinant human $GABA_A$ receptor subtypes. K_i values are the geometric mean ± SEM of three independent determinations. [b] Efficacy 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 human α5β3γ2s receptor subtype transiently expressed in *Xenopus laevis* oocytes. [c] Binding selectivity for $GABA_A$ α5 receptors over GABA-A α1, α2, and α3 receptors. [d] Efficacy is the arithmetic mean ± SEM of eleven individual cells from the human α5β3γ2 receptor subtype transiently expressed in *Xenopus laevis* oocytes.

liquid chromatography (HPLC) was carried out on an Agilent 1100 series machine using a Hichrom KR100−5C8 (250 × 4.6 mm i.d.) column or an Ace 3C8 (150 × 4.6 mm i.d.) column. A flow rate of 1 mL/min was used with an injection volume of 5 μL and detection was by UV at λ_{254} nm.

6,6-Dimethyl-3-methanesulfonyl-1-(pyrazol-3-yl)-6,7-dihydro-2-benzothiophen-4(5*H*)-one (6). To a stirred solution of 6,6-dimethyl-3-methylthio-1-(pyrazol-3-yl)-6,7-dihydro-2-benzothiophen-4(5*H*)-one (**5**) (3.0 g, 10.2 mmol) in CH_2Cl_2: dioxane (250 mL, 4:1) at −78 °C was added *m*-CPBA (5.1 g (70% w/w), 20.5 mmol) portionwise. The mixture was stirred at −78 °C for 30 min and then at room temperature for 1 h. After this time more *m*-CPBA (1.4 g (70% w/w), 5.6 mmol) was added and the mixture stirred at room-temperature overnight. After this time the mixture was poured into $NaHCO_3$ (sat., 50 mL) and the organic layer separated. The organic phase was washed with more $NaHCO_3$ (sat., 2 × 50 mL), dried ($MgSO_4$), and evaporated. The residue was triturated twice with diethyl ether and the resultant colorless solid (3.1 g, 94%) collected by filtration. mp 213−216 °C. Anal. ($C_{14}H_{16}N_2O_3S_2$) C, H, N. HRMS (ES$^+$) Observed: 325.0665, Calculated $C_{14}H_{17}N_2O_3S_2$ [M + H]$^+$: 325.0665. ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.12 (6H, s), 2.55 (2H, s), 2.98 (2H, s), 3.55 (3H, s), 6.58 (1H, d, J = 2.6 Hz), 7.69 (1H, d, J = 2.4 Hz).

6,6-Dimethyl-3-ethylthio-1-(pyrazol-3-yl)-6,7-dihydro-2-benzothiophen-4(5*H*)-one (7). To a solution of **6** (100 mg, 3.1 mmol) in THF (10 mL) was added sodium ethanethiolate (52 mg, 6.2 mmol) and the mixture stirred at room temperature for 15 min. After this time water (20 mL) and EtOAc (25 mL) were added and the organic layer separated and dried (Na_2SO_4). The solvent was evaporated and the resultant solid tritrated with diethyl ether to afford the title compound (60 mg, 63%) as a pale yellow solid. mp 182−184 °C. Anal. ($C_{15}H_{18}N_2OS_2$) C, H, N. HRMS (ES$^+$) Observed: 307.0938, Calculated $C_{15}H_{19}N_2OS_2$ [M + H]$^+$: 307.0939. ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.07 (6H, s), 1.48 (3H, t, J = 7.4 Hz), 2.43 (2H, s), 2.85 (2H, s), 3.10 (2H, q, J = 7.3 Hz), 6.49 (1H, br s,), 7.65 (1H, d, J = 2.3 Hz).

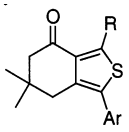
6,6-Dimethyl-3-(1,1-dimethylethyl)thio-1-(pyrazol-3-yl)-6,7-dihydro-2-benzothiophen-4(5*H*)-one (11). The title compound was prepared in a simlar manner to that described for **7**, using sodium 2-methyl-2-propanethiolate. Purification was achieved by column chromatography on silica gel, eluting with petrol (60/80):EtOAc (1:1), to afford **11** as a yellow solid. Yield 48%. mp 220−222 °C. Anal. ($C_{17}H_{22}N_2OS_2$) C, H, N. HRMS (ES$^+$) Observed: 335.1260, Calculated $C_{17}H_{23}N_2OS_2$ [M + H]$^+$: 335.1252. ^{1}H NMR (360 MHz, DMSO-d_6) δ 1.00 (6H, s), 1.45 (9H, s), 2.39 (2H, s), 2.88 (2H, s), 6.54 (1H, br s), 7.87 (1H, br s), 13.07(1H, br s). MS (ES$^+$) 335 (MH)$^+$.

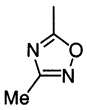
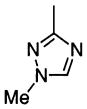
General Procedure for the Synthesis of the 6,7-Dihydro-2-benzothiophen-4(5*H*)-ones 8−10 and 14−15. To a suspension of **6** (50 mg, 0.15 mmol) in EtOH (2 mL) was added NaOH (77 μL of a 4 N solution, 0.31 mmol) followed by the appropriate thiol (0.31 mmol). The mixture was stirred at room temperature for 1 h (compounds **8**−**10**) or heated at 70 °C for 72 h (compounds **14** and **15**). The mixture was diluted with MeOH:H_2O:1 N HCl (5:5:1) and then poured onto a C-18 Bond Elut cartridge (prewashed with MeOH followed by water). The cartridge was eluted with MeOH:H_2O (8 mL) (1:1 → 1:0), and the product fractions were evaporated. The residue was triturated with diethyl ether to give the thioether as a solid.

6,6-Dimethyl-3-propylthio-1-(pyrazol-3-yl)-6,7-dihydro-2-benzothiophen-4(5*H*)-one (8). Yield 20%. mp 181−182 °C. Anal. ($C_{16}H_{20}N_2OS_2$) C, H, N. MS (ES$^+$) 321 [M + H]$^+$. ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.07 (6H, s), 1.10 (3H, t, J = 7.3 Hz), 1.86 (2H, sextet, J = 7.3 Hz), 2.43 (2H, s), 2.85 (2H, s), 3.05 (2H, t, J = 7.3 Hz), 6.48 (1H, d, J = 2.3 Hz), 7.64 (1H, d, J = 2.3 Hz).

3-Butylthio-6,6-dimethyl-1-(pyrazol-3-yl)-6,7-dihydro-2-benzothiophen-4(5*H*)-one (9). Yield 49%. mp 149−150 °C. Anal. ($C_{17}H_{22}N_2OS_2$) C, H, N. MS (ES$^+$) 335 [M + H]$^+$. ^{1}H NMR

Table 2. Binding Affinity and Efficacy of the C1-Heteroaryl-thiophenes



			K_i (nM) human $GABA_A$ αxβ3γ2 receptors [a]					Efficacy [b]
No.	Ar	R	α5	α1	α2	α3	Sel. [c]	α5
26		SMe	1.7 ± 0.3	45 ± 3	26 ± 4	25 ± 4	15-26	-31 ± 2
27		SMe	5.8 ± 1.2	165 ± 34	31 ± 7	33 ± 4	5-28	-40 ± 5
28		SMe	9.1 ± 0.9	172 ± 28	43 ± 4	35 ± 8	4-19	-20 ± 5
29		SMe	15 ± 2	175 ± 43	161 ± 31	159 ± 39	11-12	
30		SMe	>300	>300	>300	>300	-	
32		SMe	85 ± 14	>300	>300	>300	>4	
34		$S(CH_2)_2OH$	0.5 ± 0.1	3.6 ± 0.1	5.2 ± 1.1	10 ± 1	7-20	-39 ± 3
36		SMe	37 ± 8	>300	>300	185 ± 3	5->8	
39		SMe	2.2 ± 0.2	61 ± 16	44 ± 10	47 ± 10	20-28	-24 ± 2
41		SMe	19 ± 6	267 ± 66	49 ± 10	102 ± 32	3-14	
43		$S(CH_2)_2OH$	1.6 ± 0.4	20 ± 2	16 ± 2	20 ± 1	10-13	-38 ± 2
44		$S(CH_2)_3OH$	4.7 ± 0.7	79 ± 7	48 ± 6	48 ± 2	10-17	+25 ± 10
45			1.4 ± 0.2	24 ± 7	39 ± 11	28 ± 3	17-28	-5 ± 2

[a] Displacement of [^{3}H]Ro 15−1788 binding from recombinant human $GABA_A$ receptor subtypes. K_i values are the geometric mean ± SEM of three independent determinations. [b] Efficacy 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 human α5β3γ2s receptor subtype transiently expressed in *Xenopus laevis* oocytes. [c] Binding selectivity for $GABA_A$ α5 receptors over $GABA_A$ α1, α2, and α3 receptors.

(360 MHz, $CDCl_3$) δ 0.96 (3H, t, J = 7.4 Hz), 1.07 (6H, s), 1.52 (2H, sextet, J = 7.4 Hz), 1.82 (2H, pentet, J = 7.6 Hz), 2.43 (2H, s), 2.85 (2H, s), 3.08 (2H, t, J = 7.4 Hz), 6.48 (1H, d, J = 2.3 Hz), 7.64 (1H, d, J = 2.3 Hz).

6,6-Dimethyl-3-(1-methylethyl)thio-1-(pyrazol-3-yl)-6,7-dihydro-2-benzothiophen-4(5*H*)-one (10). Yield 50%. mp 195−198 °C. Anal. ($C_{16}H_{20}N_2OS_2$·0.1(H_2O)) C, H, N. HRMS (ES^+) Observed: 321.1086, Calculated $C_{16}H_{21}N_2OS_2$ $[M + H]^+$: 321.1095. ^{1}H NMR (360 MHz, DMSO-d_6) δ 0.99 (6H, s), 1.42 (6H, d, J = 6.7 Hz), 2.37 (2H, s), 2.84 (2H, s), 3.50−3.58 (1H, m), 6.50 (1H, d, J = 2.2 Hz), 7.85 (1H, br s), 13.02 (1H, br s).

6,6-Dimethyl-1-(pyrazol-3-yl)-3-(thiazol-2-yl)thio-6,7-dihydro-2-benzothiophen-4(5*H*)-one (14). Yield 13%. mp 195−198 °C. Anal. ($C_{16}H_{15}N_3OS_3$) C, H, N. HRMS (ES^+) Observed: 362.0442, Calculated $C_{16}H_{16}N_3OS_3$ $[M + H]^+$: 362.0456. ^{1}H NMR (360 MHz, DMSO-d_6) δ 1.03 (6H, s), 2.47 (2H, s), 2.87 (2H, s), 6.48−6.50 (1H, m), 7.83−7.85 (1H, m), 8.04−8.08 (2H, m), 13.03 (1H, br s).

6,6-Dimethyl-1-(pyrazol-3-yl)-3-(thien-2-yl)thio-6,7-dihydro-2-benzothiophen-4(5*H*)-one (15). Yield 47%. mp 226−228 °C. Anal. ($C_{17}H_{16}N_2OS_3$·0.1(H_2O)) C, H, N. MS (ES^+) 361 $[M + H]^+$. ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.09 (6H, s), 2.46

Table 3. Efficacy of **2** and **43** in L(tk⁻) Cells Expressing Different Human $GABA_A$ Receptor Subtypes

	efficacy at human $GABA_A$ $\alpha x\beta 3\gamma 2$ receptors (%)[a]			
compound	α1	α2	α3	α5
DMCM(**2**)	−71 ± 2	−53 ± 3	−62 ± 2	−57 ± 1
43	−21 ± 2[b]	−1 ± 2[b]	−3 ± 1[b]	−51 ± 2

[a] Maximum modulation of the current produced by DMCM or **43** 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 [**43**] = 100 nM from human $\alpha x\beta 3\gamma 2$ (x = 1, 2, or 3) receptor subtypes stably expressed in mammalian fibroblast L(tk⁻) cells.

(2H, s), 2.83 (2H, s), 6.40 (1H, d, J = 2.3 Hz), 7.13 (1H, dd, J = 5.3 and 3.6 Hz), 7.43 (1H, dd, J = 3.6 Hz), 7.57−7.61 (2H, m).

6,6-Dimethyl-3-(2-hydroxyethyl)thio-1-(pyrazol-3-yl)-6,7-dihydro-2-benzothiophen-4(5*H*)-one (12). To a suspension of **6** (100 mg, 0.31 mmol) in EtOH (5 mL) were added 2-mercaptoethanol (27 μL, 0.39 mmol) and NaOH (82 μL, 0.33 mmol). The mixture was stirred for 90 min then partitioned between water (20 mL), HCl (1 N, 1 mL) and EtOAc (20 mL). The organic phase was separated, dried (Na_2SO_4), and evaporated. The residue was triturated with diethyl ether to afford the title compound (96 mg, 96%) as a pale yellow solid. mp 182−185 °C. Anal. ($C_{15}H_{18}N_2O_2S_2 \cdot 0.3(H_2O)$) C, H, N. HRMS ($ES^+$) Observed: 323.0885, Calculated $C_{15}H_{19}N_2O_2S_2$ $[M + H]^+$: 323.0888. ^1H NMR (360 MHz, DMSO-d_6) δ 1.00 (6H, s), 2.37 (2H, s), 2.83 (2H, s), 3.16 (2H, t, J = 6.4 Hz), 3.70−3.80 (2H, m), 5.08−5.20(1H, m), 6.49 (1H, d, J = 3.3 Hz), 7.84 (1H, br s), 13.04 (1H, br s).

6,6-Dimethyl-3-(3-hydroxypropyl)thio-1-(pyrazol-3-yl)-6,7-dihydro-2-benzothiophen-4(5*H*)-one (13). The title compound was prepared in a simlar manner to that described for **12**, using 3-mercapto-1-propanol. Yield 91%. mp 172−175 °C. Anal. ($C_{16}H_{20}N_2O_2S_2$) C, H, N. MS (ES^+) 337 $[M + H]^+$. ^1H NMR (360 MHz, DMSO-d_6) δ 1.00 (6H, s), 1.88 (2H, pentet, J = 6.9 Hz), 2.37 (2H, s), 2.84 (2H, s), 3.09 (2H, t, J = 7.2 Hz), 3.51−3.56 (2H, m), 4.66 (1H, t, J = 5.3 Hz), 6.49 (1H, br s), 7.84 (1H, br s), 13.00 (1H, br s).

6,6-Dimethyl-3-methanesulfinyl-1-(pyrazol-3-yl)-6,7-dihydro-2-benzothiophen-4(5*H*)-one (16). To a stirred solution of **5** (200 mg, 0.69 mmol) in CH_2Cl_2:dioxane (9 mL, 3:1) at −78 °C was added *m*-CPBA (169 mg (70% w/w), 0.69 mmol) portionwise. After addition the mixture was stirred at −78 °C for 1 h, then diluted with CH_2Cl_2 (10 mL) and poured into $NaHCO_3$ (sat., 10 mL). The organic layer was separated, dried (Na_2SO_4), and evaporated. The residue was triturated with diethyl ether and the resultant colorless solid (106 mg, 50%) collected by filtration. mp 206−210 °C. HRMS (ES^+) Observed: 308.0660, Calculated $C_{14}H_{16}N_2O_2S_2$ $[M]^+$: 308.0653. ^1H NMR (360 MHz, $CDCl_3$) δ 1.09 (3H, s), 1.11 (3H, s), 2.42 (1H, d, J = 17 Hz), 2.52 (1H, d, J = 17 Hz), 2.95 (2H, s), 3.01 (3H, s), 6.54 (1H, s), 7.68 (1H, s).

6,6-Dimethyl-3-methoxy-1-(pyrazol-3-yl)-6,7-dihydro-2-benzothiophen-4(5*H*)-one (17). To a solution of **16** (80 mg, 0.26 mmol) in MeOH (3 mL) was added a solution of sodium methoxide in MeOH (1.0 mL of a 0.5 M solution, 0.5 mmol). The soution was heated at 70 °C for 24 h then cooled to room temperature and partitioned between water (20 mL) and EtOAc (2 × 20 mL). The combined organic layers were dried (Na_2SO_4) and evaporated. The residue was chromatographed on silica gel, eluting with CH_2Cl_2:MeOH (95:5) to give the title compound (11 mg, 15%) as a pale yellow solid. mp 202−204 °C. Anal. ($C_{14}H_{16}N_2O_2S.0.1(H_2O)$) C, H, N. MS ($ES^+$) 277 [M + H]$^+$. ^1H NMR (360 MHz, DMSO-d_6) δ 0.98 (6H, s), 2.27 (2H, s), 2.79 (2H, s), 4.03 (3H, s), 6.54 (1H, br s), 7.81 (1H, br s), 12.92 (1H, br s).

6,6-Dimethyl-3-isopropoxy-1-(pyrazol-3-yl)-6,7-dihydro-2-benzothiophen-4(5*H*)-one (18). In the same way as described for **17** using sodium isopropoxide in 2-propanol and **6** the title compound (40 mg, 43%) was isolated as a yellow solid. mp 196−198 °C. Anal. ($C_{16}H_{20}N_2O_2S \cdot 0.3(H_2O)$) C, H, N. MS ($ES^+$) 305 $[M + H]^+$. ^1H NMR (360 MHz, $CDCl_3$) δ 1.06 (6H, s), 1.51 (6H, d, J = 6.1 Hz). 2.36 (2H, s), 2.80 (2H, s), 4.55−4.62 (1H, m), 6.45 (1H, br s), 7.62 (1H, d, J = 2.3 Hz).

6,6-Dimethyl-3-methylamino-1-(pyrazol-3-yl)-6,7-dihydro-2-benzothiophen-4(5*H*)-one (19). To **16** (116 mg, 0.38 mmol) was added a solution of methylamine in MeOH (33% (w/v); 10 mL, 80 mmol). The soution was heated at 100 °C for 2 h in a sealed tube then cooled to room temperature. The solvent was evaporated and the residue chromatographed on silica gel, eluting with CH_2Cl_2:MeOH (95:5) to give the title compound (92 mg, 88%) as an orange solid. mp 240−243 °C. Anal. ($C_{14}H_{17}N_3OS \cdot 0.3(H_2O)$) C, H, N. MS ($ES^+$) 276 $[M + H]^+$. ^1H NMR (360 MHz, CDCl3) δ 1.06 (6H, s), 2.32 (2H, s), 2.72 (2H, s), 3.06 (3H, d, J = 5.2 Hz), 6.41 (1H, br s), 7.60 (1H, d, J = 2.3 Hz), 8.58 (1H, br s).

6,6-Dimethyl-3-isopropylamino-1-(pyrazol-3-yl)-6,7-dihydro-2-benzothiophen-4(5*H*)-one (20). In the same way as described for **19** using isopropylamine in EtOH and heating for 24 h the title amine (57 mg, 58%) was isolated as an orange solid. mp 215−218 °C. Anal. ($C_{16}H_{21}N_3OS$) C, H, N. MS (ES^+) 308 $[M + H]^+$. ^1H NMR (360 MHz, $CDCl_3$) δ 1.06 (6H, s), 1.35 (6H, d, J = 6.3 Hz), 2.32 (2H, s), 2.71 (2H, s), 3.50−3.60 (1H, m), 6.40 (1H, d, J = 2.2 Hz), 7.60 (1H, d, J = 2.4 Hz), 8.64−8.70 (1H, m).

6,6-Dimethyl-3-(2-hydroxyethyl)amino-1-(pyrazol-3-yl)-6,7-dihydro-2-benzothiophen-4(5*H*)-one (21). To a solution of **16** (100 mg, 0.33 mmol) in EtOH (3 mL) was added ethanolamine (1.0 mL, 16 mmol). The soution was heated at 100 °C for 2.5 h then cooled to room temperature and partitioned between water (40 mL) and EtOAc (3 × 25 mL). The combined organic layers were dried (Na_2SO_4) and evaporated. The residue was chromatographed on silica gel, eluting with CH_2Cl_2:MeOH (90:10) to give the title compound (57 mg, 58%) as a pale yellow solid. mp 227−230 °C. Anal. ($C_{15}H_{19}N_3O_2S.0.2(H_2O)$) C, H, N. MS ($ES^+$) 306 $[M + H]^+$. ^1H NMR (360 MHz, DMSO-d_6) δ 0.99 (6H, s), 2.24 (2H, s), 2.68 (2H, s), 3.28−3.34 (2H, m), 3.60−3.65 (2H, m), 6.36 (1H, d, J = 2.2 Hz), 7.74 (1H, d, J = 2.3 Hz), 8.66−8.76 (1H, m).

6,6-Dimethyl-3-ethyl-1-(pyrazol-3-yl)-6,7-dihydro-2-benzothiophen-4(5*H*)-one (22) and 6,6-Dimethyl-1-(pyrazol-3-yl)-6,7-dihydro-2-benzothiophen-4(5*H*)-one (23). To a stirred solution of **16** (122 mg, 0.4 mmol) in THF at −10 °C was added ethylmagnesium bromide (0.79 mL of a 1.0 M solution in THF, 0.79 mmol). The mixture was stirred at −10 °C for 1 h then NH_4Cl (sat., 1 mL) was added. The cooling bath was removed and the mixture stirred at room temperature for 10 min. The mixture was partitioned between EtOAc (15 mL) and water (15 mL). The organic layer was separated, dried (Na_2SO_4), and evaporated. The residue was chromatographed on silica gel, eluting with EtOAc:CH_2Cl_2 (6:1). The fractions containing 6,6-dimethyl-3-ethyl-1-(pyrazol-3-yl)-6,7-dihydro-2-benzothiophen-4(5*H*)-one (**22**) (less polar spot on TLC) were combined and evaporated, and the residue was triturated with Et_2O. **22** was isolated as a pale yellow solid (6 mg, 6%). mp 152−155 °C. HRMS (ES^+) Observed: 274.1141, Calculated $C_{15}H_{18}N_2OS$ $[M]^+$: 274.1140. ^1H NMR (360 MHz, $CDCl_3$) δ 1.07 (6H, s), 1.34 (3H, t, J = 7.4 Hz), 2.42 (2H, s), 2.87 (2H, s), 3.30 (2H, q, J = 7.4 Hz), 6.49 (1H, d, J = 2.2 Hz), 7.65 (1H, d, J = 2.2 Hz). HPLC (50% CH_3CN; 50% 25mM aq KH_2PO_4 (containing 0.2% diethylamine)): 99% purity. Retention time: 4.96 min.

The fractions containing 6,6-dimethyl-1-(pyrazol-3-yl)-6,7-dihydro-2-benzothiophen-4(5*H*)-one (**23**) (more polar spot on TLC) were combined and evaporated, and the residue was triturated with Et_2O. **23** was isolated as a colorless solid (15 mg, 15%). mp 160−163 °C. HRMS (ES^+) Observed: 247.0898, Calculated $C_{13}H_{15}N_2OS$ $[M + H]^+$: 247.0905. ^1H NMR (250 MHz, $CDCl_3$) δ 1.09 (6H, s), 2.46 (2H, s), 2.92 (2H, s), 6.54 (1H, d, J = 2.4 Hz), 7.67 (1H, d, J = 2.4 Hz), 8.11 (1H, s). HPLC (40% CH_3CN; 60% 25 mM aq KH_2PO_4 (containing 0.2% diethylamine)): 99% purity. Retention time: 6.10 min.

1-Bromo-6,6-dimethyl-3-methylthio-6,7-dihydro-2-benzothiophen-4(5*H*)-one (25). To a solution of 1-bromo-6,6-

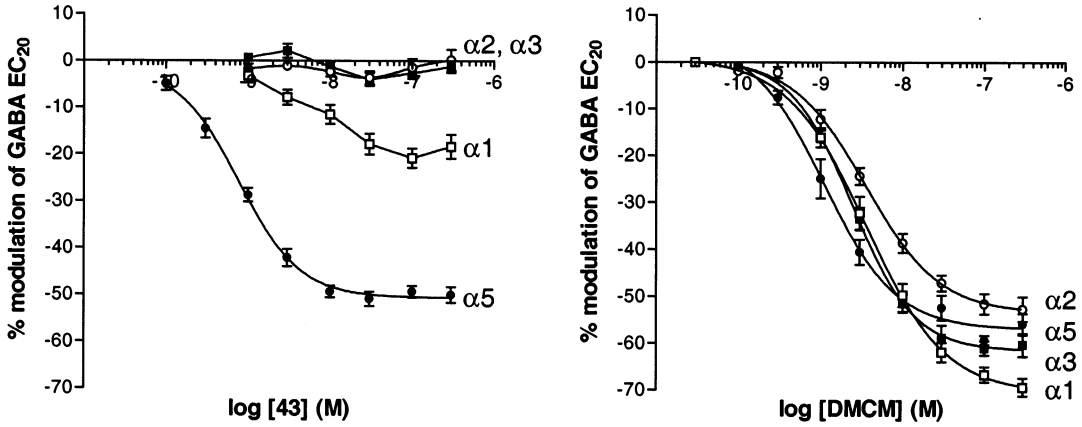


Figure 1. Efficacy of **43** and DMCM in mammalian fibroblast L(tk⁻) cells expressing human $GABA_A$ α*x*β3γ2 (*x* = 1, 2, 3, 5) receptors

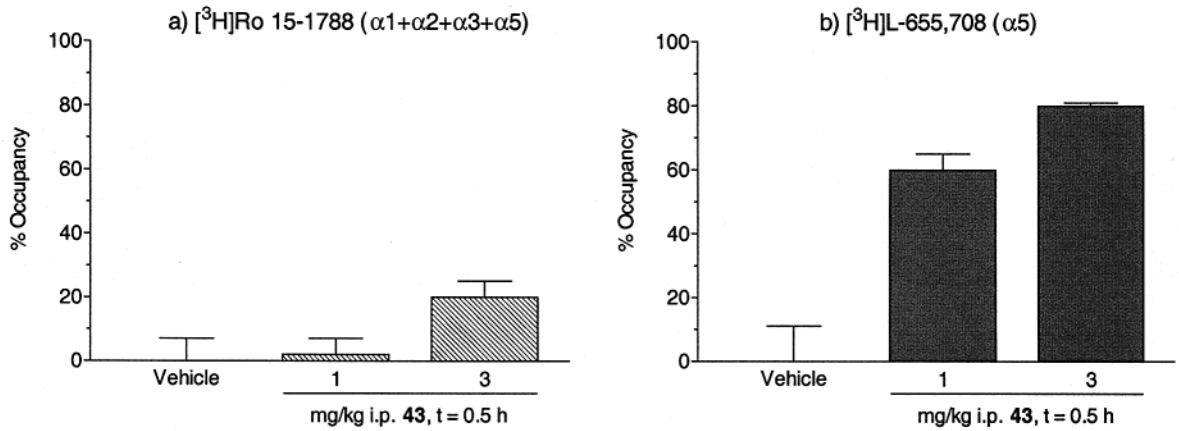


Figure 2. Occupancy of **43** at rat brain Bz sites. (a) Occupancy of the combined (i.e., α1 + α2 + α3 + α5-containing) $GABA_A$ receptor population measured using [^{3}H]Ro 15−1788. (b) Occupancy at only α5-containing $GABA_A$ receptors measured using [^{3}H]L-655,708. Rats (*n* = 5−7/group) were dosed ip with either vehicle (70% PEG 300) and 0.5 h later receptor occupancy measured. Values shown are mean ± SEM.

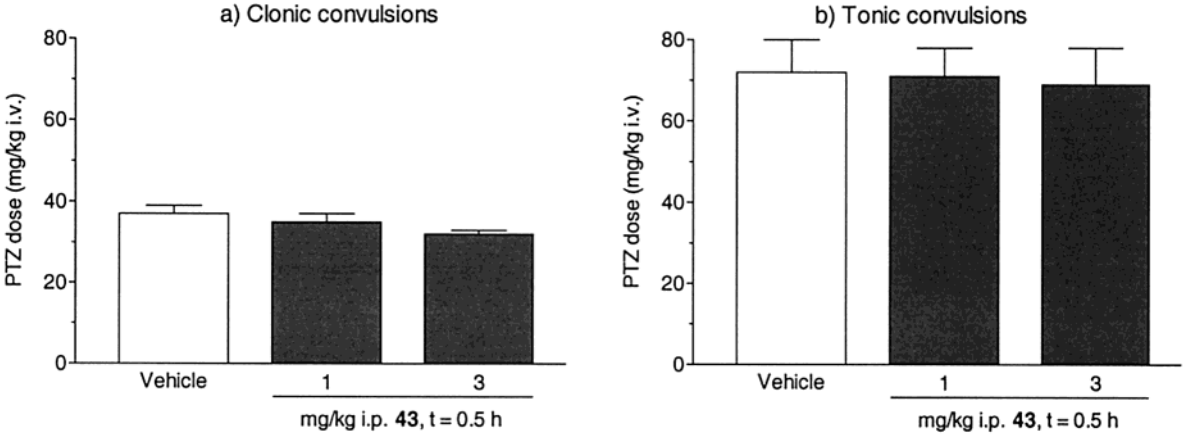


Figure 3. Lack of effect of **43** on the dose of pentylenetetrazole (PTZ) required to produce (a) clonic or (b) tonic seizures in mice. Mice (*n* = 11−12/group) were dosed ip with either vehicle (70% PEG 300) or 1 or 3 mg/kg of **43**. Thirty minutes later PTZ (15 mg/kg) was infused at a rate of 0.2 mg/min, and the time (and therefore by calculation the dose) at which clonic and tonic seizure activity occurred was recorded. Values shown are mean ± SEM. In a separate experiment (data not shown), a dose of 3 mg/kg of **43** corresponds to an occupancy of α5-containing $GABA_A$ receptors (measured using [^{3}H]L-655,708) of 91 ± 4%.

dimethyl-3-methanesulfonyl-6,7-dihydro-2-benzothiophen-4(5*H*)-one (**24**) (2 g, 5.9 mmol) in THF (70 mL) was added sodium methanethiolate (0.9 g, 13 mmol) portionwise. The mixture was stirred at room temperature for 72 h then partitioned between EtOAc (100 mL) and water (100 mL). The organic layer was separated, dried ($MgSO_4$), and evaporated. The residue was triturated with isohexane and the title compound (1.1 g, 58%) isolated as a yellow solid. mp 114−116 °C. Anal. ($C_{11}H_{13}BrOS_2$) C, H. HRMS (ES$^+$) Observed (^{79}Br): 304.9673, Calculated $C_{11}H_{14}BrOS_2$ [M (^{79}Br) + H]$^+$: 304.9669. ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.00 (6H, s), 2.37 (2H, s), 2.54 (2H, s), 2.56 (3H, s).

6,6-Dimethyl-3-methylthio-1-(pyridin-2-yl)-6,7-dihydro-2-benzothiophen-4(5*H*)-one (26). A solution of **25** (700 mg, 2.3 mmol) and 2-(tri-*n*-butylstannyl)pyridine (1.27 g, 3.45 mmol) in dioxane (70 mL) was degassed with nitrogen for 15

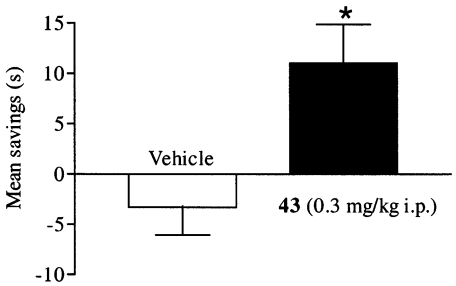


Figure 4. Enhanced performance of rats dosed with thiophene **43** in the delayed 'matching-to-place' water maze test. **43** was administered intraperitoneally (ip) at a dose of 0.3 mg/kg in a 70% poly(ethylene glycol) 300 (PEG 300) aqueous solution. Vehicle-treated animals were dosed ip with a 70% PEG 300 aqueous solution. Ten drug-treated and 10 vehicle-treated animals were used in this test. The difference in time taken between trial 1 and trial 2 (savings) to find the hidden platform over a 10 day test period. Rats dosed with **43** made significantly higher savings (*) compared with vehicle-treated animals.

min. Tetrakis(triphenylphosphine)palladium (0) (250 mg, 0.2 mmol) was added and the mixture heated at 100 °C for 18 h. After this time the solvent was evaporated and the residue partitioned between CH_2Cl_2 (20 mL) and water (20 mL). The organic phase was separated and the aqueous layer washed once more with CH_2Cl_2 (20 mL). The combined organic layers were dried ($MgSO_4$) and evaporated. The residue was triturated with diethyl ether and the resultant pale yellow solid collected by filtration. This solid was then triturated with CH_2Cl_2 to afford the title compound (266 mg) as a colorless solid. The filtrate was chromatographed on silica gel, eluting with isohexane:EtOAc (2:1), to afford the title thiophene (137 mg) as a colorless solid. Both batches of **26** were identical by ^{1}H NMR. Total mass = 403 mg; 58% yield. mp 217−220 °C. Anal. ($C_{16}H_{17}NOS_2$) C, H, N. HRMS (ES^+) Observed: 304.0831, Calculated $C_{16}H_{18}NOS_2$ $[M + H]^+$: 304.0830. ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.09 (6H, s), 2.45 (2H, s), 2.65 (3H, s), 2.93 (2H, s), 7.16−7.19 (1H, m), 7.47 (1H, d, J = 8 Hz), 7.72−7.76 (1H, m), 8.60−8.61 (1H, m).

6,6-Dimethyl-3-methylthio-1-(pyridin-3-yl)-6,7-dihydro-2-benzothiophen-4(5*H*)-one (27). A solution of **25** (410 mg, 1.3 mmol) and 3-(tri-*n*-butylstannyl)pyridine (740 mg, 2.0 mmol) in dioxane (15 mL) was degassed with nitrogen for 30 min. Tetrakis(triphenylphosphine)palladium(0) (350 mg, 0.3 mmol) was added and the mixture heated at 100 °C for 10 h. After this time the solvent was evaporated and the residue partitioned between EtOAc (20 mL) and water (20 mL). The organic phase was separated, dried (Na_2SO_4), and evaporated. The residue was chromatographed on silica gel, eluting with hexane:EtOAc (1:1), to afford the title compound (115 mg, 28%) as a colorless solid. mp 227−230 °C. Anal. ($C_{16}H_{17}NOS_2$·0.1(H_2O)) C, H, N. HRMS (ES^+) Observed: 304.0831, Calculated $C_{16}H_{18}NOS_2$ $[M + H]^+$: 304.0830. ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.04 (6H, s), 2.44 (2H, s), 2.63 (3H, s), 2.76 (2H, s), 7.34−7.40 (1H, m), 7.70−7.75 (1H, m), 7.47 (1H, d, J = 8 Hz), 8.56−8.60 (1H, m), 8.69−8.70 (1H, m).

6,6-Dimethyl-3-methylthio-1-(pyridin-4-yl)-6,7-dihydro-2-benzothiophen-4(5*H*)-one (28). A solution of **25** (374 mg, 1.2 mmol), 4-pyridylboronic acid (605 mg, 4.9 mmol) and Na_2CO_3 (782 mg, 7.4 mmol) in ethylene glycol dimethyl ether (25 mL) and water (10 mL) was degassed with nitrogen for 40 min. After this time tetrakis(triphenylphosphine)palladium (0) (300 mg, 0.26 mmol) was added and the mixture heated at reflux for 5 h. More 4-pyridylboronic acid (100 mg, 0.8 mmol) was added and heating continued for a further 3 h. The solution was cooled to room temperature, and K_2CO_3 (sat., 10 mL) was added to the solution. The mixture was extracted with EtOAc (2 × 30 mL), and the combined organic layers were dried (Na_2SO_4) and evaporated. The residue was chromatographed on silica gel, eluting with hexane:EtOAc (1:1) to afford the title compound (164 mg, 44%) as a pale yellow solid. mp 230−233 °C. Anal. ($C_{16}H_{17}NOS_2$·0.1(H_2O)) C, H, N. HRMS (ES^+) Observed: 304.0830, Calculated $C_{16}H_{18}NOS_2$ $[M + H]^+$: 304.0830. ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.05 (6H, s), 2.45 (2H, s), 2.64 (3H, s), 2.83 (2H, s), 7.33 (2H, dd, J = 4.6, 1.6 Hz), 8.83 (2H, dd, J = 4.6, 1.6 Hz).

6,6-Dimethyl-3-methylthio-1-(thiazol-5-yl)-6,7-dihydro-2-benzothiophen-4(5*H*)-one (29). In the same way as described for **26** using 5-(tri-*n*-butylstannyl)thiazole and dichlorobis(triphenylphosphine)palladium, the title compound (20 mg, 20%) was isolated as a yellow solid. mp 138−140 °C. HRMS (ES^+) Observed: 309.0318, Calculated $C_{14}H_{15}NOS_3$ $[M]^+$: 309.0316. ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.07 (6H, s), 2.44 (2H, s), 2.62 (3H, s), 2.75 (2H, s), 7.90 (1H, s), 8.80 (1H, s). HPLC (55% CH_3CN; 45% 25mM aq KH_2PO_4 (containing 0.2% diethylamine)): >99% purity. Retention time: 7.71 min.

6,6-Dimethyl-3-methylthio-1-phenyl-6,7-dihydro-2-benzothiophen-4(5*H*)-one (30). A solution of **25** (400 mg, 1.3 mmol), benzeneboronic acid (190 mg, 1.5 mmol), Na_2CO_3 (1 N, 20 mL), and tetrakis(triphenylphosphine)palladium(0) (300 mg, 0.26 mmol) in toluene (20 mL) and EtOH (10 mL) was heated at reflux for 18 h. The mixture was cooled to room temperature, evaporated, and partitioned between HCl (1 N) and EtOAc. The organic phase was separated, washed with water and K_2CO_3 (sat.), and then dried ($MgSO_4$). The solvent was evaporated then chromatographed on silica gel, eluting with hexane:EtOAc (9:1 → 4:1) to afford the title compound (130 mg, 33%) as a colorless solid. mp 143−145 °C. Anal. ($C_{17}H_{18}OS_2$) C, H. HRMS (ES^+) Observed: 303.0882, Calculated $C_{17}H_{19}OS_2$ $[M + H]^+$: 303.0877. ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.02 (6H, s), 2.43 (2H, s), 2.62 (3H, s), 2.77 (2H, s), 7.42−7.44 (5H, m).

6,6-Dimethyl-3-methylthio-1-(pyrrol-2-yl)-6,7-dihydro-2-benzothiophen-4(5*H*)-one (32). In the same way as described for **28** using 1-(*tert*-butyloxycarbonyl)pyrrol-2-yl boronic acid, 6,6-dimethyl-3-methylthio-1-(1-(*tert*-butyloxycarbonyl)-pyrrol-2-yl)-6,7-dihydro-2-benzothiophen-4(5*H*)-one (**31**) (80 mg, 20%) was isolated as a pale yellow solid. mp 165−167 °C. Anal. ($C_{20}H_{25}NO_3S_2$) C, H, N. MS (ES^+) 392 $[M + H]^+$. ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.00 (6H, s), 1.42 (9H, s), 2.37 (2H, s), 2.45 (2H, s), 2.57 (3H, s), 6.23−6.26 (2H, m), 7.41 (1H, s), 8.80 (1H, t, J = 2.2 Hz).

31 (80 mg, 0.2 mmol) was dissolved in trifluoroacetic acid (5 mL) and allowed to stand at 20 °C for 30 min. The solvent was removed in vacuo and the residue dissolved in EtOAc. The solution was washed with 2 N Na_2CO_3, water, and brine. The organic layer was separated, dried (Na_2SO_4), and evaporated. The residue was chromatographed on silica gel, eluting with EtOAc:hexane (1:4), to afford the title pyrrole (35 mg, 59%) as a yellow solid. mp 195−197 °C. Anal. ($C_{15}H_{17}NOS_2$) C, H, N. MS (ES^+) 292 $[M + H]^+$. ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.04 (6H, s), 2.40 (2H, s), 2.59 (3H, s), 2.73 (2H, s), 6.30−6.33 (2H, m), 6.85−6.88 (1H, m), 8.23 (1H, br s).

6,6-Dimethyl-3-methanesulfinyl-1-(pyridin-2-yl)-6,7-dihydro-2-benzothiophen-4(5*H*)-one (33). 26 (250 mg, 0.83 mmol) was dissolved in CH_2Cl_2 (25 mL) and cooled to −78 °C. A solution of *m*-CPBA (286 mg of 50−55% technical grade, 0.83 mmol) in CH_2Cl_2 (5 mL) was added and the mixture warmed to −50 °C. After stirring for 1 h at −50 °C more CH_2Cl_2 (20 mL) was added and the mixture washed with $NaHCO_3$ (sat., 20 mL) and brine (20 mL). The organic layer was separated, dried (Na_2SO_4), and evaporated. The residue was chromatographed on silica gel, eluting with isohexane:EtOAc (1:1) → CH_2Cl_2:MeOH (95:5), to afford the sulfoxide (209 mg, 79%) as a colorless solid. mp 165−167 °C. HRMS (ES^+) Observed: 320.0769, Calculated $C_{16}H_{18}NO_2S_2$ $[M + H]^+$: 320.0779. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.09 (3H, s), 1.12 (3H, s), 2.44 (1H, d, J = 18 Hz), 2.53 (1H, d, J = 18 Hz), 3.00 (3H, s), 3.03−3.04 (2H, m), 7.25−7.28 (1H, m), 7.53−7.55 (1H, m), 7.77−7.81 (1H, m), 8.66−8.68 (1H, m). HPLC (70% CH_3CN; 30% H_2O (containing 0.1% TFA)): >99% purity. Retention time: 3.82 min.

6,6-Dimethyl-3-(2-hydroxyethyl)thio-1-(pyridin-2-yl)-6,7-dihydro-2-benzothiophen-4(5*H*)-one (34). To a suspension of **33** (150 mg, 0.47 mmol) in EtOH (5 mL) were added 2-mercaptoethanol (37 μL, 0.52 mmol) and NaOH (130 μL, 0.52

mmol). The mixture was stirred at room temperature for 1 h then the solvent evaporated. The residue was partitioned between water (20 mL) and EtOAc (20 mL). The organic phase was separated, dried ($MgSO_4$), and evaporated. The residue was chromatographed on silica gel, eluting with isohexane: EtOAc (1:1 → 0:1), to afford the title compound (96 mg, 61%) as a colorless solid. mp. 175−177 °C. Anal. ($C_{17}H_{19}NO_2S_2$) C, H, N. HRMS (ES^+) Observed: 334.0919, Calculated $C_{17}H_{20}NO_2S_2$ $[M + H]^+$: 334.0935. ^1H NMR (400 MHz, $CDCl_3$) δ 1.09 (6H, s), 2.46 (2H, s), 2.93 (2H, s), 3.34 (2H, t, $J = 6.0$ Hz), 4.01 (2H, t, $J = 6.0$ Hz), 7.17−7.20 (1H, m), 7.47−7.49 (1H, m), 7.75 (1H, dt, $J = 7.8$, 1.9 Hz), 8.60−8.61 (1H, m).

6,6-Dimethyl-3-methylthio-1-(3-methyl-1,2,4-oxadiazol-5-yl)-6,7-dihydro-2-benzothiophen-4(5*H*)-one (36). To a solution of 6,6-dimethyl-3-methylthio-4-oxo-4,5,6,7-tetrahydro-2-benzothiophene-1-carboxylic acid (**35**) (1.0 g, 3.7 mmol) in dioxane (90 mL) was added 1,1′-carbonyldiimidazole (0.6 g, 3.7 mmol) and the mixture stirred at 20 °C for 30 min. Acetamide oxime (0.41 g, 5.5 mmol) was added and the mixture heated at 100 °C for 48 h. The solution was evaporated to dryness and the residue chromatigraphed on silica gel, eluting with EtOAc:hexane (2:3), to yield the oxadiazole (0.1 g, 9%) as a pale yellow solid. mp 177−180 °C. Anal. ($C_{14}H_{16}N_2O_2S_2$) C, H, N. MS (ES^+) 309 $[M + H]^+$. ^1H NMR (250 MHz, $CDCl_3$) δ 1.11 (6H, s), 2.45−2.47 (5H, s), 2.65 (3H, s), 3.11 (2H, s).

6,6-Dimethyl-3-methylthio-4-oxo-4,5,6,7-tetrahydro-2-benzothiophene-1-carbothioamide (38). H_2S was bubbled through a solution of 6,6-dimethyl-3-methylthio-4-oxo-4,5,6,7-tetrahydro-2-benzothiophene-1-carbonitrile (**37**) (5.0 g, 20 mmol) and Et_3N (3 mL) in pyridine (50 mL) for 30 min. After this time the solution was allowed to stand at room temperature overnight. Nitrogen was bubbled through the solution which was subsequently diluted with CH_2Cl_2. The mixture was washed with water (2×), HCl (2×) and brine. The organic layer was separated, dried (Na_2SO_4), and evaporated. The title compound (3.9 g, 68%) was isolated as a yellow solid and used in the following reaction without further purification. mp 238−240 °C. Anal. ($C_{12}H_{15}NOS_3$) C, H, N. HRMS (ES^+) Observed: 286.0412, Calculated $C_{12}H_{16}NOS_3$ $[M + H]^+$: 286.0394. ^1H NMR (360 MHz, DMSO-d_6) δ 0.99 (6H, s), 2.34 (2H, s), 2.61 (3H, s), 2.93 (2H, s), 8.68 (1H, br s), 9.82 (1H, br s).

6,6-Dimethyl-3-methylthio-1-(thiazol-2-yl)-6,7-dihydro-2-benzothiophen-4(5*H*)-one (39). A solution of **38** (500 mg, 1.7 mmol) in EtOH (5 mL) was heated with chloroacetaldehyde (0.5 mL of a 50% (w/v) aqueous solution, 3.2 mmol) at reflux for 18 h. After this time the solvent was evaporated and the residue partitioned between between EtOAc (20 mL) and water (20 mL). The organic layer was separated, dried (Na_2SO_4), and evaporated. The residue was chromatographed on silica gel, eluting with hexane:EtOAc (3:1) to afford the title thiazole (350 mg, 65%) as a pale yellow solid. mp 149−151 °C. HRMS (ES^+) Observed: 310.0394, Calculated $C_{14}H_{16}NOS_3$ $[M + H]^+$: 310.0394. ^1H NMR (360 MHz, $CDCl_3$) δ 1.12 (6H, s), 2.46 (2H, s), 2.64 (3H, s), 2.90 (2H, s), 7.35 (1H, d, $J = 3.6$ Hz), 7.81 (1H, d, $J = 3.6$ Hz). HPLC (70% CH_3CN; 30% H_2O (containing 0.1% TFA)): >99% purity. Retention time: 6.66 min.

6,6-Dimethyl-3-methylthio-1-(1-methyl-1,2,4-triazol-3-yl)-6,7-dihydro-2-benzothiophen-4(5*H*)-one (41). A solution of **37** (5.0 g, 20 mmol) in saturated ethanolic hydrogen chloride solution (500 mL) was stirred at 20 °C for 15 h. The solvent was removed in vacuo and the residue triturated with EtOAc. The resultant ethyl 6,6-dimethyl-3-methylthio-4-oxo-4,5,6,7-tetrahydro-2-benzothiophene-1-carboximidoate hydrochloride (**40**) (2.5 g, 38%), which was isolated as a colorless solid, was collected by filtration and dried under vacuum. mp 134−136 °C. MS (ES^+) 298 $[M + H]^+$. ^1H NMR (250 MHz, DMSO-d_6) δ 1.00 (6H, s), 1.44 (3H, t, $J = 7.0$ Hz), 2.45 (2H, s), 2.67 (3H, s), 3.00 (2H, s), 4.52 (2H, q, $J = 7.0$ Hz).

40 (1.0 g, 3.4 mmol) was dissolved in EtOH (60 mL), and methylhydrazine (160 mg, 3.4 mmol) was added. The solution was heated at 50 °C for 7 h then evaporated to dryness. The residue was dissolved in formic acid (20 mL) and the solution heated at 100 °C for 16 h. The solvent was then removed in vacuo and the residue partitioned between CH_2Cl_2 and saturated K_2CO_3 solution. The organic layer was separated, dried ($MgSO_4$), and evaporated. The crude residue was chromatographed on silica gel, eluting with EtOAc, to afford the triazole (150 mg, 15%) as a yellow solid. mp 146−148 °C. Anal. ($C_{14}H_{17}N_3OS_2$) C, H, N. MS (ES^+) 308 $[M + H]^+$. ^1H NMR (250 MHz, $CDCl_3$) δ 1.05 (6H, s), 2.44 (2H, s), 2.63 (3H, s), 2.74 (2H, s), 3.96 (3H, s), 7.98 (1H, s).

6,6-Dimethyl-3-methanesulfonyl-1-(thiazol-2-yl)-6,7-dihydro-2-benzothiophen-4(5*H*)-one (42). To a stirred solution of **39** (1.44 g, 4.7 mmol) in CH_2Cl_2:dioxane (60 mL, 5:1) was added *m*-CPBA (1.7 g (95% w/w), 9.3 mmol) at room temperature. The mixture was stirred for 2 h then more *m*-CPBA (0.42 g (95% w/w), 2.3 mmol) was added. The mixture was stirred for a further 2 h then washed with Na_2CO_3 (sat., 50 mL). The organic phase was separated, dried (Na_2SO_4), and evaporated. The residue was chromatographed on silica gel, eluting with isohexane:EtOAc (3:1 → 1:1) to afford the sulfone (1.33 g, 84%) as a colorless solid. ^1H NMR (400 MHz, $CDCl_3$) δ 1.16 (6H, s), 2.59 (2H, s), 3.02 (2H, s), 3.56 (3H, s), 7.51 (1H, d, $J = 3.3$ Hz), 7.95 (1H, d, $J = 3.3$ Hz).

6,6-Dimethyl-3-(2-hydroxyethyl)thio-1-(thiazol-2-yl)-6,7-dihydro-2-benzothiophen-4(5*H*)-one (43). In the same way as described for **12** using **42**. Yield 91%. mp 175−177 °C. Anal. ($C_{15}H_{17}NO_2S_3 \cdot 0.2(H_2O)$) C, H, N. HRMS ($ES^+$) Observed: 340.0510, Calculated $C_{15}H_{18}NO_2S_2$ $[M + H]^+$: 340.0500. ^1H NMR (360 MHz, $CDCl_3$) δ 1.12 (6H, s), 2.47 (2H, s), 2.90 (2H, s), 3.32 (2H, t, $J = 5.9$ Hz), 4.01 (2H, t, $J = 5.9$ Hz), 7.36 (1H, d, $J = 3.2$ Hz), 7.82 (1H, d, $J = 3.2$ Hz).

6,6-Dimethyl-3-(3-hydroxypropyl)thio-1-(thiazol-2-yl)-6,7-dihydro-2-benzothiophen-4(5*H*)-one (44). In the same way as described for **43** using 3-mercapto-1-propanol. Yield 75%. mp 136−139 °C. Anal. ($C_{16}H_{19}NO_2S_3$) C, H, N. MS (ES^+) 354 $[M + H]^+$. ^1H NMR (360 MHz, DMSO-d_6) δ 1.05 (6H, s), 1.89 (2H, pentet, $J = 6.8$ Hz), 2.44 (2H, s), 2.91 (2H, s), 3.15 (2H, t, $J = 7.2$ Hz), 3.54 (2H, q, $J = 5.8$ Hz), 4.69 (1H, t, $J = 5.2$ Hz), 7.82 (1H, d, $J = 3.3$ Hz), 7.88 (1H, d, $J = 3.3$ Hz).

6,6-Dimethyl-1-(thiazol-2-yl)-3-(thiazol-2-yl)thio-6,7-dihydro-2-benzothiophen-4(5*H*)-one (45). In the same way as described for **14** using **42** and stirring at room temperature for 20 h. Yield 88%. mp 200−202 °C. Anal. ($C_{16}H_{14}N_2OS_4.0.3(H_2O)$) C, H, N. MS ($ES^+$) 379 $[M + H]^+$. ^1H NMR (360 MHz, $CDCl_3$) δ 1.14 (6H, s), 2.50 (2H, s), 2.91 (2H, s), 7.34 (1H, d, $J = 3.2$ Hz), 7.57 (1H, d, $J = 3.5$ Hz), 7.79 (1H, d, $J = 3.2$ Hz), 8.00 (1H, d, $J = 3.3$ Hz).

Biological Methods

Radioligand Binding Studies. L(tk⁻) cells expressing human recombinant $GABA_A$ receptors containing $\beta3$ and $\gamma2$ subunits in combination with various α subunits were harvested and binding performed as described by Hadingham et al.[39] The displacement of [^{3}H]Ro 15−1788 binding by the test compounds was measured in $GABA_A$ receptors containing either an α1-, α2-, α3-, or α5-subunit and from the IC_{50} the K_i was calculated assuming respective K_D values of [^{3}H]Ro 15−1788 binding of 0.92, 1.05, 0.58, and 0.45 nM at the α1-, α2-, α3-, and α5-subtypes. Nonspecific binding was defined by the inclusion of 10 μM flunitrazepam for the α1-, α2-, α3-, and α5-subtypes. The percentage inhibition of [^{3}H]Ro 15−1788, the IC_{50} and the K_i values were calculated using ActivityBase (IDBS).

Electrophysiology. (a) Voltage Clamp Recording of *Xenopus laevis* Oocytes Transiently Transfected with the Human GABA-A α5 Receptor Subtype. Oocytes were isolated from adult female *Xenopus laevis* frogs which had been anaesthetized by immersion for 30 to 45 min in a solution of 0.4% ethyl *m*-aminobenzoate (MS 222 or Tricaine) in accordance with the UK Animals (Scientific Procedures) Act 1986. Oocytes were then transfected with human $GABA_A$ receptor cDNA encoding one α5 plus $\beta3$ and $\gamma2$s subunits engineered into the expression vector pCDM8 or pcDNAI/Amp injected directly into the nucleus using a Drummond Scientific Instruments 100 μL syringe attached to a micromanipulator. Injected oocytes were then transferred into incubation medium (1 L of regular MBS, 2 mL of penicillin (10,000 units/mL) and

streptomycin (10 mg/mL), 50 mg of gentamycin, and 2 mM Na^+pyruvate).

Electrophysiological recordings were made 2 to 4 days after cDNA injection since the efficacy values determined for standard compounds on day 1 were variable. If an oocyte expressed the receptor subtype with an atypically high EC_{20} value then this cell was rejected from analysis. The approximate ideal EC_{20} value for α5 receptor subunits in oocytes was 1−6 μM. Cells were superfused with MBS in a chamber of approximately 50 μL volume. Glass recording microelectrodes were fabricated on a Brown Flaming electrode puller and the tips were backfilled to approximately 10 mm with an Agar solution containing KCl (2 M) solution. The electrode tips were broken back to give a resistance of 1 to 2 MΩ in MBS solution. Oocytes were impaled with these microelectrodes using micromanipulators and then voltage-clamped at −60 mV using an Axoclamp 2A/2B or Geneclamp amplifier. The change in current following drug application was then plotted on a chart recorder.

For each oocyte the expression of receptors was first checked by a 30 s to 1 min application of 3 mM GABA in MBS. GABA currents ranged typically from 0.3 to 3 μA, and cells with currents <0.3 μA were rejected. Following a high concentration of GABA the oocyte was perfused with MBS for 8 to 10 min to facilitate recovery from desensitization. The concentration of GABA required to activate a response which was 20% of the maximum amplitude (EC_{20}) was then determined. The test compound was applied at a concentration approximately equivalent to 100× the K_i value. Efficacy of the test compounds was then determined as the percentage change in the current activated by the EC_{20} concentration of GABA according to the equation: Efficacy (%) = ((current with test compound/control current) × 100) − 100.

(b) Whole Cell Patch-Clamp of L(tk⁻) Cells Stably Transfected with Human GABA-A Receptors. Experiments were performed on L(tk⁻) cells expressing human cDNA combinations α1β3 γ2s, α2β3 γ2s, α3β3 γ2s, and α5β3γ2s. Glass cover-slips containing the cells in a monolayer culture were transferred to a Perspex chamber on the stage of Nikon Diaphot inverted microscope. Cells were continuously perfused with a solution containing 124 mM NaCl, 2 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 1.25 mM KH_2PO_4, 25 mM $NaHCO_3$, 11 mM D-glucose, at pH 7.2, and observed using phase-contrast optics. Patch-pipets were pulled with an approximate tip diameter of 2 μm and a resistance of 4 MΩ with borosilicate glass and filled with 130 mM CsCl, 10 mM HEPES, 10 mM EGTA, 3 mM Mg^+-ATP, pH adjusted to 7.3 with CsOH. Cells were patch-clamped in whole-cell mode using an Axopatch-200B patch-clamp amplifier. Drug solutions were applied by a double-barreled pipet assembly, controlled by a stepping motor attached to a Prior manipulator, enabling rapid equilibration around the cell. Increasing GABA concentrations were applied for 5 s pulses with a 30 s interval between applications. Curves were fitted using a nonlinear square-fitting program to the equation $f(x) = B_{max}/[1 + (EC_{50}/x)^n]$ where x is the drug concentration, EC_{50} is the concentration of drug eliciting a half-maximal response, and n is the Hill coefficient. Allosteric modulation of GABA receptors was measured relative to a GABA EC_{20}, individually determined for each cell to account for differences in GABA affinity.

In Vivo Binding. In vivo occupancy of the $GABA_A$ receptor subtypes was determined using the method described by Atack et al.[33] Rats or mice were dosed intraperitoneally at 1 and 10 mL/kg, respectively, with either **43** in 70% PEG 300, 70% PEG 300 (to determine total radioligand binding) or 5 mg/kg bretazenil in PEG 300 (to determine nonspecific radioligand binding). To determine the occupancy at $GABA_A$ α1-, α2-, α3-, and α5-subunit-containing receptors, rats were given an intravenous (iv) dose of a 15 μCi/mL solution of [^{3}H]Ro 15−1788 (DuPont NEN, 1 mL/kg) 3 min prior to killing by stunning with decapitation. Occupancy at $GABA_A$ α5-subunit-containing receptors was determined using [^{3}H]L-655,708 as a 11 μCi/mL solution dosed at 1 mL/kg, which was administered iv 1 min prior to killing. Thirty minutes after drug administration the animals were killed, as described above, and the brains rapidly removed. Whole brain ([^{3}H]Ro 15−1788) or forebrain ([^{3}H]L-655,708) were removed, weighed, and rapidly homogenized in 10 times the volume of ice cold buffer (10 mM KH_2PO_4, 100 mM KCl pH 7.4) using a Polytron PT2100 homogenizer, and 300 μL of the homogenate was filtered though presoaked Whatman GF/B filters using a filtration manifold (Hoefer Scientific Instruments) and washed twice with 5 mL of the ice cold buffer. Filters were then placed in 10 mL of scintillation fluid (Hydrofluor, National Diagnostics), and the level of radioactivity present was determined using a liquid scinitillation counter (Beckman LS6500).

Specific binding was defined as radioactivity in vehicle or compound-treated animals minus radioactivity in bretazenil-treated animals.

Percentage occupancy = 100 − (binding in drug group/binding in vehicle group) × 100.

Proconvulsant Activity. The proconvulsant potential of **43** was determined using the method described by Roberts and Keith.[34] The convulsant used in this study was pentylenetetrazole which was dosed at 15 mg/mL with an infusion rate of 0.2 mL/min. This rate was chosen to ensure that drug-naive mice reached the terminal convulsion sign within 1 min. Compound **43** was dosed ip at a concentration of 3 mg/kg in 70% PEG 300.

Water Maze Test. The Morris water maze consisted of a white, circular fiber-glass pool, of diameter 2 m, filled with an opaque mixture of water and white dye (E308 opacifier, Rohm and Haas (UK) Limited) maintained at 26−28 °C. It was located in the center of a sound-attenuated room and was illuminated by overhead fluorescent strip lights. A light gray curtain was hung around the pool from above, such that it almost touched the sides upon which eight laminated high contrast black and white patterned pictures (42 cm × 30 cm) were attached to serve as spatial 'extra-maze' cues. A direction 'north' was arbitrarily determined and the pool was divided into four equal quadrants, 'northeast', 'southeast', 'southwest', and 'northwest'. The curtain was split and overlapped at points north, south, east, and west to allow access to the pool in order to place rats into the water. A round platform of diameter 10 cm was submerged 2 cm below the surface of the water rendering it invisible at surface level. A closed-circuit CCD video camera fitted with a wide-angle lens was mounted directly above the center of the pool and was connected (via a VCR) to an image analyzer (VP 200, HVS Image Ltd, UK) which digitized the image. The dark heads of the hooded lister rats provided a high contrast image against the white colored water, which could be tracked by the image analyzer. The digital information was relayed to a PC running 'HVS Water 2020' a software package supplied by HVS Image, UK. This software provided a multitude of measures including latency to reach the platform, length of path taken, swimming speed, and time spent in areas of the pool.

Before the experiments began the male hooded Lister rats were trained in the water maze task by being given four trials each day for 8−10 days. During these trials the submerged platform was placed in a different location each day, but remained constant for the duration of each day. During this training phase, the rats learned the procedural rules of the water maze delayed 'matching-to-place' task: (i) there is an escape platform hidden in the pool, (ii) the location of the hidden platform changes each day, but it is consistent within the day, and (iii) the extra maze spatial cues can be used to locate the platform position. On trial 1 the latency to find the platform is relatively long, but is shorter on trials 2, 3, and 4, demonstrating that the animals' memory for the platform location improves each time it escapes to the hidden platform. The improvement in the animals' memory was quantified by subtracting the trial 2 latency from the trial 1 latency to give the savings score. For each daily trial the rat was taken from the home cage and placed into the water maze at one of the four quasi-randomly determined locations ('north', 'east', 'south', or 'west') with its head facing, and almost touching, the pool wall. Trials began when the rat was released by the experi-

menter and ended when the rat climbed onto the platform and the mean escape latency was recorded. The maximum trial length was 60 s. If by that time the rat had not climbed on to the platform, the trial ended automatically; the experimenter intervened and placed the rat on the platform, and an escape latency of 60 s was recorded. The rat remained on the platform for 30 s (inter-trial interval, ITI). At the end of the ITI, the rat was placed into the pool again, but at a different location, and upon release the next trial began. This procedure was repeated until four trials had been completed. Before compound treatment commenced, animals were assigned to treatment groups in such a way so as to ensure that the level of performance (using mean savings) during the training phase was not significantly different. Rats were dosed ip with either vehicle (70%/30% PEG 300/water) or **43** (0.3 mg/kg), 30 min before commencing the trials. The compound treatment phase lasted for 5 days. It was identical to the methodology described above with the exception that a 4 h delay period was inserted between trial 1 and trial 2. Statistically significant differences were determined using two-way ANOVA.

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Supporting Information Available: Data showing the mean swim speeds of rats dosed with **43** (0.3 mg/kg ip) and vehicle (70% PEG 300) in the delayed 'matching-to-place' water maze test. This material is available free of charge via the Internet at http://pubs.acs.org.

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