Potent 4-Arylalkyl-Substituted 3-Isothiazolol $GABA_A$ Competitive/Noncompetitive Antagonists: Synthesis and Pharmacology

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The $GABA_A$ agonists muscimol (**1**), 4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol (THIP, gaboxadol, **3**), and the partial $GABA_A$ agonist 5-(4-piperidyl)-3-isoxazolol (4-PIOL, **6a**) and their respective 3-isothiazolol analogues thiomuscimol (**2**), thio-THIP (**4**), and thio-4-PIOL (**7a**) are ligands at the $GABA_A$ orthosteric (recognition) site. The structure−activity relationships (SARs) between these structures are key elements of a 3D-pharmacophore model for $GABA_A$ agonists and competitive antagonists [Frølund, B.; Jørgensen, A. T.; Tagmose, L.; Stensbøl, T. B.; Vestergaard, H. T.; Engblom, C.; Kristiansen, U.; Sanchez, C.; Krogsgaard-Larsen, P.; Liljefors, T. *J. Med. Chem.* **2002**, *45*, 2454−2468]. Prompted by this model, we now report the synthesis and SAR of a series of analogues of **7a**, in which the 4-position of the 3-isothiazolol was substituted by alkyl or bulky aromatic groups such as naphthylmethyl and diphenylalkyl groups (**7b**−**h**). The compounds have been pharmacologically characterized using receptor binding assays and two-electrode voltage-clamped *Xenopus* oocytes expressing $\alpha_1\beta_3\gamma_{2S}$- and $\alpha_4\beta_3\delta$-containing receptors. The compounds show SARs comparable with those of **6b**−**h** but are generally 5−15 times more potent. The 2-naphthylmethyl, the 1-bromo-2-naphthylmethyl, and the 3,3-diphenylpropyl analogues, compounds **7e**, **7f**, and **7h**, respectively, show affinity in the low-nanomolar range (K_i 2−10 nM). Interestingly, **7e** and **7h** exhibited a mixed antagonist profile consisting of a noncompetitive component in the picomolar range and a competitive component at concentrations above 1 nM. This unique profile was shown not to be due to either use dependence or kinetic effects. This antagonist profile of **7e** and **7h** was particularly pronounced at $\alpha_4\beta_3\delta$-containing $GABA_A$ receptors, which showed three- and 10-fold selectivity for **7h** and **6h**, respectively.

Introduction

The major inhibitory neurotransmitter 4-aminobutyric acid (GABA) exerts its effect in the central nervous system through two classes of receptors, the ionotropic $GABA_A$ and $GABA_C$ receptors and the metabotropic $GABA_B$ receptors. The GABA neurotransmitter system is involved in a wide range of physiological and pathological processes, and ligands for the heteromeric $GABA_A$ receptor complex have attracted considerable interest as potential therapeutics in the treatment of anxiety, seizures, schizophrenia, depression, and sleep disorders.[1]

The $GABA_A$ receptors belong to a superfamily of ligand-gated ion channels that also includes the nicotinic acetylcholine, the glycine, and the serotonin ($5\text{-}HT_3$) receptors. The $GABA_A$ receptor is a transmembrane allosteric protein complex composed of five subunits. So far, 16 $GABA_A$ receptor subunits have been identified and divided into seven groups (α_{1-6}, β_{1-3}, γ_{1-3}, δ, ϵ, π, and θ). Although a high number of different subunit compositions are possible, only a limited number has been identified, the $\alpha_1\beta_2\gamma_2$ apparently being the most abundant in the human central nervous system.[2,3]

Muscimol (**1**)[4] and 4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol (THIP, gaboxadol, **3**)[4,5] (Figure 1) are both standard $GABA_A$ receptor agonists. Recently, THIP has in in vivo studies been shown to act as a functionally selective extrasynaptic $GABA_A$ agonist with high potency and relative efficacy at the extrasynaptically expressed $\alpha_4\beta_3\delta$. THIP is currently in phase III clinical development as a hypnotic.

The affinity of the sulfur analogue of THIP (thio-THIP, **4**) is more than 300 times lower than that of THIP itself,[6] whereas the 3-isoxazolol ring in muscimol can be replaced by an isothiazolol ring to give thiomuscimol (**2**) without significant loss of $GABA_A$ receptor affinity.[7] The affinity of thio-4-PIOL (**7a**), on the other hand, is about an order of magnitude higher than that of **6a**.[8]

These observations have formed part of the basis for the hypothesis previously proposed for different binding modes in the receptor binding cavity for muscimol, THIP, and 4-PIOL.[9,10] Moreover, according to the resulting 3D-pharmacophore model, a cavity exists in the vicinity of the 4-position of the 3-isoxazolol ring of 4-PIOL but not of the corresponding position in muscimol or THIP.[9] Exploration of this cavity has led to a series of potent competitive $GABA_A$ antagonists exemplified by compounds **6e**, **f**, and **h**.[10,11]

To validate and further extend this hypothesis/model, we here describe the synthesis and pharmacological characterization of a series of thio-4-PIOL analogues (**7b**−**h**) in which the 4-position of the 3-isothiazolol is substituted by alkyl and bulky aromatic groups such as naphthylmethyl and diphenylalkyl groups.

Results

Chemistry. In the synthesis previously described for thio-4-PIOL (**7a**), the 3-isothiazolol moiety was formed by oxidative cyclization of the corresponding β-thioamide.[8] In this study, the 3-isothiazolol moiety was synthesized as shown in Scheme 1, using a strategy previously described for the synthesis of thio-THIP (**4**).[12] The (*E*)-isomer of the α,β-unsaturated amide, **9**, was synthesized using *N*-benzyl-protected 4-formylpiperidine

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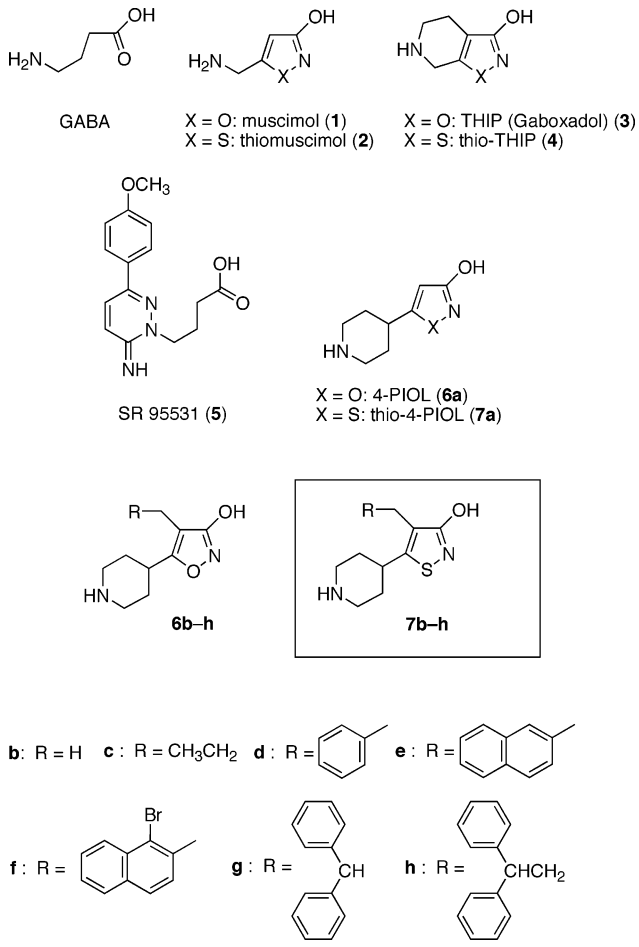


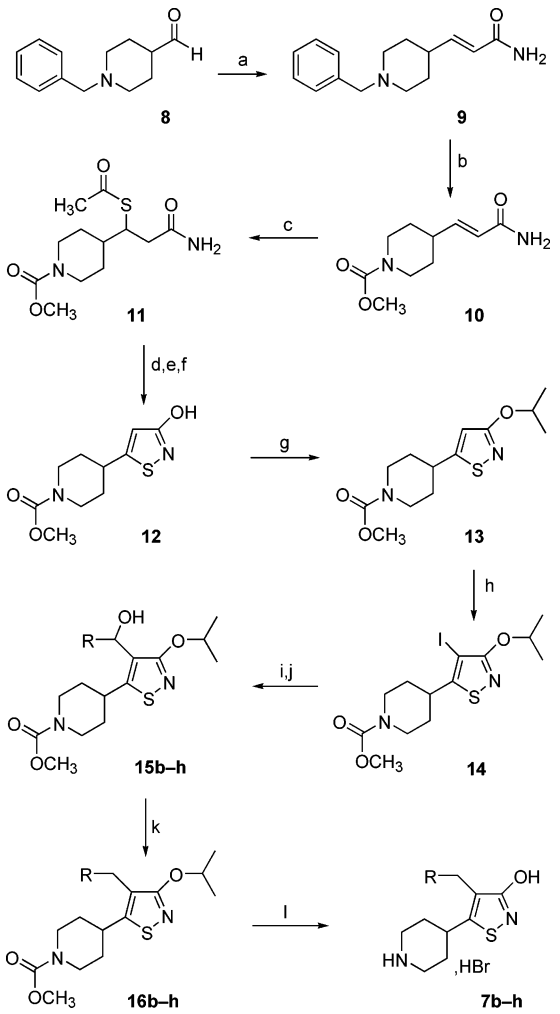
Figure 1. Structures of GABA, the GABA$_A$ agonists muscimol (**1**), thiomuscimol (**2**), THIP (**3**), Thio-THIP (**4**), the GABA$_A$ antagonist SR 95531 (**5**), the low-efficacy partial GABA$_A$ agonists 4-PIOL (**6a**) and thio-4-PIOL (**7a**), the 4-PIOL analogues **6b**−**h**, and the new 3-isothiazolols (**7b**−**h**).

aldehyde, **8**, and triethylphosphonoacetamide. Sulfur was introduced by a conjugated addition of thioacetic acid to **10** giving thioester **11** in good yield. Compound **11** was hydrolyzed to the corresponding thiol, oxidized to the disulfide, which was then cyclized using sulfuryl chloride, to give N-protected thio-4-PIOL, **12**.

With the use of a previously reported metal−iodine exchange reaction,[10] the iodinated analogue **14** underwent iodine−magnesium exchange using either ethylmagnesium chloride or bromide followed by reaction with the appropriate electrophile to give the hydroxy compounds **15b**−**h** (Scheme 1). The methyl group in compound **7b** was introduced as illustrated in Scheme 1 using DMF as the electrophile followed by reduction to give the hydroxymethyl compound **15b**. The benzylic hydroxy group was subsequently removed by ionic hydrogenation using trifluoroacetic acid and triethylsilane in dichloromethane to give the compounds **16b**−**h**. Deprotection to give the target compounds **7b**−**h** was accomplished by treatment with hydrogen bromide in acetic acid.

In Vitro Pharmacology. The compounds were pharmacologically characterized in receptor binding studies based on rat brain membrane preparations and on human $\alpha_1\beta_3\gamma_{2S}$ and $\alpha_4\beta_3\delta$ GABA$_A$ receptors expressed in *Xenopus* oocytes using two-electrode voltage-clamp electrophysiology. The affinities for GABA$_A$ and GABA$_B$ receptor sites, using [^{3}H]muscimol and [^{3}H]GABA, respectively, were determined using methods described previously.[11] Like **7a**, all of the 3-isothiazolol

Scheme 1[a]



[a] Reagents: (a) triethylphosphonoacetamide, NaH, DME; (b) $ClCO_2CH_3$; (c) CH_3COSH, EtOAc; (d) aq NaOH; (e) 35% aq H_2O_2, 45 °C; (f) SO_2Cl_2, $ClCH_2CH_2Cl$; (g) *i*-PrBr, K_2CO_3, DMF, 0 °C; (h) ICl, HOAc, H_2O, 80 °C; (i) EtMgBr, THF, −30 °C; (j) RCHO (R, see Figure 1), THF, 0 °C; for compound **14b**, (1) DMF, THF, 0 °C and room temperature, (2) $NaBH_4$, MeOH, 0 °C and room temperature; (k) TFA, Et_3SiH, CH_2Cl_2, 0 or 50 °C; (l) 33% HBr, HOAc, 65 °C.

analogues (**7b**−**h**) synthesized in the present study showed affinity selectively for the GABA$_A$ receptor sites (Table 1) and exhibited similar structure−activity relationships (SARs) as described for the corresponding 3-isoxazolol analogues **6b**−**h** (Table 1).[10,11] Alkyl groups such as methyl and propyl groups as well as a benzyl group are tolerated (compounds **7b**−**d**) showing affinities for the GABA$_A$ receptor sites comparable to or slightly higher than that of **7a**. Addition of two phenyl groups to the terminal carbon of **7c**, as in the 3,3-diphenylpropyl analogue **7h**, gave a 46-fold increase in affinity relative to the propyl analogue **7c**. The 2,2-diphenylethyl analogue **7g** shows affinity comparable to that of **7c**. In accordance with the results from the 3-isoxazolol series,[11] introduction of a bromo substituent in the 1-position of the naphthyl ring system of compound **7e** affording compound **7f** provided an enhanced affinity relative to that of the 2-naphthylmethyl analogue, **7e**. Except for the 2,2-diphenylethyl analogue **7g**, the affinities of the 3-isothiazolol series show a 5−15-fold higher affinity for the GABA$_A$ receptor sites compared to that of the 3-isoxazolol series (Table 1). The 1-bromo-substituted analogue, **7f**, showing a K_i value of 1.8 nM, is the compound of highest affinity in this study.

Table 1. Receptor Binding and in Vitro Electrophysiological Data

	K_i (nM) ($pK_i \pm$ SEM)[a]	
	[^{3}H]muscimol binding[b]	electrophysiology[c]
5	74	240
6a[d]	9100	110000
6b[d]	10000	26000
6c[d]	6600	4600
6d[d]	3800	4000
6e[d]	49	370
6f[e]	10	42
6g[d]	360	810
6h[d]	68	20
7a	1870 (5.73 ± 0.04)	25704 (4.59 ± 0.16)
7b	2200 (5.66 ± 0.01)	2880 (5.54 ± 0.08)
7c	440 (6.35 ± 0.02)	230 (6.64 ± 0.06)
7d	331 (6.48 ± 0.05)	465 (6.33 ± 0.09)
7e	5.2 (8.28 ± 0.07)	22 (7.66 ± 0.04)
7f	1.8 (8.75 ± 0.14)	3.0 (8.53 ± 0.09)
7g	388 (6.41 ± 0.09)	720 (6.14 ± 0.15)
7h	9.6 (8.02 ± 0.17)	1.2 (8.92 ± 0.04)

[a] K_i values were calculated from pK_i values found in brackets. [b] Standard receptor binding on rat brain synaptic membranes, $n \geq 3$. [c] Two-electrode voltage-clamp recordings on *Xenopus* oocytes expressing $\alpha_1\beta_3\gamma_{2S}$ $GABA_A$ receptor subunits $n = 4$. [d] Ref 10. [e] Ref 11.

The pharmacological profile of the compounds was studied using a two-electrode voltage-clamp technique on human $\alpha_1\beta_3\gamma_{2S}$ $GABA_A$ receptors expressed in *Xenopus* oocytes. At fixed concentrations of compounds, **7b**−**h**, the effect on a GABA concentration−response curve was investigated. As shown for the corresponding 3-isoxazolol analogues,[10,11] all of the new compounds were characterized as antagonists and did induce a rightward shift of the GABA concentration−response curve, suggesting competitive antagonism. However, compounds **7e** and **7h** behaved as both competitive and noncompetitive antagonists. Thus, in addition to a rightward shift of the dose−response curve, both compounds significantly reduced the maximum response to GABA to approximately 60% (relative to the maximum GABA response in the absence of the test compound). With the use of **7h** as a probe, this mixed antagonism was characterized in further details. As illustrated in Figure 2a, two distinct and separable mechanisms appeared to affect the response to GABA at $\alpha_1\beta_3\gamma_{2S}$-containing receptors. At very low concentrations, in the picomolar range, a dose-dependent reduction in the response to GABA was seen. The reduction was saturable in the sense that increasing the doses of **7h** did not lead to reduction of the maximum GABA responses below 60% of the control response. At concentrations above 1 nM, **7h** shifted the remaining GABA concentration−response curve parallel rightward in agreement with classical competitive interaction theory.

This unusual pharmacological profile observed for compound **7e** and **7h** might be the consequence of a slow on-rate or some degree of use dependence. To test for a possible use dependence, the consequence of repeated administration of agonist in the presence of antagonists was examined. Furthermore, the impact of an antagonist pretreatment time as a marker for on-rate was characterized. In both experiments, no differences were detected between the responses ruling out the two scenarios mentioned above (Figure 3).

Compound **7h** was further characterized at $\alpha_4\beta_3\delta$-containing $GABA_A$ receptors. This receptor population, which is exclusively extrasynaptically located, has previously been shown to be particularly sensitive to $GABA_A$ agonists[13] and is now thought to mediate the pharmacological effects of THIP (gaboxadol), which is under development as a hypnotic. As observed for the $\alpha_1\beta_3\gamma_{2S}$ receptor subtype, a mixed competitive/noncompetitive antagonist profile was seen, where the suppression of the maximal response to GABA is even more pronounced (E_{max} 40%) (Figure 2b). In line with previous observations at the $\alpha_4\beta_3\delta$ receptor subtype, the potency of compound **7h** is 3-fold higher than at the $\alpha_1\beta_3\gamma_{2S}$ receptor subtype, with approximate K_i values for the competitive component of 0.35 and 1.0 nM, respectively.

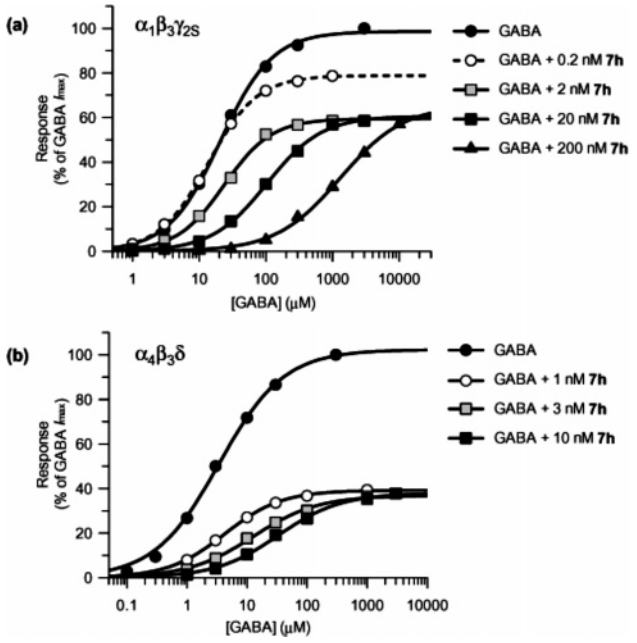


Figure 2. (a) GABA concentration−response curves (full lines) in the absence and presence of a fixed concentration (2, 20, or 200 nM) of **7h** obtained from the same *Xenopus* oocyte expressing $\alpha_1\beta_3\gamma_{2S}$ $GABA_A$ receptor subunits. Data were normalized with respect to the maximum response (GABA I_{max}) and subsequently fitted to eq 2. The dotted curve represents data from another latter oocyte exposed to 0.2 nM **7h** + variable GABA concentrations. From the latter oocyte, a practically identical GABA concentration−response curve was obtained, permitting a direct comparison between the data sets. (b) GABA concentration−response curves in the absence and presence of a fixed concentration (1, 3, or 10 nM) of **7h** obtained from the same *Xenopus* oocyte expressing $\alpha_4\beta_3\delta$ $GABA_A$ receptor subunits. Data were normalized with respect to the maximum response (GABA I_{max}) and subsequently fitted to eq 2.

Since a discrepancy of the pharmacological profiles of the 3-isothiazolol analogues of 4-PIOL and the corresponding 3-isoxazolol analogues seems to exist, two 3-isoxazolol analogues, **6e** and **6h**, were studied in detail at the $\alpha_1\beta_3\gamma_{2S}$ receptor subtype. As previously observed, the 3-isoxazolol analogue **6e** showed a pure competitive antagonist effect, whereas the 3-isoxazolol analogue **6h** in addition to the rightward shift of the GABA concentration−response curve exhibited a weak suppression of the maximal effect of GABA (E_{max} 90%). On the basis of this observation, the effect of the 3-isoxazolol analogue **6h** at the $\alpha_4\beta_3\delta$ receptor subtype was examined as well. As observed on the $\alpha_1\beta_3\gamma_{2S}$ receptor subtype, the 3-isoxazolol analogue showed a shift of the GABA dose−response curve, and in agreement with data for **7h**, a more pronounced suppression of the maximal effect of GABA (E_{max} 70%) was seen. Furthermore, the 3-isoxazolol analogue **6h** exhibits a 10-fold higher potency at the $\alpha_4\beta_3\delta$ than at the $\alpha_1\beta_3\gamma_{2S}$ receptor subtype (K_i = 2.3 and 26 nM, respectively) consistent with a more pronounced tendency for subtype selectivity than that shown for **7h**.

The benzyl analogue, **7d**, and the highly potent 1-bromo-2-naphthylmethyl analogue, **7f**, slightly altered the E_{max} of GABA, whereas the rest of the series of compounds in the

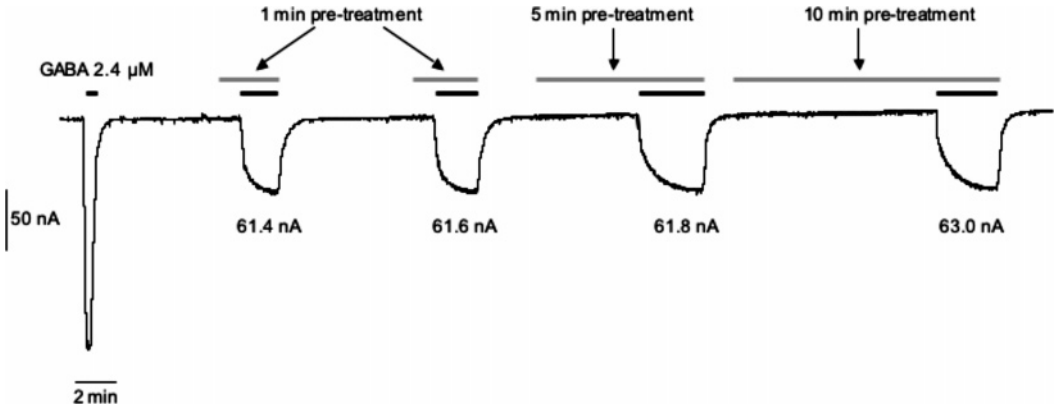


Figure 3. Effect of **7h** on the response to 2.4 μM GABA (black bar). The figure displays traces from a voltage-clamped *Xenopus* oocyte expressing $\alpha_1\beta_3\gamma_{2S}$ GABA$_A$ receptor subunits. The oocyte was clamped at −70 mV, and **7h** (grey bar) was added with different pretreatment periods (1, 5, and 10 min).

present study were shown to be purely competitive antagonists. K_i values for the competitive component of the tested compounds in this study are given in Table 1 and show a fairly good correlation to the obtained binding affinities.

Discussion

Previous SAR studies have disclosed that the 3-isothiazolol analogue of muscimol (**1**), thiomuscimol (**2**), is slightly weaker than muscimol, thio-THIP (**4**) is markedly less active than THIP (**3**). Thio-4-PIOL (**7a**), however, was shown to be a partial GABA$_A$ agonist slightly more potent and efficacious than the parent compound, 4-PIOL (**6a**).

A summary of these and other SAR studies led to a hypothesis of the binding mode of heterocyclic GABA$_A$ agonists and provided a 3D-pharmacophore model for this group of compounds.[9,10,14] On the basis of this model, we have previously described a series of 3-isoxazolol analogues of 4-PIOL (**6a**) as competitive GABA$_A$ antagonists. Introduction of bulky aromatic substituents, such as 3,3-diphenylpropyl and 2-naphthylmethyl, in the 4-position of the 3-isoxazolol ring of 4-PIOL provided **6h** and **6e**, respectively, showing markedly higher affinity for, and more potent effects at, the GABA$_A$ receptors than the parent compound. The results of these SAR studies strongly suggest the existence of a large cavity in the receptor binding site capable of accommodating these large substituents, with specific sites for ligand interactions. All of these analogues were shown to be moderate to potent competitive antagonists at the GABA$_A$ receptor.

Now, a series of 3-isothiazolol analogues structurally related to the 3-isoxazolol analogues mentioned above have been synthesized and pharmacologically characterized. In general, a similar SAR as seen for the corresponding 3-isoxazolol series was observed, although the 3-isothiazolol analogues generally showed higher affinity and potency as compared to that of the 3-isoxazolol analogues. The 2-naphthylmethyl, the 1-bromo-2-naphthylmethyl, and the 3,3-diphenylpropyl analogues, compounds **7e**, **7f**, and **7h**, showed affinities and potencies in the low-nanomolar range considerably higher than that of the standard competitive GABA$_A$ antagonist SR 95531 (Table 1).[15,16]

The higher affinities of the 3-isothiazolol compounds **7a**−**h** as compared to the corresponding 3-isoxazolol compounds **6a**−**h** (Table 1) may be rationalized by the higher lipophilicities and the lower desolvation energies of the sulfur analogues. The log *P* values for 3-isoxazolol and 3-isothiazolol are calculated to be 0.87 and 2.24, respectively, by using Crippen's fragmentation method and 0.65 and 2.24, respectively, by using the ClogP algorithm. The free energies of hydration of the anions of 3-isoxazolol and 3-isothiazolol are calculated to be −77.9 and −75.7 kcal/mol, respectively, indicating a significantly lower desolvation energy for the sulfur compound. Although this rationalizes the higher affinities of the thio-4-PIOL analogues **7a**−**h** as compared to those of the corresponding 4-PIOL analogues, it cannot explain the virtually identical affinities of muscimol (**1**) and thiomuscimol (**2**) and the significantly (300-fold) lower affinity of thio-THIP (**4**) in comparison to that of THIP (**3**).

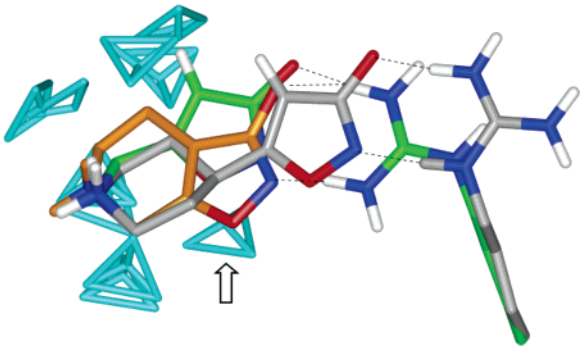


Figure 4. Pharmacophore model for GABA$_A$ receptor ligands showing the proposed binding modes of muscimol (**2**, green carbon atoms), 4-PIOL (**6a**, gray carbon atoms), and THIP (**3**, orange carbon atoms). The cyan-colored tetrahedrons represent sterically "forbidden" volumes (receptor essential volumes). Dashed lines indicate hydrogen bonds. Some hydrogens are removed for clarity.

We have previously reported a pharmacophore model for GABA$_A$ receptor ligands based on muscimol (**2**) and 4-PIOL (**6a**).[9] The main features of this model are that the 3-isoxazolol rings of the two compounds do not overlap in their proposed binding modes and that the two compounds interact with different conformations of an arginine residue located at the GABA$_A$ recognition site. By docking THIP (**3**) into this pharmacophore model it is revealed that the 3-isoxazolol ring in THIP (**3**) occupies yet another position in the receptor cavity as shown in Figure 4. The docking to the pharmacophore model was done by requiring overlap of the protonated amino groups of all three compounds, hydrogen bonding by the isoxazolol group of THIP (**3**) to one of the arginine conformers, and avoidance of previously identified sterically "forbidden" receptor volumes (receptor essential volumes) represented by tetrahedrons in Figure 4.

In contrast to the ring oxygen in 4-PIOL (**6a**), the ring oxygens in muscimol (**1**) and THIP (**3**) are both in the vicinity of a previously identified sterically "forbidden" volume indicated

by an arrow in Figure 4. The ring oxygen in THIP (**3**) is significantly closer to the forbidden volume than the corresponding oxygen in muscimol (**1**). The replacement of the ring oxygen by the much larger sulfur atom is expected to decrease the affinity of thio-THIP (**4**) significantly more than that of thiomuscimol (**2**). Thus, the expected affinity increase of thiomuscimol (**2**) and thio-THIP (**4**) in comparison to that of muscimol (**1**) and THIP (**3**) due to favorable lipophilicities and desolvation energies is counteracted by an affinity decrease due to steric repulsions between the sulfur atom and a sterically "forbidden" volume. This affinity decrease is significantly larger for thio-THIP (**4**) than for thiomuscimol (**2**). Thus, the different affinity changes due to an oxygen−sulfur exchange in muscimol (**1**), THIP (**3**), and 4-PIOL (**6a**) may be rationalized on the basis of different positions of their 3-isoxazolol rings in the receptor cavity as proposed by the pharmacophore model.

Taken into account that thio-4-PIOL (**7a**) exhibits a higher efficacy than 4-PIOL (**6a**) and that the methyl and ethyl analogues of 4-PIOL show a weak agonist effect,[10,17] it was expected that the corresponding thio-4-PIOL analogues would be partial agonists of higher affinity and efficacy. Actually, the methyl and propyl analogues, **7b** and **7c**, did show higher affinity but were without detectable efficacy.

Interestingly, the 2-naphthylmethyl and the 3,3-diphenylpropyl 3-isothiazolol analogues, **7e** and **7h**, did show a markedly different pharmacological profile than previously shown for the corresponding 3-isoxazolol analogues at the synaptic $\alpha_1\beta_3\gamma_{2S}$ receptor subtype. As expected for a competitive antagonist a rightward shift of the GABA concentration−response curve was observed, but in addition the compounds did cause a reduction of the maximal response of GABA down to 60%. The apparent dual behavior could be separated into two components, a noncompetitive saturable component at subnanomolar concentrations and a competitive component at low-nanomolar concentrations. A similar antagonist profile was shown for the compound at the extrasynaptic $\alpha_4\beta_3\delta$ receptor subtype where the noncompetitive effect was even more pronounced (E_{max} 40%). At this receptor subtype a minor reduction of the maximal effect of GABA (E_{max} 70%) was seen for the 3-isoxazolol analogue **6e**.

It is very unexpected that compound **6e**, **7e**, and **7h** in addition to a competitive component possess a noncompetitive component. Since these compounds are highly lipophilic one might expect that part of the noncompetitive action could be due to slow on-rate either to the orthosteric (recognition) site or to a site located within the chloride channel. However, since experiments with variation in pretreatment time or repeated administration showed no indication of use dependence or slow on-rate, these explanations may be rejected. The insurmountable inhibition of GABA responses may therefore be the consequence of interaction with a site, an amino acid residue, located outside the ion channel and probably very close to the orthosteric binding site. In agreement with this interpretation are binding data, which all point toward a purely competitive interaction. If the secondary binding site is located very close to the orthosteric binding site, and since the potency at this site is within the picomolar range, it might be possible to selectively label it at very low concentrations of either radioactively labeled compounds or photoaffinity probes based on the structures of **7e** and **7h**.

Conclusion

In conclusion, we have synthesized and pharmacologically characterized a series of analogues of the partial $GABA_A$ agonist thio-4-PIOL (**7a**) with substituents, such as alkyl and more bulky aromatic groups, in the 4-position of the 3-isothiazolol ring. The 3-isothiazolol analogues exhibit similar SARs as shown for the corresponding 3-isoxazolol analogues but show 5−15-fold higher affinity and potency at the $GABA_A$ receptor. The 2-naphthylmethyl, the 1-bromo-2-naphthylmethyl, and the 3,3-diphenylpropyl analogues, compounds **7e**, **7f**, and **7h**, respectively, show affinity in the low-nanomolar range (K_i 2−10 nM). In addition, the 3-isothiazolol analogue **7e** exhibit 3-fold selectivity for the extrasynaptic $\alpha_4\beta_3\delta$ receptor subtype over the synaptic $\alpha_1\beta_3\gamma_{2S}$ receptor subtype, whereas a 10-fold selectivity was shown for the corresponding 3-isoxazolol compound **6e**.

Like the 3-isoxazolol analogues most of the 3-isothiazolol analogues were shown to be competitive antagonists. However, the antagonist effects of **7e** and **7h** were shown to consist of a competitive and a noncompetitive component operating in different concentration ranges. In the picomolar range, a noncompetitive saturable component prevails reducing the maximal response of GABA, whereas at concentrations in the nanomolar range, a competitive component becomes significant. These results indicate an additional high-affinity binding site in or near the orthosteric site and will be subject of further studies.

Experimental Section

Chemistry. General Procedures. All reactions involving air-sensitive reagents were performed under a N_2 atmosphere using the syringe−septum technique, and glassware was flame-dried in vacuo prior to use. Compounds were visualized on TLC (silica gel 60 F_{254} plates; Merck) using UV light and $KMnO_4$ spraying reagent. 2,4-Dinitrophenylhydrazin (DNP) was used to visualize aldehydes and ninhydrin to visualize secondary and primary amines. Compounds containing the 3-isothiazolol moiety were visualized using $FeCl_3$ spraying reagent. Column chromatography (CC) was performed on Millipore A/S silica gel 60A (70−200 μm) and dry column vacuum chromatography (DCVC) was performed on silica gel 60A (35−70 μm) from Millipore A/S. All solvents and reagents were purchased from commercial sources and used without further purification except for DME, DMF, and THF, which were stored over 4 Å molecular sieves. Melting points were determined in open capillary tubes and are uncorrected. NMR spectra were recorded on a 300 MHz Varian Gemini spectrometer in $CDCl_3$ solutions using TMS as an internal standard or in CD_3OD solutions. Elemental analyses were carried out at the Analytical Research Department, H. Lundbeck A/S, Denmark or by J. Theiner, Microanalytical Laboratory, Institute of Physical Chemistry, University of Vienna, Austria and are within ±0.4% of the calculated values unless otherwise stated.

(*E*)-3-(1-Benzyl-4-piperidyl)-2-propenamide (9). NaH (60%, 2.72 g, 67.9 mmol) was added slowly to a suspension of diethylphosphonoacetamide (13.25 g, 67.9 mmol) in DME (150 mL) followed by dropwise addition of a solution of compound **8**[18] (5.52 g, 27.2 mmol) in DME (6 mL). The reaction mixture was left for 90 min at room temperature and then quenched with H_2O (60 mL). The pH was adjusted to 2, and the acidic phase was washed with Et_2O (3 × 100 mL). The pH was adjusted to 10 precipitating the product. The alkaline H_2O phase was filtered and extracted with Et_2O (3 × 100 mL). The combined organic phases were dried and evaporated in vacuo and combined with the filtered product. Recrystallization (EtOAc) afforded **9** as colorless crystals (4.9 g, 70%): mp 153.5−154 °C. ^{1}H NMR ($CDCl_3$): δ 7.37−7.22 (5H, m), 6.83 (1H, dd, J = 15.6 Hz, J = 6.8 Hz), 5.80 (1H, dd, J = 15.6 Hz, J = 1.2 Hz), 5.49 (2H, bs), 3.52 (2H, s), 2.97−2.87 (2H, m), 2.23−1.80 (3H, m), 1.79−1.67 (2H, m), 1.60−1.43 (2H, m). ^{13}C NMR ($CDCl_3$): δ 168.09, 150.11, 138.05, 129.31, 128.29, 127.16, 120.89, 63.25, 53.09, 38.29, 30.82. Anal. ($C_{15}H_{20}N_2O$) C, H, N.

(*E*)-3-(1-Methoxycarbonyl-4-piperidyl)-2-propenamide (10). Methyl chloroformate (3 mL, 38.7 mmol) was added quickly through a condenser to compound **9** (5 g, 19.4 mmol). The reaction was left for 3 h, evaporated in vacuo, and added to H_2O (75 mL). The aqueous phase was extracted with EtOAc (10 × 100 mL). The combined organic phases were dried and evaporated giving a white solid. Recrystallization (EtOAc) afforded **10** (2.91 g, 71%) as colorless crystals: mp 149−151.5 °C. ^{1}H NMR ($CDCl_3$): δ 6.82 (1H, dd, J = 15.6 Hz, J = 6.6 Hz), 5.83 (1H, dd, J = 15.6 Hz, J = 1.5 Hz), 5.69 (1H, bs), 4.16 (2H, bs), 3.70 (3H, s), 2.92−2.77 (2H, m), 2.40−2.13 (2H, m), 1.82−1.70 (2H, m), 1.47−1.25 (2H, m). ^{13}C NMR ($CDCl_3$): δ 167.87, 156.00, 149.31, 121.13, 52.56, 43.48, 38.23, 30.53. Anal. ($C_{10}H_{16}N_2O_3$) C, H, N.

3-Acetylsulfanyl-3-(1-methoxycarbonyl-4-piperidyl)propanamide (11). To a suspension of compound **10** (1.1 g, 5.18 mmol) was added thioacetic acid (410 μL, 5.70 mmol). The reaction was left for 3 days at room temperature followed by evaporation in vacuo, and recrystallization (EtOAc) gave **11** (1.15 g, 77%) as colorless crystals: mp 125−126 °C. ^{1}H NMR ($CDCl_3$): δ 6.08−5.88 (2H, m), 4.16 (2H, bs), 3.89−3.76 (1H, m), 3.68 (3H, s), 2.80−2.43 (4H, m), 2.35 (3H, s), 1.96−1.63 (3H, m) 1.40−1.09 (2H, m). ^{13}C NMR ($CDCl_3$): δ 195.60, 172.76, 155.85, 52.45, 45.43, 43.77, 43.66, 39.29, 38.80, 30.64, 29.39, 28.33. Anal. ($C_{12}H_{20}N_2O_4S$) C, H, N.

5-(1-Methoxycarbonyl-4-piperidyl)isothiazol-3-ol (12). A solution of NaOH (1.1 g, 27.7 mmol) in H_2O (10 mL) was added to compound **11** (4.0 g, 13.9 mmol), and the reaction was stirred for 4 h at room temperature. The pH was adjusted to 5 with 2 M H_2SO_4, and the acidic mixture was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic phases were dried and evaporated in vacuo affording a pale yellow solid. The solid was suspended in H_2O (20 mL), heated to 45 °C, and H_2O_2 (35%, 660 μL, 6.8 mmol) was added dropwise. The reaction was left for 24 h at 45 °C followed by evaporation in vacuo giving a white foam, which was dissolved in $ClCH_2CH_2Cl$ (18 mL). SO_2Cl_2 (0.56 mL, 6.89 mmol) was added slowly to the solution, and the reaction was left for 24 h at room temperature before adding additional SO_2Cl_2 (0.27 mL, 3.35 mmol). Stirring was continued for 8 h after which additional SO_2Cl_2 (0.27 mL, 3.35 mmol) was added. The reaction was left for 16 h followed by evaporation in vacuo. The residue was added H_2O (50 mL) and extracted with EtOAc (5 × 50 mL). The combined organic phases were dried and evaporated. Recrystallization (EtOAc) afforded **12**[8] (1.41 g, 42%) as light brown crystals. ^{1}H NMR ($CDCl_3$): δ 6.33 (1H, s), 4.23 (2H, bs), 3.72 (3H, s), 3.07−2.83 (3H, m), 2.00 (2H, bd, J = 12.3 Hz), 1.67 (2H, dq, J = 12.3 Hz, J = 4.5 Hz).

3-Isopropoxy-5-(1-methoxycarbonyl-4-piperidyl)isothiazole (13). To a solution of **12** (1.12 g, 4.62 mmol) in dry DMF (30 mL) was added K_2CO_3 (703 mg, 5.08 mmol), and the mixture was stirred at 60 °C for 30 min. Isopropyl bromide (650 μL, 6.93 mmol) was added to the mixture, and stirring was continued at 60 °C for 20 h. The reaction mixture was added to H_2O (30 mL) and extracted with petroleum ether (80−100 °C) (4 × 15 mL). The combined organic phases were dried and evaporated in vacuo. CC [toluene−EtOAc (2:1)] gave **13** (731 mg, 56%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$): δ 6.31 (1H, s), 5.21−5.06 (1H, m), 4.21 (2H, bs), 3.71 (3H, s), 3.03−2.82 (3H, m), 1.98 (2H, bd, J = 12.6 Hz), 1.71−1.51 (2H, m), 1.40−1.30 (6H, m). ^{13}C NMR ($CDCl_3$): δ 172.61, 168.43, 155.89, 109.15, 70.94, 52.55, 43.62, 36.26, 32.77, 21.92. Anal. ($C_{13}H_{20}N_2O_3S$) C, H, N, S.

4-Iodo-3-isopropoxy-5-(1-methoxycarbonyl-4-piperidyl)isothiazole (14). To a solution of compound **13** (840 mg, 2.95 mmol) in HOAc (5 mL), a solution of ICl (0.22 mL, 4.43 mmol) in HOAc (7 mL) was added dropwise followed by addition of H_2O (20 mL). The reaction was stirred at 80 °C for 18 h. The reaction mixture was added to $Na_2S_2O_4$ (s) and evaporated. The residue was dissolved in H_2O (40 mL) and extracted with Et_2O (3 × 40 mL). The combined organic phases were washed with 2% aq $Na_2S_2O_4$ (3 × 50 mL), dried, and evaporated in vacuo. CC [toluene−EtOAc (4:1)] gave **14** (987 mg, 82%) as a light brown oil. ^{1}H NMR ($CDCl_3$): δ 5.15 (1H, hep, J = 6.3 Hz), 4.28 (2H, bs), 3.72 (3H, s), 3.08 (1H, dt, J = 12 Hz, J = 3.6 Hz), 2.90 (2H, bt, J = 12.6 Hz), 2.03 (2H, bd, J = 12.6 Hz), 1.66−1.48 (2H, m), 1.40 (6H, d, J = 6.3 Hz). ^{13}C NMR ($CDCl_3$): δ 170.20, 166.86, 159.31, 155.88, 72.51, 67.31, 52.63, 43.81, 39.33, 31.54, 21.87. Anal. ($C_{13}H_{19}IN_2O_3S$) C, H, I, N, S.

4-Hydroxymethyl-3-Isopropoxy-5-(1-methoxycarbonyl-4-piperidyl)isothiazole (15b). To a solution of **14** (1.02 g, 2.49 mmol) in dry THF cooled to −30 °C was added EtMgBr (1 M, 2.49 mmol) dropwise at −30 °C. The reaction was left at 0 °C for 2.5 h after which a solution of dry DMF (0.38 mL, 4.99 mmol) in dry THF was added dropwise at 0 °C. Stirring was continued at room temperature overnight. The reaction was quenched with aq saturated NH_4Cl (20 mL) and extracted with EtOAc (3 × 20 mL). The combined organic phases were dried, evaporated in vacuo, and purified by DCVC [petroleum ether → EtOAc] affording a yellow oil (668 mg). The yellow oil was dissolved in MeOH (25 mL), cooled to 0 °C, and added to $NaBH_4$ (0.12 g, 3.21 mmol) in small portions. Stirring was continued 20 min at 0 °C and 2.5 h at room temperature followed by evaporation in vacuo. The residue was added to H_2O (30 mL) and extracted with EtOAc (3 × 30 mL). The combined EtOAc phases were dried and evaporated in vacuo. DCVC [petroleum ether → EtOAc] gave **15b** as a colorless oil (551 mg, 70%). ^{1}H NMR ($CDCl_3$): δ 5.15 (1H, hep, J = 6.3 Hz), 4.48 (1H, s), 4.23 (2H, bs), 3.69 (3H, s), 3.19 (1H, tt, J = 11.9, J = 3.6 Hz), 3.20−2.69 (3H, m), 1.97 (2H, bd, J = 12.3 Hz), 1.58 (2H, dq, J = 12.5 Hz, J = 4.1 Hz), 1.36 (6H, d, J = 6.3 Hz). ^{13}C NMR ($CDCl_3$): δ 169.45, 166.18, 155.36, 120.57, 71.12, 54.55, 52.43, 43.74, 35.69, 32.87, 21.95. Anal. ($C_{14}H_{22}N_2O_4S$) C, H, N, S.

4-(1-Hydroxypropyl)-3-isopropoxy-5-(1-methoxycarbonyl-4-piperidyl)isothiazole (15c). To a solution of **14** (750 mg, 1.83 mmol) in dry THF (6 mL), cooled to −30 °C, was added EtMgBr (1 M, 1.83 mmol) dropwise at −30 °C. The reaction was left at 0 °C for 3 h after which a solution of propionaldehyde (0.13 mL, 1.83 mmol) in dry THF (0.75 mL) was added dropwise at 0 °C. Stirring was continued at room temperature overnight. The reaction was quenched with saturated NH_4Cl (15 mL) and extracted with EtOAc (3 × 15 mL). The combined organic phases were dried and evaporated in vacuo. DCVC [petroleum ether → EtOAc] gave **15c** (464 mg, 74%) as a light yellow oil. ^{1}H NMR ($CDCl_3$): δ 5.19 (1H, hep, J = 6.2), 4.57 (1H, bt, J = 6.9 Hz), 4.24 (2H, bs), 3.70 (3H, s), 3.23 (1H, tt, J = 12.1 Hz, J = 3.6 Hz), 3.09 (1H, bs), 2.95−2.74 (2H, m), 2.20−1.83 (3H, m), 1.80−1.66 (1H, m), 1.65−1.47 (2H, m), 1.37 (6H, d, J = 6.6 Hz), 0.93 (3H, t, J = 7.5 Hz). ^{13}C NMR ($CDCl_3$): δ 167.43, 165.32, 155.37, 123.00, 71.29, 69.12, 52.45, 43.88, 35.69, 33.43, 30.13, 22.04, 10.44. Anal. ($C_{16}H_{26}N_2O_4S$) C, H, N, S.

3-Isopropoxy-5-(1-methoxycarbonyl-4-piperidyl)-4-(phenylhydroxymethyl)isothiazole (15d) was synthesized as described for **15c** using **14** (400 mg, 0.97 mmol) in dry THF (4 mL), EtMgBr (1 M, 0.97 mmol), and benzaldehyde (99 μL, 0.97 mmol). The crude product was purified by DCVC [toluene → toluene−EtOAc (1:1)] affording **15d** (214 mg, 56%) as a white foam. ^{1}H NMR ($CDCl_3$): δ 7.36−7.21 (5H, m), 5.88 (1H, s), 5.16 (1H, hep, J = 6.2 Hz), 4.15 (2H, bs), 3.67 (3H, s), 3.15 (1H, tt, J = 12 Hz, J = 3.6 Hz), 2.86−2.58 (2H, m), 1.88 (1H, bd, J = 12.9 Hz), 1.67 (1H, bd, J = 12.9 Hz), 1.59−1.36 (2H, m), 1.33 (3H, d, J = 6.2 Hz), 1.22 (3H, d, J = 6.2 Hz). ^{13}C NMR ($CDCl_3$): δ 169.11, 165.81, 155.78, 142.75, 128.19, 127.26, 125.67, 122.88, 71.64, 68.28, 52.48, 43.81, 35.69, 33.31, 32.85, 21.92, 21.71.

3-Isopropoxy-5-(1-methoxycarbonyl-4-piperidyl)-4-(hydroxy-(2-naphthyl)methyl)isothiazole (15e) was synthesized as described for **15c** using **14** (300 mg, 0.73 mmol) in dry THF (3 mL), EtMgBr (1 M, 0.73 mmol), and 2-formylnaphthalene (114 mg, 0.73 mmol). The crude product was purified by CC [toluene−EtOAc (4:1)] affording **15e** (208 mg, 65%) as a white solid: mp 131−135 °C. ^{1}H NMR: δ 7.87−7.75 (4H, m), 7.52−7.40 (3H, m), 6.02 (1H, d, J = 7.5 Hz), 5.20 (1H, hep, J = 6.3 Hz), 4.15 (2H, bs), 3.68 (3H, s), 3.27 (1H, bd, J = 7.5 Hz), 3.22−3.08 (1H, m), 2.82−2.57 (2H, m), 1.95−1.82 (1H, m), 1.80−1.70 (1H, m), 1.63−1.45 (2H, m), 1.34 (3H, d, J = 6.3 Hz), 1.21 (3H, d, J = 6.3 Hz). ^{13}C NMR

($CDCl_3$): δ 169.29, 166.01, 155.84, 139.93, 133.15, 132.79, 128.23, 128.04, 127.71, 126.38, 126.12, 124.43, 124.31, 122.34, 71.91, 68.84, 52.61, 43.89, 35.92, 33.40, 22.08, 21.87. Anal. ($C_{24}H_{28}N_2O_4S$) C, H, N.

4-((1-Bromo-2-naphthyl)hydroxymethyl)-3-isopropoxy-5-(1-methoxycarbonyl-4-piperidyl)isothiazole (15f) was synthesized as described for **15c** by using **14** (300 mg, 0.73 mmol) in dry THF (3 mL), EtMgBr (1 M, 0.73 mmol), and 1-bromo-2-formylnaphthalene (172 mg, 0.73 mmol). The crude product was purified by DCVC [toluene → toluene−EtOAc (1:1)] affording **15f** (248 mg, 65%) as a white foam. ^{1}H NMR ($CDCl_3$): δ 8.30 (1H, d, J = 8.4 Hz), 7.84−7.76 (3H, m), 7.62−7.47 (2H, m), 6.37 (1H, bd, J = 4.2 Hz), 5.18 (1H, hep, J = 6.2 Hz), 4.06 (2H, bs), 3.83 (1H, bs), 3.63 (3H, s), 3.06−2.93 (1H, m), 2.72−2.53 (1H, m), 2.52−2.37 (1H, m), 1.78 (1H bd, J = 12.6 Hz), 1.58 (1H, bd, J = 12.6 Hz), 1.48−1.34 (2H, m), 1.31 (3H, d, J = 6.2 Hz), 1.27 (3H, d, J = 6.2 Hz). ^{13}C NMR ($CDCl_3$): δ 169.75, 165.95, 155.74, 139.33, 133.96, 132.09, 128.04, 127.60, 127.40, 127.28, 126.78, 125.42, 122.52, 121.22, 71.72, 69.21, 52.46, 43.82, 43.70, 35.85, 33.00, 21.91, 21.85.

4-(2,2-Diphenyl-1-hydroxethyl)-3-isopropoxy-5-(1-methoxycarbonyl-4-piperidyl)isothiazole (15g) was synthesized as described for **15c** by using **14** (480 mg, 1.17 mmol) in dry THF (4 mL), EtMgBr (1 M, 1.17 mmol), and 2,2-diphenylethanal (210 μL, 1.17 mmol). The crude product was purified by CC [toluene−EtOAc (4:1)] affording **15g** (254 mg, 45%) as a white foam: mp 66−70 °C. ^{1}H NMR ($CDCl_3$): δ 7.49−7.44 (2H, m), 7.42−7.35 (2H, m), 7.31−7.24 (1H, m), 7.17−7.05 (3H, m), 7.03−6.80 (2H, m), 5.38−5.21 (1H, m), 4.52 (1H, d, J = 10.2 Hz), 4.32−3.90 (2H, m), 3.68 (3H, s), 3.04 (1H, d, J = 9.9 Hz), 2.74 (1H, bt, J = 12.9 Hz), 2.60−2.44 (2H, m), 1.83 (1H, bd, J = 13.1 Hz), 1.50 (3H, d, J = 6 Hz), 1.47−1.31 (4H, m), 1.11 (1H, dq, J = 12.8 Hz, J = 4.4 Hz), 0.71 (1H, bd, J = 13.1 Hz). ^{13}C NMR ($CDCl_3$): δ 168.83, 165.46, 155.84, 141.85, 141.03, 129.07, 128.66, 128.60, 128.41, 126.87, 126.74, 120.97, 71.92, 70.28, 57.34, 52.59, 43.95, 43.90, 35.87, 33.86, 32.72, 22.25, 22.18. Anal. ($C_{27}H_{32}N_2O_4S\cdot^1/_4H_2O$) C, H, N.

4-(3,3-Diphenyl-1-hydroxypropyl)-3-isopropoxy-5-(1-methoxycarbonyl-4-piperidyl)isothiazole (15h) was synthesized as described for **15c** by using **14** (400 mg, 0.97 mmol) in dry THF (4 mL), EtMgBr (1 M, 0.97 mmol), and 3,3-diphenylpropanal (205 mg, 0.97 mmol). The crude product was purified by DCVC [toluene → toluene−EtOAc (1:1)] affording **15h** (230 mg, 48%) as a white foam. ^{1}H NMR ($CDCl_3$): δ 7.34−7.14 (10H, m), 5.23 (1H, hep, J = 6.3 Hz), 4.52−4.42 (1H, m), 4.24−3.96 (3H, m), 3.67 (3H, s), 2.81 (1H, bs), 2.73−2.60 (1H, m), 2.59−2.30 (4H, m), 1.73 (1H, bd, J = 13.2 Hz), 1.52 (1H, bd, J = 13.2), 1.47−1.28 (8H, m). ^{13}C NMR ($CDCl_3$): δ 167.70, 165.93, 155.76, 144.45, 144.01, 129.04, 128.62, 128.23, 128.04, 127.83, 126.47, 126.42, 125.29, 122.80, 71.78, 65.43, 52.54, 52.50, 47.40, 43.71, 43.58, 42.88, 35.39, 32.90, 32.50, 22.09, 22.06.

3-Isopropoxy-5-(1-methoxycarbonyl-4-piperidyl)-4-methylisothiazole (16b). To a solution of **15b** (447 mg, 1.48 mmol) in dry CH_2Cl_2 (5 mL) was added triethylsilane (380 μL, 2.37 mmol), and the solution was cooled to 0 °C. TFA (2.8 mL) was added dropwise at 0 °C, and the reaction was allowed to warm to room temperature followed by heating to 50 °C. Stirring was continued at 50 °C for 5 h. The reaction was quenched with H_2O (7 mL) and extracted with CH_2Cl_2 (3 × 7 mL). The combined CH_2Cl_2 phases were dried and evaporated in vacuo. The residue was purified by DCVC [petroleum ether → EtOAc] affording **16b** as a colorless oil (258 mg; 58%). ^{1}H NMR ($CDCl_3$): δ 5.15 (1H, hep, J = 6.3 Hz), 4.26 (2H, bs), 3.71 (3H, s), 3.00 (1H, tt, J = 12 Hz, J = 3.8 Hz), 2.88 (2H, bt, J = 12 Hz), 1.98 (3H, s), 1.97−1.86 (2H, m), 1.58 (2H, dq, J = 12.6 Hz, J = 4.4 Hz), 1.36 (6H, d, J = 6.3 Hz). ^{13}C NMR ($CDCl_3$): δ 167.43, 165.25, 156.03, 117.69, 71.24, 53.07, 44.51, 36.55, 32.72, 22.70, 10.48.

3-Isopropoxy-5-(1-methoxycarbonyl-4-piperidyl)-4-propylisothiazole (16c) was synthesized as described for **16b** by using **15c** (446 mg, 1.30 mmol), triethylsilane (330 μL, 2.08 mmol), TFA (2.5 mL), and 2 h of reaction time at 50 °C. The crude product was purified by DCVC [petroleum ether → EtOAc] affording **16c** as a yellow oil (370 mg, 87%). ^{1}H NMR ($CDCl_3$): δ 5.15 (1H, hep, J = 6.1 Hz), 4.26 (2H, bs), 3.72 (3H, s), 3.00 (1H, tt, J = 12.1 Hz, J = 3.7 Hz), 2.94−2.74 (2H, m), 2.43−2.33 (2H, m), 2.04−1.82 (2H, m), 1.68−1.44 (4H, m), 1.35 (6H, d, J = 6.3 Hz), 0.93 (3H, t, J = 7.4 Hz). ^{13}C NMR ($CDCl_3$): δ 166.73, 165.11, 155.49, 121.76, 70.60, 52.52, 43.95, 35.81, 33.08, 26.85, 22.43, 22.01, 13.87.

4-Benzyl-3-isopropoxy-5-(1-methoxycarbonyl-4-piperidyl)-isothiazole (16d) was synthesized as described for **16b** by using **15d** (214 mg, 0.55 mmol), triethylsilane (140 μL, 0.88 mmol), TFA (1 mL), and 2 h of reaction time at 50 °C. The crude product was purified by DCVC [toluene→ toluene−EtOAc (1:1)] affording **16d** (179 mg, 87%) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 7.29−7.11 (5H, m), 5.16 (1H, hep, J = 6.1 Hz), 4.18 (2H, bs), 3.80 (2H, s), 3.69 (3H, s), 3.00 (1H, dt, J = 12.4 Hz, J = 3.9 Hz), 2.77 (2H, bt, J = 12.4 Hz), 1.76 (2H, bd, J = 12.4), 1.51 (2H, dq, J = 12.4 Hz, J = 3.9 Hz), 1.31 (6H, d, J = 6.1 Hz). ^{13}C NMR ($CDCl_3$): δ 67.08, 67.05, 155.82, 139.73, 128.35, 128.19, 126.13, 120.48, 71.00, 60.26, 52.51, 43.88, 35.99, 32.74, 30.42, 21.91.

3-Isopropoxy-5-(1-methoxycarbonyl-4-piperidyl)-4-(2-naphthylmethyl)isothiazole (16e) was synthesized as described for **16b** by using **15e** (197 mg, 0.45 mmol), triethylsilane (110 μL, 0.72 mmol), TFA (0.8 mL), and 2 h of reaction time at 50 °C. Crude **16e** (173 mg, 94%) was a yellow oil and pure according to NMR. ^{1}H NMR ($CDCl_3$): δ 7.82−7.69 (3H, m), 7.54 (1H, s), 7.48−7.37 (2H, m), 7.30 (1H, dd, J = 8.6, J = 1.7 Hz), 5.19 (1H, hep, J = 6.2), 4.16 (2H, bs), 3.96 (2H, s), 3.67 (3H, s), 3.10−2.98 (1H, m), 2.74 (2H, bt, J = 12.5 Hz), 1.77 (2H, bd, J = 12 Hz), 1.60−1.38 (2H, m), 1.32 (6H, d, J = 6.2 Hz). ^{13}C NMR ($CDCl_3$): δ 167.35, 167.12, 155.84, 137.15, 133.44, 132.05, 128.04, 127.61, 127.40, 126.90, 126.23, 126.09, 125.41, 120.24, 71.10, 52.54, 43.85, 36.02, 32.72, 30.57, 21.95.

4-((1-Bromo-2-naphthyl)methyl)-3-isopropoxy-5-(1-methoxycarbonyl-4-piperidyl)isothiazole (16f) was synthesized as described for **16b** by using **15f** (248 mg, 0.48 mmol), triethylsilane (120 μL, 0.76 mmol), TFA (0.9 mL), and 2 h of reaction time at 50 °C. The crude product was purified by DCVC [toluene → toluene−EtOAc (2:1)] affording **16f** (204 mg, 85%) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 8.33 (1H, d, J = 8.4 Hz), 7.78 (1H, d, J = 8.1 Hz), 7.67 (1H, d, J = 8.7 Hz), 7.59 (1H, t, J = 7.7 Hz), 7.49 (1H, d, J = 7.5 Hz), 7.12 (1H, d, J = 8.4 Hz), 5.20 (1H, hep, J = 6 Hz), 4.18 (2H, s), 4.12 (2H, bs), 3.66 (3H, s), 3.03 (1H, dt, J = 11.9 Hz, J = 3.6 Hz), 2.70 (2H, bt, J = 12.3 Hz), 1.71 (2H, bd, J = 12.9 Hz), 1.57−1.38 (2H, m), 1.31 (6H, d, J = 6 Hz). ^{13}C NMR ($CDCl_3$): δ 168.31, 167.10, 155.78, 137.05, 133.27, 132.32, 128.05, 127.59, 127.53, 127.29, 127.08, 126.17, 123.54, 119.50, 71.14, 52.46, 43.80, 35.97, 32.63, 31.52, 21.92.

4-(2,2-Diphenyl-1-ethyl)-3-isopropoxy-5-(1-methoxycarbonyl-4-piperidyl)isothiazole (16g) was synthesized as described for **16b** by using **15g** (269 mg, 0.61 mmol), triethylsilane (160 μL, 1.0 mmol), TFA (1.1 mL), and 2 h of reaction time at 50 °C. Crude **16g** (221 mg, 78%) was a yellow oil and pure according to NMR. ^{1}H NMR ($CDCl_3$): δ 7.37−7.10 (10H, m), 5.23 (1H, hep, J = 6.2 Hz), 4.24 (1H, t, J = 8.0 Hz), 4.08 (2H, bs), 3.67 (3H, s), 3.12 (2H, d, J = 8.0 Hz), 2.64−2.49 (2H, m), 2.37−2.24 (1H, m), 1.39 (6H, d, J = 6.2 Hz), 1.30−1.15 (4H, m). ^{13}C NMR ($CDCl_3$): δ 167.33, 166.86, 155.88, 144.42, 128.37, 128.22, 126.38, 119.35, 70.95, 52.55, 49.82, 43.98, 35.76, 33.05, 32.11, 22.09.

4-(3,3-Diphenyl-1-propyl)-3-isopropoxy-5-(1-methoxycarbonyl-4-piperidyl)isothiazole (16h) was synthesized as described for **16b** by using **15h** (210 mg, 0.42 mmol), triethylsilane (110 μL, mmol), TFA (0.8 mL), and 2 h of reaction time at 50 °C. The crude product was purified by DCVC [toluene → toluene−EtOAc (1:1)] affording **16h** (184 mg, 91%) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 7.32−7.12 (10H, m), 5.17 (1H, hep, J = 6.2 Hz), 4.13 (2H, bs), 3.88 (1H, bt, J = 7.5 Hz), 3.69 (3H, s), 2.72−2.20 (7H, m), 1.70 (2H, bd, J = 12.6), 1.54−1.30 (8H, m). ^{13}C NMR ($CDCl_3$): δ 167.15, 165.82, 155.78, 144.59, 128.47, 127.82, 126.24, 121.33, 70.80, 52.51, 50.48, 43.75, 35.51, 34.36, 32.61, 23.19, 22.03.

4-Methyl-5-(4-piperidyl)isothiazol-3-ol Hydrobromide (7b). Compound **16b** (258 mg, 0.86 mmol) was added to 33% HBr in HOAc (16 mL) and stirred for 72 h at 65 °C. The reaction mixture was evaporated followed by coevaporation from MeOH−toluene (1:1) three times. The residue was recrystallized from EtOH affording **7b** (78 mg, 32%) as light gray crystals; mp >200 °C. ^{1}H NMR (CD_3OD): δ 3.55−3.45 (2H, m), 3.44−3.31 (1H, m), 3.25−3.12 (2H, m), 2.26−2.14 (2H, m), 2.03 (3H, s), 1.91−1.74 (2H, m). ^{13}C NMR (CD_3OD): δ 170.21, 163.16, 118.96, 45.09, 34.88, 30.19, 10.27. Anal. ($C_9H_{15}BrN_2OS$) C, H, Br, N, S.

5-(4-Piperidyl)-4-propylisothiazol-3-ol Hydrobromide (7c) was synthesized as described for **7b** by using **16c** (370 mg, 1.13 mmol) and 33% HBr in HOAc (21 mL). The crude product was purified by recrystallization (EtOH) affording **7c** (203 mg, 58%) as light yellow crystals: mp >200 °C. ^{1}H NMR (CD_3OD): δ 3.54−3.45 (2H, m), 3.44−3.32 (1H, m), 3.19 (2H, dt, J = 13.1 Hz, J = 3 Hz), 2.53−2.41 (2H, m), 2.25−2.10 (2H, m), 1.94−1.74 (2H, m), 1.57 (2H, hex, J = 7.5 Hz), 0.96 (3H, t, J = 7.4 Hz). ^{13}C NMR (CD_3OD): δ 170.03, 163.82, 123.53, 45.10, 34.80, 31.21, 28.10, 23.56, 14.30. Anal. ($C_{11}H_{19}BrN_2OS$) C, H, Br, N, S.

4-Benzyl-5-(4-piperidyl)isothiazol-3-ol Hydrobromide (7d) was synthesized as described for **7b** by using **16d** (179 mg, 0.48 mmol) and 33% HBr in HOAc (10 mL). The crude product was purified by recrystallization (EtOH) affording **7d** (93 mg, 55%) as light gray crystals: mp >200 °C. ^{1}H NMR (CD_3OD): δ 7.29−7.09 (5H, m), 3.88 (2H, s), 3.45−3.33 (3H, m), 3.08 (2H, dt, J = 13.1 Hz, J = 3.0 Hz), 1.96 (2H, bd, J = 13.4 Hz), 1.74 (2H, dq, J = 13.4, J = 3.9). ^{13}C NMR (CD_3OD): δ 170.32, 165.76, 141.33, 129.71, 129.42, 127.46, 122.63, 45.01, 35.00, 31.29, 30.77. Anal. ($C_{15}H_{19}BrN_2OS$) C, H, Br, N, S.

4-(2-Naphthylmethyl)-5-(4-piperidyl)isothiazol-3-ol Hydrobromide (7e) was synthesized as described for **7b** by using **16e** (173 mg, 0.42 mmol) and 33% HBr in HOAc (10 mL). The crude product was purified by recrystallization (EtOH) affording **7e** (44 mg, 26%) as light brown crystals: mp >200 °C. ^{1}H NMR (CD_3OD): δ 7.82−7.73 (3H, m), 7.65 (1H, s), 7.47−7.35 (3H, m), 4.05 (2H, s), 3.50−3.32 (3H, m), 3.05 (2H, dt, J = 13.1 Hz, J = 3.0 Hz), 1.95 (2H, bd, J = 14.1 Hz), 1.83−1.65 (2H, m). ^{13}C NMR (CD_3OD): δ 170.39, 166.06, 138.83, 135.16, 133.78, 129.40, 128.77, 128.62, 128.08, 127.33, 126.69, 122.40, 44.94, 35.03, 31.47, 30.74. Anal. ($C_{19}H_{21}BrN_2OS\cdot^2/_5H_2O$), Calcd: Br, 19.37. Found: Br, 20.20.

4-((1-Bromo-2-naphthyl)methyl)-5-(4-piperidyl)isothiazol-3-ol Hydrobromide (7f) was synthesized as described for **7b** by using **16f** (204 mg, 0.41 mmol) and 33% HBr in HOAc (10 mL). The crude product was purified by recrystallization (EtOH) affording **7f** (109 mg, 56%) as light brown crystals: mp > 200 °C. ^{1}H NMR (CD_3OD): δ 8.32 (1H, d, J = 8.6 Hz), 7.85 (1H, d, J = 8.1 Hz), 7.79 (1H, d, J = 8.4 Hz), 7.67−7.59 (1H, m), 7.57−7.49 (1H, m), 7.22 (1H, d, J = 8.6 Hz), 4.25 (2H, s), 3.42−3.33 (3H, m), 2.97 (2H, dt, J = 13.1 Hz, J = 2.9 Hz), 1.96 (2H, bd, J = 13.8 Hz), 1.84−1.66 (2H, m). ^{13}C NMR (CD_3OD): δ 170.24, 166.79, 138.42, 135.04, 133.81, 129.47, 129.22, 128.97, 128.23, 128.18, 127.65, 124.38, 121.39, 45.02, 35.22, 32.80, 30.65. Anal. ($C_{19}H_{20}Br_2N_2OS\cdot1.5H_2O$) C, H, Br, N, S.

4-(2,2-Diphenyl-1-ethyl)-5-(4-piperidyl)isothiazol-3-ol Hydrobromide (7g) was synthesized as described for **7b** by using **16g** (221 mg, 0.48 mmol) and 33% HBr in HOAc (11 mL). The crude product was purified by recrystallization (EtOH) affording **7g** (56 mg, 26%) as light gray crystals: mp >210 °C. ^{1}H NMR (CD_3OD): δ 7.31−7.22 (8H, m), 7.21−7.13 (2H, m), 4.45 (1H, bs), 4.30 (1H, t, J = 7.8 Hz), 3.29−3.18 (4H, m), 2.89 (2H, bdt, J = 12.9 Hz, J = 3 Hz), 2.76 (1H, btt, J = 11.9, J = 3.8 Hz), 1.56−1.26 (4H, m). ^{13}C NMR (CD_3OD): δ 170.27, 165.37, 146.18, 129.57, 129.52, 127.55, 121.52, 50.98, 45.15, 34.92, 33.07, 31.15. Anal. ($C_{22}H_{25}BrN_2OS\cdot^2/_5H_2O$) C, H, Br, N, S.

4-(3,3-Diphenyl-1-propyl)-5-(4-piperidyl)isothiazol-3-ol Hydrobromide (7h) was synthesized as described for **7b** by using **16h** (184 mg, 0.38 mmol) and 33% HBr in HOAc (10 mL). The crude product was purified by recrystallization (EtOH) affording **7h** (94 mg, 53%) as colorless crystals: mp >200 °C. ^{1}H NMR (CD_3OD): δ 7.29 (8H, d, J = 4.2 Hz), 7.22−7.14 (2H, m), 3.91 (1H, t, J = 7.7 Hz), 3.40 (2H, bd, J = 12.9 Hz), 2.95−2.72 (3H, m), 2.52−2.41 (2H, m), 2.39−2.26 (2H, m), 1.97 (2H, bd, J = 13.6 Hz), 1.71 (2H, dq, J = 13.6 Hz, J = 3.5 Hz). ^{13}C NMR (CD_3OD): δ 170.43, 164.35, 146.39, 129.71, 129.19, 127.44, 123.21, 52.09, 45.11, 35.25, 34.72, 30.58, 24.61. Anal. ($C_{23}H_{27}BrN_2OS\cdot^1/_4H_2O$) C, H, Br, N, S.

Pharmacology. The receptor binding technique for determining the affinities for GABA$_A$ and GABA$_B$ receptors was performed using rat brain membrane preparations using either [^{3}H]muscimol or [^{3}H]GABA as the radioligands as described previously.[11] Cloning and sequencing of cDNAs encoding human α_1, α_4, β_3, γ_{2S}, and δ GABA$_A$ receptor subunit proteins have been described elsewhere.[1,19−21] α_1 encoding cDNA was engineered into a pCDM8 vector (Invitrogen, San Diego, CA) and α_4, β_3, γ_{2S}, and δ into a pcDNAI/Amp vector (Invitrogen). DNA was a kind gift from Dr. Paul Whiting, Merck Sharp & Dohme, Terlings Park, Harlow, U.K. Large scale cDNA preparation and purification was undertaken using a QIAGEN Plasmid Maxi kit (QIAGEN GmbH, Hilden, Germany). Plasmids were linearized using HpaI and XbaI restriction enzymes for $\alpha_1/\alpha_4/\delta$ and β_3/γ_{2S} cDNAs, respectively, and transcribed and capped in vitro (mMessage mMachine T7 kit, Ambion, Inc., Austin, TX). The RNAs were precipitated with LiCl and redissolved in sterile RNase-free water, diluted to a concentration of 0.2 μg/μL, and divided into portions that were stored at −80 °C. cRNA was kindly supplied by Jan Egebjerg and Lene Heding, Department of Molecular Genetics, H. Lundbeck A/S.

An adult female *Xenopus laevis* was anaesthetized by immersion in a 0.4% (w/v) 3-aminobenzoic acid ethyl ester solution (Sigma) for 15−20 min. Through an incision in the abdominal wall, 2−3 ovarian lobes were removed and stage V and VI oocytes manually defolliculated with watchmaker's fine forceps. After mild collagenase treatment (type IA (Sigma), 0.5 mg/mL, for 6 min) to remove remaining follicle cells, each oocyte was injected with 23.0 nL of cRNA encoding human $\alpha_1\beta_3\gamma_{2S}$ GABA$_A$ receptor subunits (0.2 μg/μL in a subunit ratio of 1:1:1)). Oocytes were incubated for at least 24 h in modified Barth's saline [88 mM NaCl, 1 mM KCl, 15 mM HEPES, 2.4 mM $NaHCO_3$, 0.41 mM $CaCl_2$, 0.82 mM $MgSO_4$, 0.3 mM $Ca(NO_3)_2$] supplemented with 2 mM sodium pyruvate, 0.1 U/L penicillin and 0.1 μg/L streptomycin and filtered through nitrocellulose.

Oocytes were placed in a 60 μL bath and perfused with Ringer [115 mM NaCl, 2.5 mM KCl, 10 mM HEPES, 1.8 mM $CaCl_2$, 0.1 mM $MgCl_2$, pH 7.5]. Cells were impaled with agar-plugged 0.5−1 MΩ electrodes containing 3 M KCl and voltage clamped at −70 mV by a GeneClamp 500B amplifier (Axon Instruments). The cells were continuously perfused with Ringer buffer at 4−6 mL/min, and the drugs were applied in the perfusate. GABA-containing solutions were applied until the peak of the response was observed, usually after 30 s or less. A 4−8 min wash-out period between GABA applications was allowed in order to minimize desensitization. To ensure complete binding, antagonists were preapplied alone for 1 min before their coapplication with GABA. The competitive GABA$_A$ receptor antagonists were tested with respect to their ability to shift a GABA concentration−response curve rightward when present at a fixed concentration. K_i values for these antagonists were derived from coanalysis of data obtained from each individual oocyte in the absence and presence of antagonist by use of the Waud equation:

$$E = [E_{max}[L]^n]/[EC_{50}{}^n(1 + [I]/K_i)^n + [L]^n] \quad (1)$$

where E_{max} is the maximum response, [L] is the concentration of the ligand, EC_{50} is the concentration of the ligand eliciting 50% of the maximal response, n is the slope factor, and [I] is the (fixed) antagonist concentration. Geometric mean values of the K_i values were calculated.

For antagonists exhibiting a mixed competitive/noncompetitive behavior, an approximate K_i value for the competitive component of the profile was estimated. This was accomplished by fitting only data points from the initial phase of the GABA concentration−

response curve in the presence of antagonist to the Waud equation, omitting data points from which a visible reduction of E_{max} becomes apparent.

For the most elaborate data sets, a quantitative analysis of the mixed competitive/noncompetitive interaction was feasible, using the following equation:

$$E = E_{max}/[1 + F_{nc}([I]/[1 + K_{nc}]) + [EC_{50}/[L]]^n(1 + [I]/K_i)^n] \quad (2)$$

where K_{nc} is a measure of the potency of the noncompetitive component and F_{nc} is an arbitrary constant controlling the reduction of E_{max}.

All experiments were performed on at least four different oocytes stemming from at least two different oocyte batches. Stock solution of GABA (1 M) were made in double-distilled water and stored in aliquots at −20 °C. 4-PIOL analogues were dissolved freshly in DMSO at a concentration of 10 mM and diluted at the appropriate concentration in Ringer buffer.

Calculations of log *P*. The calculations were performed by using Crippen's fragmentation method and the ClogP algorithm as implemented in ChemDrawUltra version 8.0.3 (CambridgeSoft).

Calculations of ΔG_{solv}. Solvated structures were obtained by full optimization at the HF/6-31+G* level employing the CPCM[22] solvation method as implemented in Gaussian98.[23]

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Supporting Information Available: Data from microanalysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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