4-Aryl-5-(4-Piperidyl)-3-Isoxazolol $GABA_A$ Antagonists: Synthesis, Pharmacology, and Structure−Activity Relationships

Bente Frølund,*,† Lars S. Jensen,† Signe I. Storustovu,‡ Tine B. Stensbøl,‡ Bjarke Ebert,‡ Jan Kehler,‡ Povl Krogsgaard-Larsen,† and Tommy Liljefors†

Department of Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Copenhagen, DK-2100 Copenhagen, Denmark, and Department of Molecular Pharmacology, Electrophysiology, and Medicinal Chemistry, H. Lundbeck A/S, DK-2500 Valby, Denmark

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A series of 4-aryl-5-(4-piperidyl)-3-isoxazolol $GABA_A$ antagonists have been synthesized and pharmacologically characterized. The *meta*-phenyl-substituted compounds **9k** and **9m** and the *para*-phenoxy-substituted compound **9l** all display high affinities (K_i = 10−70 nM) and antagonist potencies in the low nanomolar range (K_i = 9−10 nM). These potencies are significantly higher than those of previously reported 4-PIOL antagonists and considerably higher than that of the standard $GABA_A$ antagonist **SR 95531**.

Introduction

4-Aminobutyric acid (GABA), the major inhibitory neurotransmitter in the central nervous system, exerts its effect in the central nervous system through two different classes of receptors, the ionotropic $GABA_A$ and $GABA_C$ receptors and the metabotropic $GABA_B$ receptors. Especially the $GABA_A$ receptors have attracted much attention as therapeutic targets for the treatment of conditions such as anxiety, epilepsy, and sleep disorders.[1]

The $GABA_A$ receptor is a member of a superfamily of ligand-gated ion channels, which also comprises the nicotinic acetylcholine, the glycine, and the serotonin ($5\text{-}HT_3$) receptors. The $GABA_A$ receptor is a heteropentameric transmembrane allosteric protein complex, and in addition to the GABA recognition site, it contains a considerable number of separate but allosterically interacting binding sites. This is reflected in the structural diversity of compounds acting at the $GABA_A$ receptors, including important drugs such as benzodiazepines, neurosteroids, and barbiturates.

In contrast to the allosteric modulatory sites, the GABA binding site has very distinct and specific structural requirements for recognition and activation. Thus, very few different classes of structures have been reported. Within the series of compounds showing agonist activity at the $GABA_A$ receptor site are the selective $GABA_A$ agonists muscimol (**1**)[2] and 4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol (THIP, **2**),[2,3] which have been used for the characterization of the $GABA_A$ receptors (Figure 1).[4] Recently, **2** has been shown to be functionally selective for a subpopulation of $GABA_A$ receptors and is currently in clinical trials as a therapeutic for the regulation of sleep.

To date, six α, three β, three γ, and three ρ, ϵ, π, θ, and δ subunits have been identified in humans. Although a high theoretical number of different pentameric receptor isoforms are possible, only a limited number have been identified, with the $\alpha_1\beta_2\gamma_2$ apparently being the most abundant in the human central nervous system.[5,6]

We have previously reported a 3D-pharmacophore model for $GABA_A$ agonists and competitive antagonists based on the $GABA_A$ receptor ligands: **1**, **2**, and 4-PIOL (**3**).[7,8] Using this model, a series of potent and selective competitive antagonists have been developed, including compounds **4** and **5**, and the existence of a cavity of considerable dimensions in the vicinity of the 4-position of the 3-isoxazolol ring of **3** has been identified.[8,9] Most of these potent antagonists have aromatic rings linked to the 3-isoxazol moiety via a methylene group. However, in a previous study, we have shown that attaching a phenyl group directly to the 4-position of the 3-isoxazolol ring in **3** yields a compound (compound **6**) that displays an unexpectedly high affinity, more than 40-fold higher than that of the parent compound **3** and 17 times higher than that of the corresponding 4-benzyl-substituted compound.[9] In the present study, 4-phenyl-5-(4-piperidyl)-3-isoxazolol (**6**) has been used as lead compound to investigate the influence of different substituents in the *ortho*-, *meta*-, and *para*-positions of the

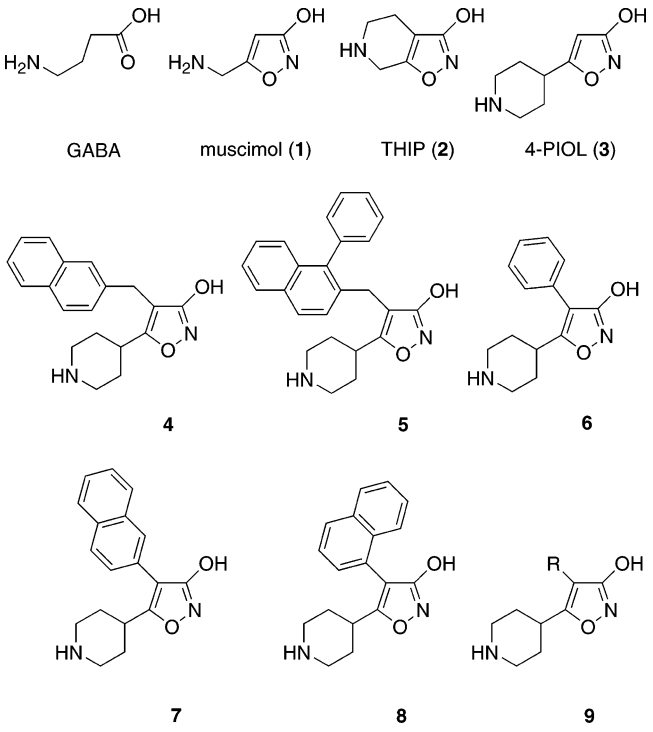

Figure 1. Structures of GABA, the $GABA_A$ agonists muscimol (**1**), THIP (**2**), the low-efficacy partial $GABA_A$ agonists 4-PIOL (**3**), the $GABA_A$ antagonists **4**−**8**, and the new 4-aryl-5-(4-piperidyl)-3-isoxazolols (**9**).

* To whom correspondence should be addressed. Phone: (+45) 35306495. Fax: (+45) 35306040. E-mail: bfr@farma.ku.dk.
† University of Copenhagen.
‡ H. Lundbeck A/S.

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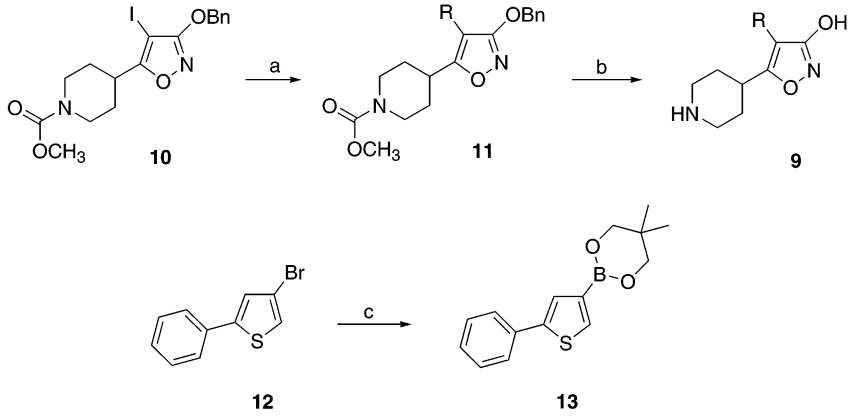
Scheme 1[a]



[a] Reagents and conditions: (a) $Pd(PPh_3)_2Cl_2$, aq K_2CO_3, $RB(OH)_2$ (for **9m**, compound **13**), DMF; (b) 33% HBr, AcOH, room temperature; (c) *n*-BuLi, THF, −78 °C, $B(O\text{-}i\text{-}pr)_3$.

4-phenyl group on the binding to the $GABA_A$ receptor. The synthesis and pharmacological properties are reported and structure−activity relationships are discussed in terms of our previously reported pharmacophore model.

Results and Discussion

Chemistry. The target compounds were synthesized as outlined in Scheme 1.

The 4-iodo analogue, **10**,[8] was used as starting material for coupling under Suzuki conditions using $Pd(PPh_3)_2Cl_2$ and the appropriate aryl boronic acid or ester to give the 4-aryl-substituted compounds **11** in high yields. The boronic acids were commercially available, except for the 5-phenyl-3-thienyl analogue, where the 5,5-dimethyl[1,3,2]dioxaborinan ester (**13**) was synthesized from the corresponding 4-bromo-5-phenylthiophene (**12**).[10] Deprotection to give the target compounds with the generic structures **9** was accomplished by treatment with hydrogen bromide in acetic acid.

In Vitro Pharmacology. The compounds were characterized in receptor binding studies using rat membrane preparations and electrophysiologically using two-electrode voltage clamp on human $\alpha_1\beta_3\gamma_{2S}$ $GABA_A$ receptors expressed in *Xenopus* oocytes. 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.[8] Like the lead compound **6**, the entire selected group of compounds showed affinity selectively for the $GABA_A$ receptor sites (Table 1). None of the compounds showed detectable affinity for the $GABA_B$ receptors at test concentrations of 100 μM.

The standard $GABA_A$ receptor antagonist **SR 95531** was included in this study for comparison.

Receptor Binding and Structure−Affinity Relationships. As mentioned in the introduction, the attachment of a phenyl group directly to the 4-position of the 3-isoxazolol ring in **3** gives compound **6**, which displays an unexpectedly high affinity compared to that of the parent compound **3** (Table 1). The position of the phenyl ring in terms of our previously reported pharmacophore model[9] is shown as ring A in Figure 2. The figure also shows the spatial relationships according to the pharmacophore model between the phenyl ring in **6** and the naphthyl rings in **5**, **7**, and **8**.

To investigate if other monocyclic aromatic ring systems other than phenyl are compatible with high affinity, the 3-thienyl analogue (**9h**) and the 3-pyridyl analogue (**9g**) were synthesized and pharmacologically characterized. The affinity of **9h** is comparable to that of **6**, whereas **9g** shows a reduction in affinity by a factor of 4. The affinity order follows the relative hydrophobicities of the ring systems as calculated by ClogP (benzene 2.14, thiophene 1.78, and pyridine 0.65; ChemDrawUltra version 10, CambridgeSoft). On this basis, further studies were confined to investigate the effects of *ortho*-, *meta*-, and *para*-substitutions in the 4-phenyl-substituted compound **6**.

Table 1. Receptor Binding and In Vitro Electrophysiology Data for Selected Compounds

		[^{3}H]muscimol binding[a]	electrophysiology[b]
cmpd	R	K_i[c] (nM) ($pK_i \pm$ SEM)	K_i[c] (nM) ($pK_i \pm$ SEM)
4-PIOL	H	9100[d]	11 000[d]
4	2-naphthylmethyl	49[d]	370[d]
5	1-phenyl-2-naphthylmethyl	21[d]	198[d]
6	phenyl	220[d]	159[d]
7	2-naphthyl	36[d]	141[d]
8	1-naphthyl	820[d]	334[d]
9a	2-chlorophenyl	851 (6.07 ± 0.11)	129 (6.89 ± 0.05)
9b	3-chlorophenyl	58 (7.24 ± 0.01)	48 (7.32 ± 0.04)
9c	4-chlorophenyl	2425 (5.62 ± 0.06)	204 (6.69 ± 0.05)
9d	3,5-dichlorophenyl	77 (7.11 ± 0.01)	68 (7.17 ± 0.08)
9e	3-nitrophenyl	234 (6.63 ± 0.02)	138 (6.86 ± 0.03)
9f	3-aminophenyl	84 (7.07 ± 0.24)	59 (7.23 ± 0.02)
9g	3-pyridyl	955 (6.02 ± 0.08)	427 (6.32 ± 0.03)
9h	3-thienyl	317 (6.50 ± 0.02)	76 (7.12 ± 0.09)
9i	4-*tert*-butylphenyl	6977 (5.16 ± 0.07)	257 (6.59 ± 0.03)
9j	4-biphenyl	195 (6.71 ± 0.11)	71 (7.15 ± 0.01)
9k	3-biphenyl	10 (8.00 ± 0.09)	10 (8.02 ± 0.04)
9l	4-phenoxyphenyl	70 (7.15 ± 0.02)	8 (8.10 ± 0.04)
9m	3-(5-phenyl)thienyl	25 (7.61 ± 0.03)	9 (8.04 ± 0.05)
SR 95531		74[d]	240[d]
9n	1-bromo-2-naphthylmethyl	10[d]	42[d]

[a] Standard receptor binding on rat brain synaptic membranes, $n = 3$. [b] Two-electrode voltage-clamp recordings on *Xenopus* oocytes expressing $\alpha_1\beta_3\gamma_{2S}$ $GABA_A$ receptor subunits, $n = 4$. [c] K_i values were calculated from pK_i values found in bracket. [d] From reference 9.

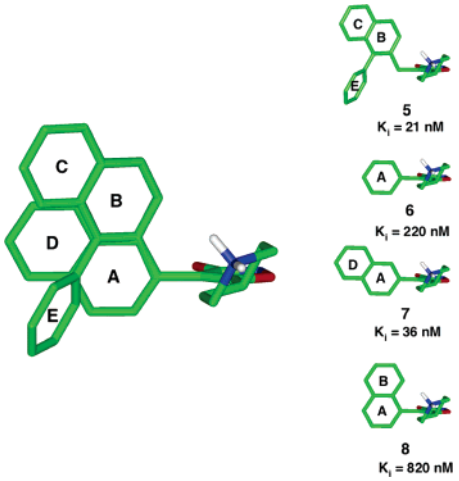


Figure 2. Superimposition of the previously deduced bioactive conformations of **5**−**8**.[9]

***ortho*-Substitution.** An *ortho*-chloro substituent (**9a**) reduces the affinity of **6** by a factor of 4 (Table 1). This is most probably due to a steric conflict between the chlorine atom and the axial hydrogen atoms in the piperidyl ring of the 4-PIOL moiety in one of the two alternative *ortho*-positions and with steric and electrostatic repulsions with the negatively charged isoxazole oxygen in the other. This is in line with the observation that the 4-(1-naphthyl) compound **8** displays a 4-fold lower affinity than **6** (Table 1), most probably due to steric repulsions between ring B in **8** (Figure 2) and the 4-PIOL moiety. Thus, substitution in the *ortho*-position is not compatible with high affinity and was, therefore, not further investigated.

***para*-Substitution.** A chloro substituent (**9c**) and a *tert*-butyl group (**9i**) in the *para*-position of the phenyl ring reduces the receptor affinity by a factor of 11 and 32, respectively. This strongly indicates that there are steric restrictions to ligand binding in the vicinity of the *para*-position in **6** and close to ring D in Figure 2. Interestingly, a phenyl substituent in the *para*-position (**9j**) gives retained affinity compared to that of **6** and a phenoxy group (**9l**) gives a 3-fold affinity increase. Because the thickness of a phenyl ring (3.4 Å) is somewhat smaller than twice the van der Waals radius of a chloro substituent (3.6−4.0 Å), the conformation of the phenyl and phenoxy substituents with respect to the phenyl ring directly attached to the 4-PIOL system may be adjusted to avoid the steric repulsions experienced by a chloro substituent and a *tert*-butyl group.

***meta*-Substitution.** According to the superimpositions in Figure 2, a *meta*-substituent may in the receptor binding site be located in the region of ring C in Figure 2. This ring corresponds to the distal naphthalene ring in **5**, which contributes to the affinity of **5** by a factor of as much as 78, indicating a potentially favorable interaction site for substituents located in this area.[8] Compound **9b** with a chloro substituent in the *meta*-position of the phenyl ring shows a 4-fold higher affinity than that of **6**. Introduction of a chloro substituent in both *meta*-positions, compound **9d**, results in an affinity comparable with that of compound **9b**. The single chloro substituent in **9b** is most likely located in the area of ring C, whereas the second chloro substituent in the dichloro compound **9d** is located in the area close to ring E. This ring corresponds to the 1-phenyl ring in compound **5** and contributes by only a factor of 2 to the affinity of **5**. On the basis of these observations, the influence of different substituents in the *meta*-position of the phenyl ring on binding to the $GABA_A$ receptor was further investigated.

As may be inferred from Figure 2, the distal phenyl group in the 3-biphenyl-substituted compound **9k** perfectly overlaps with ring C in compound **5**. As may be predicted using the pharmacophore model, the affinity of the 3-biphenyl analogue, **9k**, was shown to be the highest one of the group of compounds tested, revealing a K_i value in the low nanomolar range (10 nM, Table 1). This corresponds to an increase in affinity compared to that of **6** by a factor of 22, which nicely validates the superimpositions in Figure 2.

The 3-nitrophenyl and 3-aminophenyl analogues, compounds **9e** and **9f**, respectively, show similar affinities as that of the lead compound **6**. This indicates that the receptor region corresponding to ring C in Figure 2 and/or in the vicinity of the alternative *meta*-position (close to ring E in Figure 2) is water accessible and that the nitro and amino substituents are both solvated in the binding site. The opposite electronic effects of these two substituents on ring A and their similar influence on the affinity also indicate that the interactions between ring A and the receptor is largely of hydrophobic nature.

Based on the affinities of compounds **9h** and **9k**, a hybrid compound **9m** was designed using a 3-(5-phenyl)thienyl group. As predicted, **9m** displays an affinity similar to that of **9k**.

Electrophysiology. The pharmacological profile of the selected group of compounds was studied using a two-electrode voltage clamp on human $GABA_A$ $\alpha_1\beta_3\gamma_{2S}$ receptors expressed in *Xenopus* oocytes. As shown for structurally related 4-PIOL analogues, all of the compounds tested in the present study are competitive antagonists in that they are able to shift a GABA concentration−response curve rightward when present in a fixed concentration.[11] The electrophysiological data shows a fairly good correlation to the obtained binding affinities (Table 1). However, the 4-*tert*-butylphenyl and 4-chlorophenyl-substituted analogues, **9i** and **9c**, did show higher potency than expected from the affinity data. The 3-biphenyl-, 4-phenoxyphenyl-, and 3-(5-phenyl)thienyl-substituted analogues, **9k**, **9l**, and **9m**, showed antagonist potencies in the low nanomolar range (K_i 9−10 nM), considerably higher potencies than that of the standard $GABA_A$ antagonist 2-(3′-(carboxypropyl)-3-amino-6-(paramethoxyphenyl)pyridazinium bromide (**SR 95531**;[12] K_i 240 nM, Table 1), of the parent compound **6** (K_i 159 nM, Table 1), and of the earlier reported corresponding 1-bromo-2-naphthylmethyl analogue, **9n**,[9] of compound **3** (K_i 42 nM).

Conclusions

On the basis of our previous observation that a phenyl group directly attached to the 4-position of the 3-isoxazolol ring in **3** results in a considerable affinity increase for the $GABA_A$ receptor, a series of 4-aryl-5-(4-piperidyl)-3-isoxazolols and in particular of *ortho*-, *meta*-, and *para*-substituted 4-phenyl-5-(4-piperidyl)-3-isoxazolols have been synthesized and pharmacologically characterized by receptor binding and electrophysiology. The *meta*-phenyl-substituted compounds **9k** and **9m** and the *para*-phenoxy-substituted compound **9l** all display antagonist potencies significantly higher than that of previously reported 4-PIOL antagonists and considerably higher than that of the standard $GABA_A$ antagonist **SR 95531**. The study has furthermore provided additional information on the properties of the ligand-binding site of the $GABA_A$ receptor and also validated our previously reported pharmacophore model.

Experimental Section

Chemistry. General Procedures. All reactions involving air-sensitive reagents were performed under N_2 using syringe-septum cap techniques. Column chromatography (CC) was performed on

Merck silica gel 60 (0.06−0.200 mm). Flash column chromatography (FC) was performed using Merck silica gel 60 (0.040−0.063 mm). Dry column vacuum chromatography[13] was performed using Merck silica gel 60 (0.015−0.040 mm). Analytical thin-layer chromatography (TLC) was carried out using Merck silica gel 60 F_{254} plates. All compounds were detected as single spots on TLC plates and visualized using UV light and $KMnO_4$ spraying reagent. Compounds containing amino groups were also visualized using a ninhydrin spray reagent. Compounds containing the 3-isoxazolol ring were visualized using a $FeCl_3$ spraying reagent. Elemental analyses were performed at Analytical Research Department, H. Lundbeck A/S, Denmark, or by Mr. J. Theiner, Department of Physical Chemistry, University of Vienna, Austria, and are within ±0.4% of the calculated values, unless otherwise stated.

5-Phenyl-3-(5,5-dimethyl-[1,3,2]dioxaborinan-2-yl)-thiophene (13). A solution of 4-bromo-5-phenylthiophene (**12**;[10] 0.24 g, 1.0 mmol) in dry THF (4 mL) was stirred at −78 °C under nitrogen atmosphere, and *n*-BuLi (0.47 mL, 2.30 M, 1.1 mmol) was added and stirred for 35 min at −78 °C. Triisopropyl borate (0.38 g, 2.0 mmol) was added, and the stirring was continued for 3 h at −78 °C and then 30 min at room temperature. Acetic acid (0.15 mL, 2.2 mmol) was added, followed by neopentylglycol (0.23 g, 2.2 mmol), and the mixture was stirred for 25 min at room temperature. Dichloromethane (15 mL) was added, and the phases were separated. The organic phase was washed with a solution of saturated aqueous ammonium chloride and water 1:1 (3 × 15 mL) and saturated aqueous sodium hydrogen carbonate (2 × 15 mL) and water (2 × 15 mL), dried, and evaporated. The crude was recrystallized from petroleum ether 80−100 °C/EtOAc to give the product as gray crystals (0.11 g, 40%): mp 115−117 °C.

3-Benzyloxy-4-(2-chlorophenyl)-5-(1-methoxycarbonyl-4-piperidyl)isoxazol (11a). A mixture of **10**, 2-chlorophenyl boronic acid (141 mg, 0.9 mmol), $Pd(PPh_3)_2Cl_2$ (30 mg, 45 mmol), DMF (4 mL), and aqueous potassium carbonate (0.3 mL, 3 M, 0.9 mmol) was stirred at 70 °C for 22 h. The reaction mixture was filtrated through celite. The organic phase was washed with water (15 mL), aqueous NaOH (2 × 15 mL, 2 M), and water (15 mL), dried, and evaporated. Dry CC (toluene/EtOAc (4:1)) gave the product as yellow oil (0.15 g, 78%).

4-(2-Chlorophenyl)-5-(4-piperidyl)-3-isoxazolol Hydrobromide (9a). Compound **11a** (0.15 g, 0.35 mmol) was dissolved in a solution of HBr in AcOH (5 mL, 33%), and the mixture was stirred at room temperature for 24 h. The reaction mixture was evaporated, and the residue was recrystallized (MeOH/Et_2O) to give **9a** (95 mg, 76%) as colorless crystals: mp >210 °C. Anal. ($C_{14}H_{15}ClN_2O_2$·HBr) C, H, N.

3-Benzyloxy-4-(3-chlorophenyl)-5-(1-methoxycarbonyl-4-piperidyl)isoxazol (11b). Compound **11b** was prepared as described for **11a** using 3-chloro-phenyl boronic acid to give the product as pale yellow crystals (0.14 g, 73%): mp 150−153 °C. Anal. ($C_{23}H_{23}ClN_2O_4$) C, H, N.

4-(3-Chlorophenyl)-5-(4-piperidyl)-3-isoxazolol Hydrobromide (9b). Compound **9b** was prepared as described for **9a** using compound **11b** (0.14 g, 0.33 mmol) to give the product (89 mg, 75%) as colorless crystals: mp >210 °C. Anal. ($C_{14}H_{15}ClN_2O_2$·HBr) C, H, N.

3-Benzyloxy-4-(4-chlorophenyl)-5-(1-methoxycarbonyl-4-piperidyl)isoxazol (11c). Compound **11c** was prepared as described for **11a** using 4-chlorophenyl boronic acid to give the product as brown crystals (0.05 g, 47%): mp 155−157 °C. Anal. ($C_{23}H_{23}ClN_2O_4$) C, H, N.

4-(4-Chlorophenyl)-5-(4-piperidyl)-3-isoxazolol Hydrobromide (9c). Compound **9c** was prepared as described for **9a** using compound **11c** (0.09 g, 0.21 mmol) to give the product (45 mg, 60%) as colorless crystals: mp >210 °C. Anal. ($C_{14}H_{15}ClN_2O_2$·HBr·H_2O) C, H, N.

3-Benzyloxy-4-(3,5-dichlorophenyl)-5-(1-methoxycarbonyl-4-piperidyl)isoxazol (11d). Compound **11d** was prepared as described for **11a** using 3,5-dichlorophenyl boronic acid to give the product as brown oil (0.11 g, 74%).

4-(3,5-Dichlorophenyl)-5-(4-piperidyl)-3-isoxazolol Hydrobromide (9d). Compound **9d** was prepared as described for **9a** using compound **11d** (0.11 g, 0.24 mmol) to give the product (60 mg, 64%) as colorless crystals: mp >210 °C. Anal.($C_{14}H_{14}Cl_2N_2O_2$·HBr) C, H, N.

3-Benzyloxy-4-(3-nitrophenyl)-5-(1-methoxycarbonyl-4-piperidyl)isoxazol (11e). Compound **11e** was prepared as described for **11a** using 3-nitrophenyl boronic acid to give the product as pale yellow crystals (0.05 g, 26%): mp 171−174 °C.

4-(3-Nitrophenyl)-5-(4-piperidyl)-3-isoxazolol Hydrobromide (9e). Compound **9e** was prepared as described for **9a** using compound **11e** (0.05 g, 0.11 mmol) to give the product (17 mg, 42%) as colorless crystals: mp >210 °C. Anal. ($C_{14}H_{15}N_3O_4$·HBr·0.5H_2O) C, H, N.

3-Benzyloxy-4-(3-aminophenyl)-5-(1-methoxycarbonyl-4-piperidyl)isoxazol (11f). Compound **11f** was prepared as described for **11a** using 3-aminophenyl boronic acid to give the product as a yellow oil (0.17 g, 93%).

4-(3-Aminophenyl)-5-(4-piperidyl)-3-isoxazolol Dihydrobromide (9f). Compound **9f** was prepared as described for **9a** using compound **11f** (0.17 g, 0.42 mmol) to give the product (0.13 g, 74%) as colorless crystals: mp >210 °C. Anal. ($C_{14}H_{15}N_3O_4$·2HBr·H_2O) C, H, N.

3-Benzyloxy-4-(3-pyridyl)-5-(1-methoxycarbonyl-4-piperidyl)-isoxazol (11g). Compound **11g** was prepared as described for **11a** using 3-pyridyl boronic acid to give the product as colorless crystals (0.18 g, 99%): mp 108−111 °C.

4-(3-Pyridyl)-5-(4-piperidyl)-3-isoxazolol Hydrobromide (9g). Compound **9g** was prepared as described for **9a** using compound **11g** (0.18 g, 0.45 mmol) to give the product (0.12 g, 79%) as colorless crystals: mp >210 °C. Anal. ($C_{13}H_{15}N_3O_2$·2HBr·H_2O) C, H, N.

3-Benzyloxy-5-(1-methoxycarbonyl-4-piperidyl)-4-(3-thienyl)-isoxazol (11h). Compound **11h** was prepared as described for **11a** using 3-thienyl boronic acid to give the product as brown crystals (40 mg, 22%): mp 153−155 °C.

5-(4-Piperidyl)-4-(3-thienyl)-3-isoxazolol Hydrobromide (9h). Compound **9h** was prepared as described for **9a** using compound **11h** (40 mg, 0.1 mmol) to give the product (28 mg, 85%) as pale yellow crystals: mp >210 °C. Anal. ($C_{12}H_{14}N_2O_2S$·HBr·0.5H_2O) C, H, N.

3-Benzyloxy-4-[(1,1-dimethylethyl)phenyl]-5-(1-methoxycarbonyl-4-piperidyl)isoxazol (11i). Compound **11i** was prepared as described for **11a** using 4-(1,1-dimethylethyl)phenyl boronic acid to give the product as brown oil (0.17 g, 84%)

4-((1,1-Dimethylethyl)-phenyl)-5-(4-piperidyl)-3-isoxazolol Hydrobromide (9i). Compound **9i** was prepared as described for **9a** using compound **11f** (0.17 g, 0.38 mmol) to give the product (0.11 g, 76%) as colorless crystals: mp >210 °C. Anal. ($C_{18}H_{24}N_2O_2$·HBr) C, H, N.

3-Benzyloxy-4-(4-biphenyl)-5-(1-methoxycarbonyl-4-piperidyl)-isoxazol (11j). Compound **11j** was prepared as described for **11a** using 4-biphenyl boronic acid to give the product as a brown oil (0.21 g, 99%).

4-(4-Biphenyl)-5-(4-piperidyl)-3-isoxazolol Hydrobromide (9j). Compound **9j** was prepared as described for **9a** using compound **11j** (0.21 g, 0.44 mmol) to give the product (0.13 g, 74%) as colorless crystals: mp >210 °C. Anal. ($C_{20}H_{20}N_2O_2$·HBr·H_2O) C, H, N.

3-Benzyloxy-4-(3-biphenyl)-5-(1-methoxycarbonyl-4-piperidyl)-isoxazol (11k). Compound **11k** was prepared as described for **11a** using 3-biphenyl boronic acid to give the product as brown oil (0.15 g, 71%).

4-(3-Biphenyl)-5-(4-piperidyl)-3-isoxazolol Hydrobromide (9k). Compound **9k** was prepared as described for **9a** using compound **11k** (0.15 g, 0.32 mmol) to give the product (0.11 g, 85%) as colorless crystals: mp >210 °C. Anal. ($C_{20}H_{20}N_2O_2$·HBr·0.5H_2O) C, H, N.

3-Benzyloxy-4-(4-phenoxyphenyl)-5-(1-methoxycarbonyl-4-piperidyl)isoxazol (11l). Compound **11l** was prepared as described

for **11a** using 4-phenoxyphenyl boronic acid to give the product as brown oil (0.15 g, 69%).

4-(4-Phenoxyphenyl)-5-(4-piperidyl)-3-isoxazolol Hydrobromide (9l). Compound **9l** was prepared as described for **9a** using compound **11l** (0.10 g, 0.21 mmol) to give the product (65 mg, 74%) as colorless crystals: mp >210 °C. Anal. ($C_{20}H_{20}N_2O_3$·HBr) C, H, N.

3-Benzyloxy-4-[(5-phenyl)-3-thienyl]-5-(1-methoxycarbonyl-4-piperidyl)isoxazol (11m). Compound **11m** was prepared as described for **11a** using 5-phenyl-3-(5,5-dimethyl-[1,3,2]dioxaborinan-2-yl)thiophen to give the product as a brown oil (0.08 g, 51%).

4-[(5-Phenyl)-3-thienyl]-5-(4-piperidyl)-3-isoxazolol Hydrobromide (9m). Compound **9m** was prepared as described for **9a** using compound **11m** (0.08 g, 0.17 mmol) to give the product (40 mg, 58%) as colorless crystals: mp >202−205 °C. Anal. ($C_{18}H_{18}N_2O_2S$·HBr·1.5H_2O) C, H, N.

Experimental Pharmacology. The receptor binding technique for determining the affinities toward $GABA_A$ and $GABA_B$ receptors was determined in rat brain membrane preparations using either [^{3}H]muscimol or [^{3}H]GABA as the radioligands and performed as described previously.[8]

Cloning and sequencing of cDNAs encoding human α_1, β_3, and γ_{2S} $GABA_A$ receptor subunit proteins have been described elsewhere.[14,15] The α_1 encoding cDNA was engineered into a pCDM8 vector (Invitrogen, San Diego, CA), and β_3 and γ_{2S} was engineered 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 α_1/δ 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, 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.

The electrophysiological characterization using a two-electrode voltage clamp on human $\alpha_1\beta_3\gamma_{2S}$ $GABA_A$ receptors expressed in *Xenopus* oocytes was performed as described previously.[16]

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Supporting Information Available: Spectral data (^{1}H NMR and ^{13}C NMR) of all synthesized compounds and elemental analyses for all new target compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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