Partial $GABA_A$ Receptor Agonists. Synthesis and in Vitro Pharmacology of a Series of Nonannulated Analogs of 4,5,6,7-Tetrahydroisoxazolo[5,4-c]pyridin-3-ol

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Received April 10, 1995®

5-(4-Piperidyl)isoxazol-3-ol (4-PIOL, **10**), a structural analog of 4-aminobutanoic acid (GABA, **1**) and the $GABA_A$ agonist 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol (THIP, **5**), is a low-efficacy partial $GABA_A$ agonist. A number of compounds bioisosterically derived from **10**, including 5-(4-piperidyl)isothiazol-3-ol (**11**), 3-(4-piperidyl)isoxazol-5-ol (**12**), 5-(1,2,3,6-tetrahydropyrid-4-yl)isoxazol-3-ol (**13**), and 5-(1,2,3,6-tetrahydropyrid-4-yl)isothiazol-3-ol (**14**), were synthesized and tested as $GABA_A$ receptor ligands. Whereas none of these compounds significantly affected $GABA_B$ receptor binding or GABA uptake, they showed affinities for $GABA_A$ receptor sites in the low-micromolar range. Using cultured cerebral cortical neurons and whole-cell patch–clamp techniques, the efficacies of these compounds relative to that of the full $GABA_A$ agonist, isoguvacine (**8**) (20 μM), were determined. The relative efficacy of **11**, which has a higher receptor affinity ($IC_{50} = 1.3 \pm 0.3$ μM) than **10** ($IC_{50} = 9.3 \pm 2.6$ μM), was comparable with that of **10** (30–35%). The tetrahydropyridine analog of **10**, compound **13**, showed a markedly lower receptor affinity ($IC_{50} = 32 \pm 10$ μM) and apparently a lower relative efficacy than **10**. The corresponding unsaturated analog of **11**, compound **14**, showed a slightly weaker receptor affinity ($IC_{50} = 4.0 \pm 2.0$ μM) but a significantly higher relative efficacy (50–55%) than **11**. The 5-isoxazolol isomer of **10**, compound **12**, showed a reduced receptor affinity ($IC_{50} = 26 \pm 7$ μM) and a very low relative efficacy. Substitution of propanoic or propenoic acid moieties for the acidic heterocyclic units of these compounds gave the monocyclic amino acids **15**–**18**, which have very little or no affinity for $GABA_A$ receptor sites.

Introduction

4-Aminobutanoic acid (GABA, **1**) is the major inhibitory neurotransmitter in the mammalian central nervous system (CNS) and operates through $GABA_A$, $GABA_B$, and probably also $GABA_C$ receptors.[1-3] Dysfunctions of the GABA system have been associated with certain neurological and psychiatric disorders, and there is a growing interest in GABA receptors,[4-6] not least of which are the $GABA_A$ receptors,[3,7,8] as potential therapeutic targets. In order to pharmacologically characterize these receptors, a number of $GABA_A$ agonists bioisosterically derived from GABA, such as muscimol (**2**),[9,10] thiomuscimol (**3**),[10] and the much weaker $GABA_A$ agonist, isomuscimol (**4**),[10] have been developed (Figure 1). Furthermore, the bicyclic analog of these compounds, 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol (THIP, **5**),[10,11] 4,5,6,7-tetrahydroisothiazolo[5,4-c]pyridin-3-ol (Thio-THIP, **6**),[12] and 4,5,6,7-tetrahydroisoxazolo[3,4-c]pyridin-3-ol (Iso-THIP, **7**),[13] as well as isoguvacine (**8**)[10,11] and isonipecotic acid (**9**),[11] have been synthesized and characterized as $GABA_A$ receptor ligands. Whereas **5**, **8**, and **9** are potent and specific $GABA_A$ agonists, **6** is much weaker, and **7** is a weak $GABA_A$ antagonist.[14]

Although there is evidence of impaired function of the central GABA system in epilepsy,[5,15] the $GABA_A$ agonist **5** failed to protect baboons with photosensitive epilepsy against photically induced myoclonic responses,[16] and **5** was only marginally effective as a clinical antiepileptic agent.[17] Quite paradoxically, positron emission tomography (PET) studies on epileptic patients and normal volunteers have shown that **5** increases, rather than reduces, global brain glucose metabolism,[18,19] suggesting that $GABA_A$ antagonists rather than agonists may have therapeutic interest in epilepsy. Reduced central cholinergic neurotransmission contributes to the syndrome of Alzheimer's disease.[20] Since central cholinergic

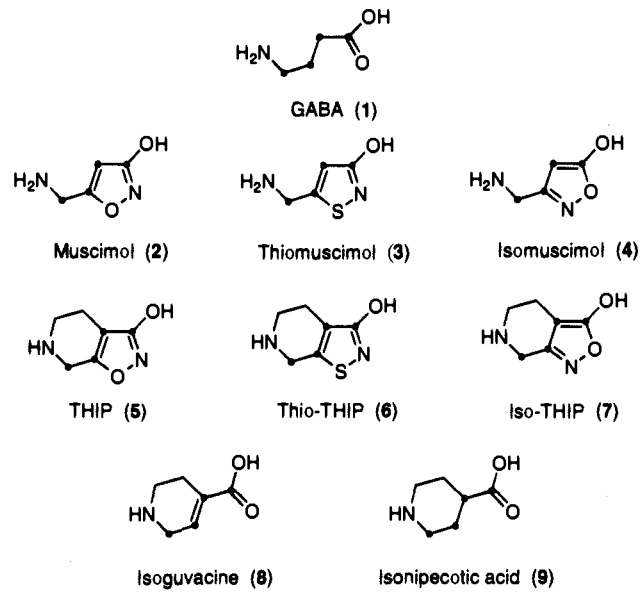

Figure 1. Structures of GABA (1), a number of $GABA_A$ agonists, and the $GABA_A$ antagonist, Iso-THIP (**7**).

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® Abstract published in *Advance ACS Abstracts*, August 1, 1995.

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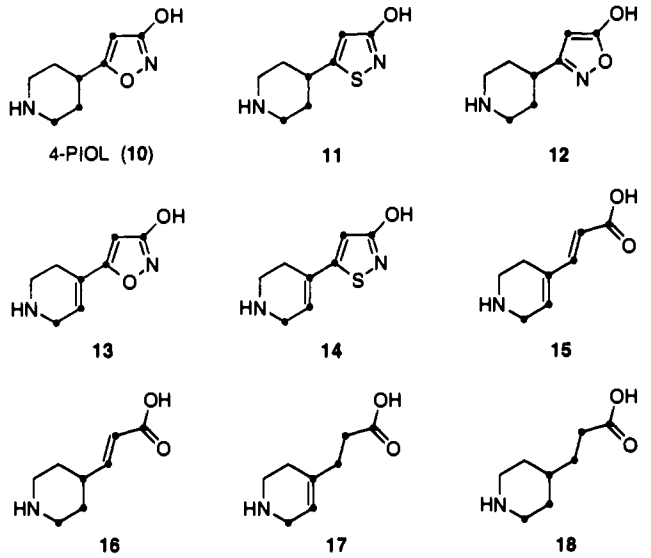


Figure 2. Structures of the low-efficacy partial $GABA_A$ agonists, 4-PIOL (**10**), and a number of new mono- and bicyclic analogs.

neurons appear to be under inhibitory GABAergic control,[21] the function of such neurons may be stimulated in a therapeutically beneficial manner by blockade of $GABA_A$ receptors.[22,23] Accumulating evidence derived from clinical studies of GABAergic drugs supports the view that activation of $GABA_A$ receptors, or distinct subtypes of such receptors, can cause psychosis in normals and stimulate psychotic symptoms in schizophrenics.[24]

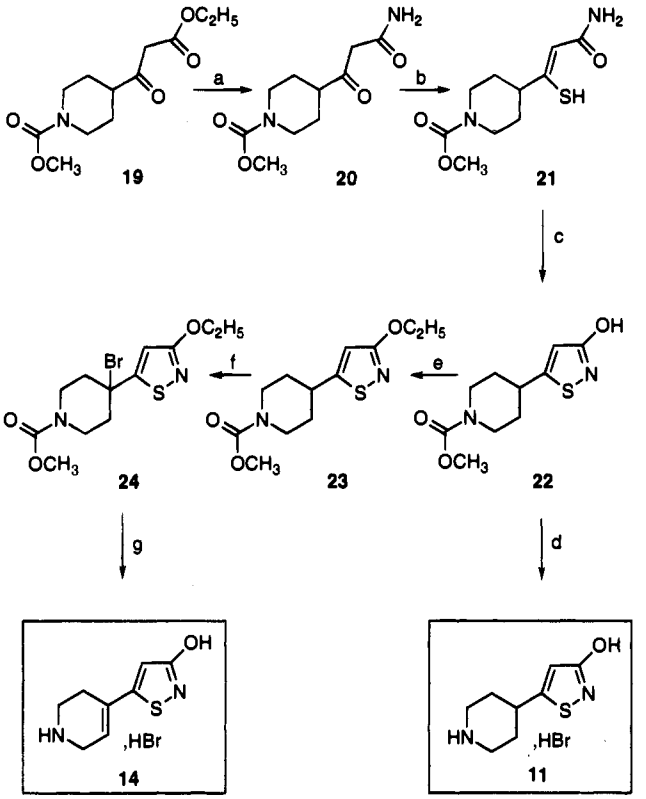
Thus, compounds capable of reducing central $GABA_A$ receptor-mediated neurotransmission may have therapeutic interest in certain CNS disorders, but $GABA_A$ antagonists may be difficult to administer safely to patients.[3] Low-efficacy partial $GABA_A$ agonists may, however, have clinical usefulness in such diseases,[3] but a prerequisite for the exploration of these therapeutic prospects is the development of a series of specific partial $GABA_A$ agonists, showing a range of efficacy levels.

We have previously described 5-(4-piperidyl)isoxazol-3-ol (4-PIOL, **10**) as a specific low-efficacy partial $GABA_A$ agonist.[25-27] We now describe the synthesis and in vitro pharmacological characterization of compounds **11**–**14**, bioisosterically derived from **10** (Figure 2). Since the monocyclic amino acids, **8** and **9**, which are amino carboxylic acid analogs of **5** (Figure 1), are approximately equipotent with **5** as $GABA_A$ agonists and equally specific,[10,11] we have also synthesized and tested the amino carboxylic acid analogs, **15**–**18**, of **10** (Figure 2).

Results

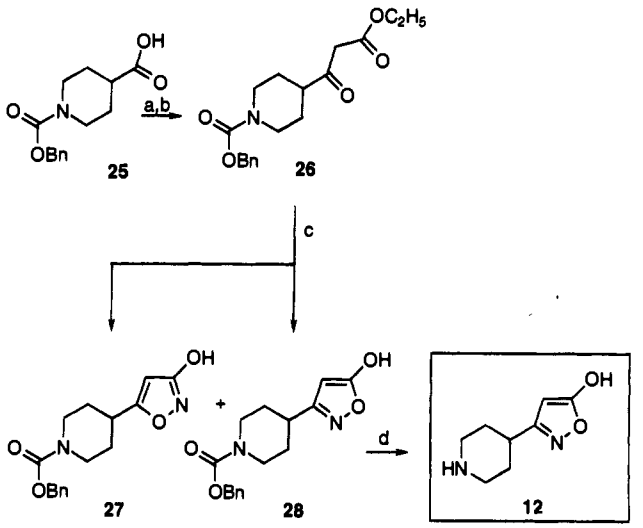
Chemistry. The thio analogs of 4-PIOL (**10**), 5-(4-piperidyl)isothiazol-3-ol (**11**) and 5-(1,2,3,6-tetrahydropyrid-4-yl)isothiazol-3-ol (**14**), were synthesized as shown in Scheme 1. Treatment of ethyl 3-[1-(methoxycarbonyl)-4-piperidyl]-3-oxopropanoate (**19**)[25] with aqueous ammonia gave the β-oxoamide **20**, which was converted into the corresponding enolized β-thioxoamide **21** by treatment with hydrogen sulfide and hydrogen chloride in ethanol. The N-protected form of **11**, compound **22**, was synthesized by oxidation of **21** with iodine in ethanol under basic conditions, and deprotection of **22** to give **11** was accomplished by treatment

Scheme 1[a]



[a] Reagents: (a) aqueous NH_3; (b) H_2S, HCl, EtOH; (c) I_2, K_2CO_3; (d) HBr, AcOH; (e) EtBr, K_2CO_3; (f) NBS; (g) HBr, H_2O.

Scheme 2[a]



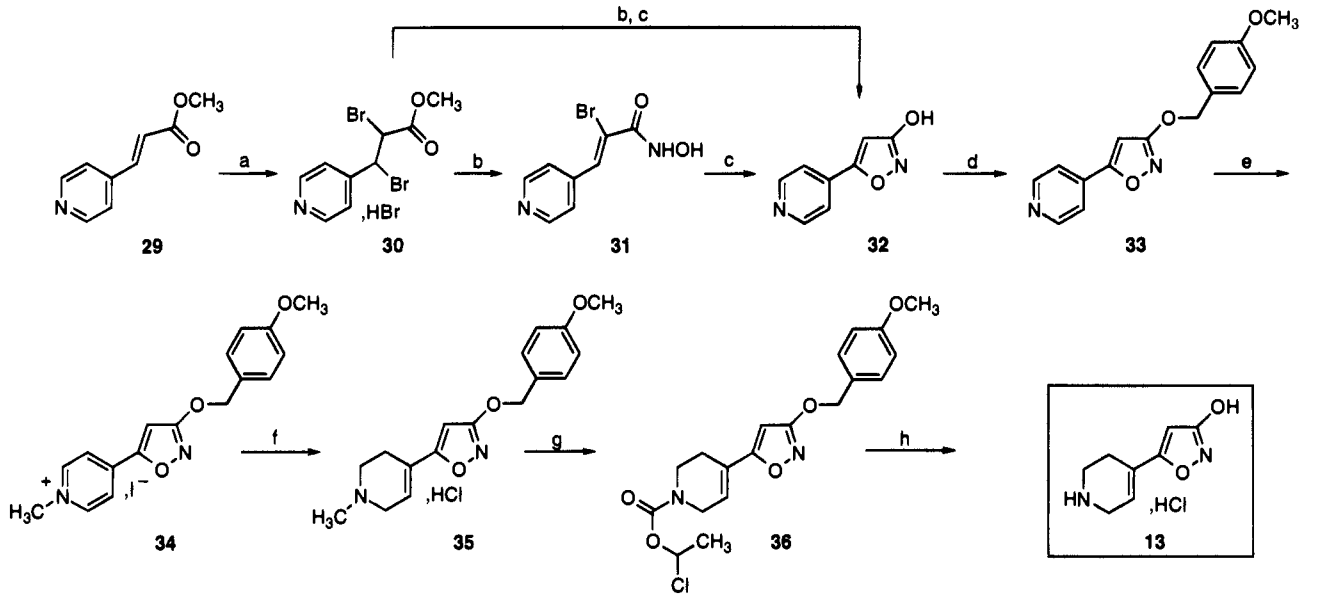
Bn = benzyl

[a] Reagents: (a) *N,N'*-thionyldiimidazole, THF; (b) [3-ethoxy-3-hydroxyacrylato(2−)-O^1,O^3]magnesate(2+), THF; (c) NH_2OH·HCl, NaOH, then concentrated HCl; (d) HBr, AcOH, then IRA-400.

with hydrogen bromide in glacial acetic acid. The O-ethylated and brominated analog of **22**, compound **24**, was dehalogenated and deprotected by treatment with 48% hydrobromic acid to give the target compound, **14**.

The 5-isoxazolol isomer, **12**, of **10** was synthesized as outlined in Scheme 2. N-protected isonipecotic acid, **25**, was converted into the β-oxo ester **26**. Treatment of **26** with hydroxylamine at pH 10 gave a separable mixture of **27** and the desired 5-isoxazolol isomer, **28**, which was deprotected to give **12** by treatment with hydrogen

Scheme 3[a]



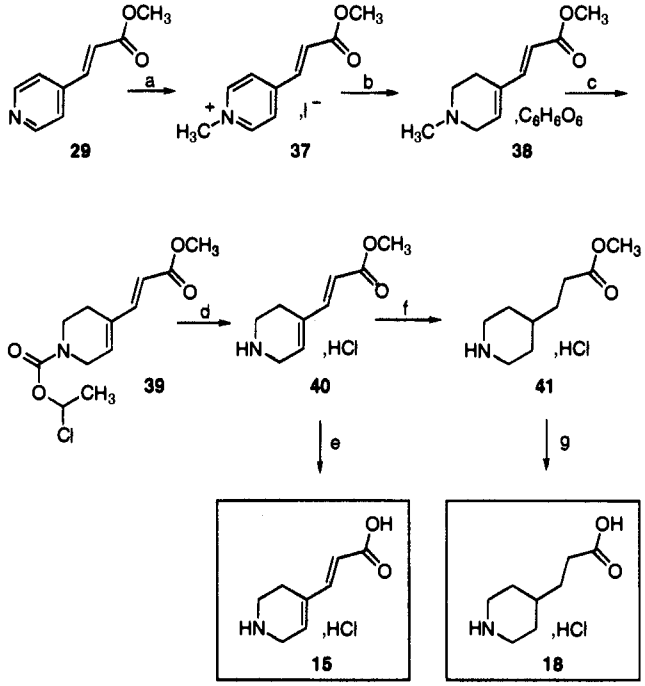
[a] Reagents: (a) HBr, AcOH, then Br_2; (b) NH_2OH·HCl, NaOH, MeOH/H_2O, 0 °C; (c) NaOH, heat, then HCl; (d) 4-CH_3OBnCl, K_2CO_3, DMF; (e) MeI, EtOH; (f) $NaBH_4$, MeOH; (g) 1,2,2,6,6-pentamethylpiperidine, $ClCOOCHClCH_3$; (h) MeOH, HCl, EtOAc.

bromide in glacial acetic acid for 3 min at room temperature.

Attempts to synthesize 5-(1,2,3,6-tetrahydropyrid-4-yl)isoxazol-3-ol (**13**) following a procedure analogous to that described for the conversion of **23** into **14** (Scheme 1) failed. Furthermore, attempts to synthesize the key intermediate, 5-(4-pyridyl)isoxazol-3-ol (**32**), by treatment of the appropriate β-oxo ester with hydroxylamine, by analogy with the conversion of **26** into a mixture of **27** and **28** (Scheme 2), were unsuccessful. Thus, only the 5-isoxazolol isomer of **32** was formed under reaction conditions (pH values, temperatures, and reaction times) identical with or similar to those described.[28-30] Treatment of propynoic acid esters[31] or 2,3-dihalogeno propanoic acid esters[32] with hydroxylamine under basic conditions has previously been shown to provide 5-substituted 3-isoxazolols in moderate yields. On the basis of this observation, we developed the reaction sequence outlined in Scheme 3 for the synthesis of compound **13**. Addition of bromine to methyl 3-(4-pyridyl)propenoate (**29**) gave a pyridinium–bromine addition product. Heating of this precipitate provided compound **30**. Reaction of **30** with hydroxylamine in the presence of base, using an improved version of a reported method,[32] gave **32** in high yields and without concomitant formation of the isomeric 5-isoxazolol product. Detailed studies of this reaction disclosed that 2-bromo-3-(4-pyridyl)propenohydroxamic acid, **31**, is an intermediate. This compound could be isolated, when the reaction was carried out at 0 °C. Heating of **31** in the presence of base gave **32**. The O-protected and quaternized analog, compound **34**, was treated with sodium borohydride to give **35**. This compound was demethylated using 1-chloroethyl chloroformate[33] in the presence of pentamethylpiperidine. Finally, compound **36** was simultaneously O- and N-deprotected by using methanolic hydrogen chloride to give **13**.

The monocyclic amino acids **15**–**18** were synthesized as outlined in Schemes 4–6. The pyridinium compound, **37** (Scheme 4), was reduced with sodium borohydride to give methyl 3-(1-methyl-1,2,3,6-tetrahydropyrid-4-yl)-propenoate (**38**), which was N-demethylated,[33] to give intermediate **40**. Compound **40** was converted into **15**

Scheme 4[a]



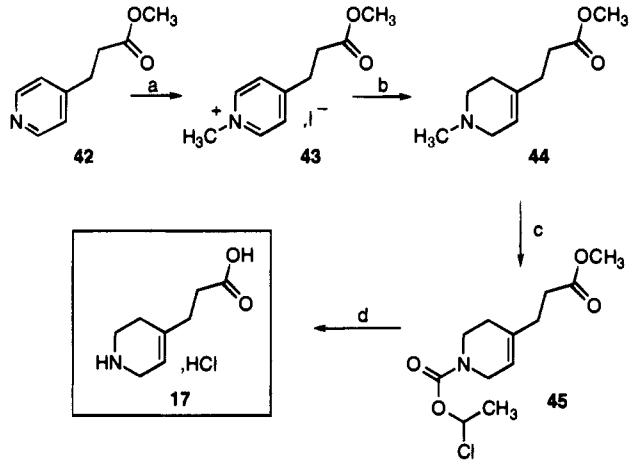
[a] Reagents: (a) MeI, EtOH; (b) $NaBH_4$, MeOH, then fumaric acid; (c) $ClCOOCHClCH_3$; (d) MeOH, HCl; (e) 1 M HCl; (f) H_2, Pd/C; (g) 4 M HCl.

and, via the hydrogenated intermediate, **41**, into 3-(4-piperidyl)propanoic acid (**18**), which has been described earlier.[34]

The propenoic analog of **18**, compound **16**, was synthesized from the β-oxo ester, **19** (Scheme 6). Raney nickel reduction of **19** and subsequent acetylation of the reduction product, **46**, gave **47**, which underwent elimination and ester hydrolysis under mild basic conditions to give intermediate **48**. Deprotection of **48** to give the target compound, **16**, was accomplished under strongly basic conditions.

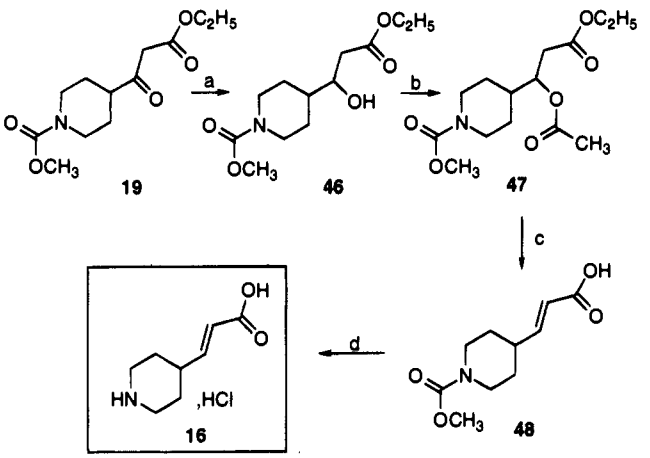
In Vitro Pharmacology. The affinities of the mono- and bicyclic analogs of 4-PIOL (**10**) under study (Figure 2) for $GABA_A$ and $GABA_B$ receptor sites and GABA uptake sites in rat brain membrane preparations were

Scheme 5[a]



[a] Reagents: (a) MeI, EtOH; (b) $NaBH_4$, MeOH; (c) ClCOOCH-$ClCH_3$; (d) MeOH, then 2 M HCl.

Scheme 6[a]



[a] Reagents: (a) Raney nickel, W_2; (b) $(Ac)_2O$; (c) 0.2 M NaOH; (d) NaOH, $MeOH/H_2O$, then HCl.

determined using [^{3}H]GABA as the radioligand. Like **10**, none of these compounds showed a detectable affinity for $GABA_B$ or GABA uptake sites (Table 1). With the exception of the amino carboxylic acid analog of **10**, compound **16**, which was a weak inhibitor of the binding of [^{3}H]GABA to $GABA_A$ receptor sites, the monocyclic compounds shown in Figure 2, quite surprisingly, did not show a significant affinity for these sites. Whereas the 5-isoxazolol (**12**) as well as the unsaturated (**13**) analogs of **10** were weaker than the parent compound as inhibitors of $GABA_A$ receptor binding, the 3-isothiazolol analogs **14** and, in particular, **11** were more potent (Table 1).

Using whole-cell patch–clamp techniques, the efficacies of **10** and the new compounds **11**–**14** as $GABA_A$ agonists relative to that of the classical $GABA_A$ agonist, isoguvacine (**8**),[10,11] were determined. We have previously shown that **10** is acting as a partial agonist at $GABA_A$ receptors in cultured hippocampal neurons.[26] Thus, **10** was shown to inhibit the current response induced by the full $GABA_A$ agonist **8** (20 μM) in a concentration-dependent manner. At high concentrations of **10** (1 mM), the net response approached the size of the response to **10** alone.[26]

In the present studies, we have shown that **10** has a similar effect at $GABA_A$ receptors in cultured neurons from cerebral cortex (Figure 3A,B).[26] The 4-PIOL analogs, **11**–**14**, showed qualitatively similar effects on cultured cortical neurons. Interestingly, however, the relative efficacies of these compounds as partial $GABA_A$ agonists ranged from levels markedly above that of **10** (Figure 3A) to significantly lower levels (Figure 3B). It should be stressed that neither potency nor efficacy of the compounds under study can be determined precisely under the present experimental conditions. On the assumption that each compound acts as a true partial agonist capable of completely displacing **8** from the $GABA_A$ receptors at high concentrations, its maximal agonist effect must lie between the agonist levels produced by a high concentration (1 mM) of the compound in the absence or presence of **8** (20 μM).

The 3-isothiazolol analog of **10**, compound **11**, which binds more tightly to $GABA_A$ receptor sites than **10** itself (Table 1), was approximately equieffective with **10**, whereas the unsaturated analog of **11**, compound **14**, was significantly more efficacious (Figures 3A and 4). In light of these relative efficacies of **11** and **14**, it was quite surprising to observe the reverse relative efficacies of **10** and the corresponding unsaturated analog, compound **13**, which is somewhat less efficacious than **10** (Figure 3B). Like **13**, the 5-isoxazolol analog of **10**, compound **12**, has a lower affinity for $GABA_A$ receptor sites than **10**. Furthermore, **12** shows a relative efficacy comparable with or, perhaps, even lower than that of compound **13** (Figures 3B and 4).

The results of receptor binding studies listed in Table 1 indicate that the new compounds, **11**–**14**, interact with $GABA_A$ receptor sites. In order to demonstrate that the partial agonist effects of **11**–**14**, shown in Figure 3A,B, are in fact also mediated by $GABA_A$ receptors, the reversal potentials of all compounds tested electrophysiologically, including the well-established $GABA_A$ receptor ligands **8**[10,11] and **10**,[26] were determined (Table 1). With the possible exception of

Table 1. pK_a Values, I/U Ratios, Receptor-Binding and Uptake Data, and Reversal Potentials

compound	pK_a values	I/U ratio	$GABA_A$ binding IC_{50} (μM)[d]	$GABA_B$ binding IC_{50} (μM)[d]	GABA uptake IC_{50} (μM)[d]	reversal potentials[d]
GABA (**1**)	4.0, 10.7	800000[a]	0.018 ± 0.003	0.030 ± 0.01	2.0 ± 0.1	nd
THIP (**5**)	4.4, 8.5	1000[a]	0.092 ± 0.003	>100	>300	nd
isoguvacine (**8**)	3.6, 9.8	200000[b]	0.022 ± 0.002	>100	>300	5.2 ± 0.3
4-PIOL (**10**)	5.3, 10.3	30000[c]	9.3 ± 2.6	>100	>300	5.9 ± 0.6
11	6.9, 10.7	5000	1.3 ± 0.3	>100	>300	5.5 ± 0.4
12	4.3, 10.6	nd	26 ± 7	>100	>300	4.3 ± 1.6
13	5.1, 9.4	nd	32 ± 10	>100	>300	5.6 ± 2.1
14	nd	nd	4.0 ± 2.0	>100	>300	5.3 ± 0.6
15	4.1, 9.8	nd	>100	>100	>300	
16	4.0, 10.8	nd	52 ± 4	>100	>300	
17	3.9, 10.4	nd	>100	>100	>300	
18	nd	nd	>100	>100	>300	

[a] Reference 3. [b] Reference 35. [c] Reference 36. nd: not determined. [d] Mean ± SEM; n = 4–5.

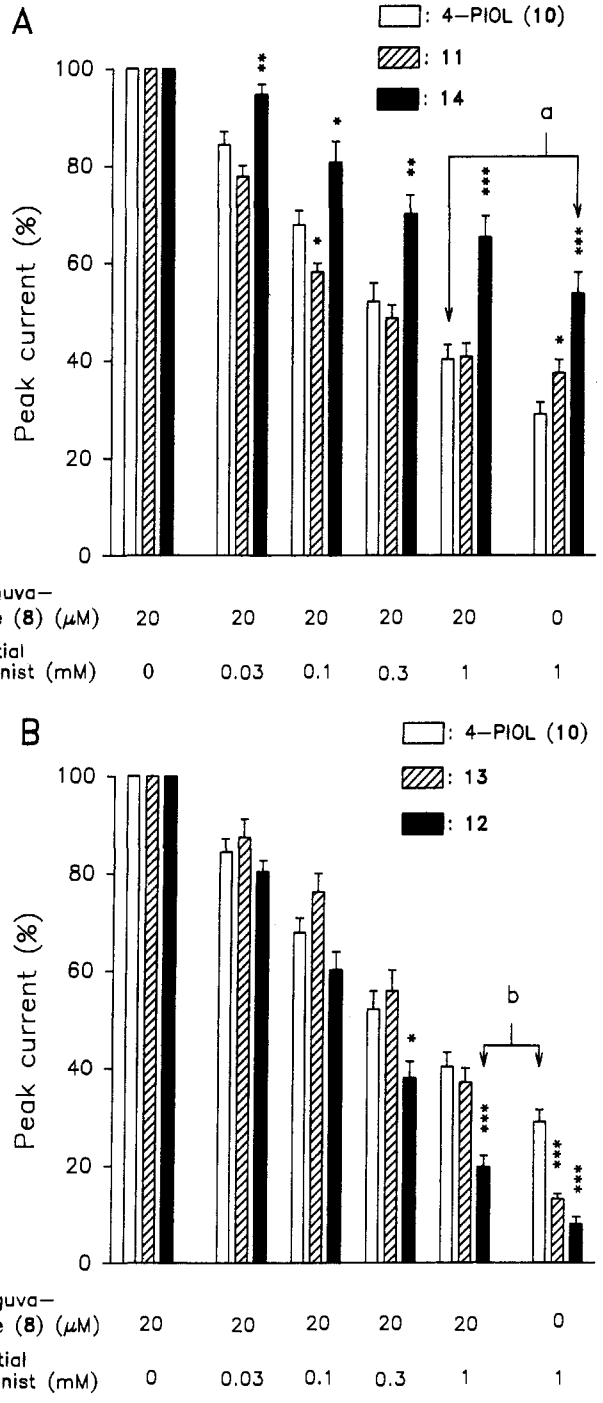


Figure 3. Effect of the partial agonists (A) 4-PIOL (**10**), 11, and **14** and (B) **10**, **12**, and **13** on the response to 20 μM isoguvacine (8). The concentrations applied are given below the histograms. The response to 20 μM 8 alone has been set as 100% (first columns on the left), and the other responses are expressed as a fraction of this. The response to 8 was progressively reduced with increasing concentrations of the respective partial agonists. The numbers of cells tested in this way with each partial agonist were as follows: **10**, 14; 11, 12; **14**, 10; **13**, 10; **12**, 10. Significant differences between **10** and each of the other partial agonists at the same concentration are marked with asterisks (*, $p < 0.05$; **, $0.01 < p < 0.05$; ***, $0.001 < p < 0.01$). (A) Footnote a: The response to 1 mM **14** was significantly larger ($p < 0.05$) than the response to the combination of 1 mM **10** and 20 μM 8. (B) Footnote b: The response to the combination of 1 mM **12** and 20 μM 8 was significantly smaller than the response to 1 mM **10** alone ($p < 0.05$).

compound **12**, these compounds showed very similar reversal potential values, indicating that their effects

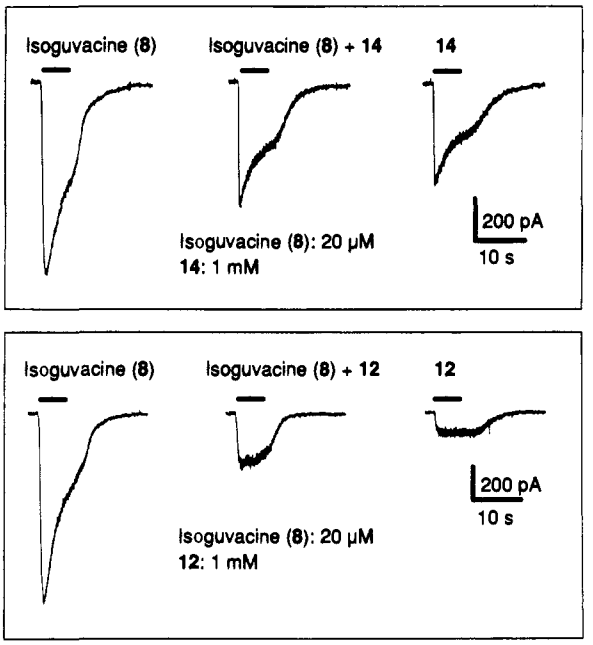


Figure 4. Examples of interaction of 1 mM **14** (upper panel) and 1 mM **12** (lower panel) with 20 μM isoguvacine (8). The traces show the change in whole-cell currents induced by the compounds. The horizontal bars mark the time of application of the compounds. Both of these partial agonists reduced the current response induced by 8 alone, and the combined responses approached the current response induced by the partial agonists alone. The relatively large responses induced by 1 mM **14** showed desensitization in all cells tested, while 1 mM **12** induced relatively smaller responses, which never desensitized.

on cultured cerebral cortex neurons involve the same ionic mechanism.

Discussion

There is a pharmacological and, perhaps, therapeutic interest in partial agonists at GABA$_A$ receptors in certain psychiatric and neurological disorders[3] (see the Introduction). A prerequisite for systematic studies of these novel pharmacological approaches is the availability of specific partial GABA$_A$ agonists showing a range of efficacy levels.

We have previously introduced THIP (**5**) as a potent and specific GABA$_A$ agonist[10,11,37] of relatively high efficacy[35,38] and 4-PIOL (**10**) as a specific low-efficacy partial GABA$_A$ agonist.[25,26] In contrast to **5**, which is systemically active in animals and man,[35,39] **10** is inactive after systemic administration to mice.[36] This difference can be explained on the basis of the difference between the pK_a(I) and pK_a(II) values and, consequently, the I/U ratios for these compounds (Table 1). Thus, the I/U ratio for **5**, i.e. the ratio between the concentrations of zwitterionic and unionized **5** in aqueous solution,[12,35] is about 1000, whereas this ratio for **10** is much higher (30 000).[36] These data may explain why **5**, but apparently not **10**, is capable of penetrating the blood–brain barrier (BBB).

Previous attempts to develop more potent analogs of **10** capable of penetrating the BBB have been unsuccessful.[25,40,41] Thus, the 2-piperidyl, 3-piperidyl, perhydroazepin-4-yl, and 3-pyrrolidinyl analogs of **10** as well as the analog in which the 3-isoxazolol nucleus was replaced by a 2-isoxazolin-3-ol unit did not interact detectably with GABA$_A$ receptor sites.[25,40,41] This structure–activity relationship (SAR) emphasizes the very

strict structural constraints imposed on agonists at $GABA_A$ receptors.[3,35,39] In the present drug design approach, we have synthesized and pharmacologically characterized a series of compounds bioisosterically derived from and structurally closely related to **10** (Figure 2). The target molecules of primary interest were selected on the basis of experience from previous medicinal chemistry projects in the $GABA_A$ agonist field.[3,35,39]

Whereas thiomuscimol (**3**) is slightly weaker than muscimol (**2**)[10] and Thio-THIP (**6**) markedly less active than **5**[12] as $GABA_A$ agonists, the 3-isothiazolols **11** and **14** (Figure 2) were shown to bind more tightly to $GABA_A$ receptor sites than **10**. Interestingly, **11** and **10** were capable of activating $GABA_A$ receptors with comparable efficacy, whereas compound **14** was significantly more efficacious (Figures 3A and 4). In contrast, the unsaturated analog of **10**, compound **13**, showed lower affinity (Table 1) and perhaps also lower efficacy than the parent compound (Figure 3B). The 5-isoxazolol analog, **12**, was the least efficacious of the active compounds, **10**–**14**, (Figures 3B and 4). This observation is interesting in light of the very low $GABA_A$ agonist potency of isomuscimol (**4**)[10] and the $GABA_A$ antagonist profile of Iso-THIP (**7**),[13] since both of these compounds contain 5-isoxazolol moieties (Figure 1).

The amino carboxylic acids, isoguvacine (**8**) and isonipecotic acid (**9**), which are derived from **5** (Figure 1), are at least as potent as the parent compound.[10,11] This observation prompted us to synthesize a series of saturated and unsaturated amino carboxylic acid analogs of **10**, compounds **15**–**18** (Figure 2). Quite surprisingly, only one of these amino acids, **16**, showed detectable, but very weak, $GABA_A$ receptor affinity (Table 1).

The SARs for the 4-PIOL analogs depicted in Figure 2 are only to a limited extent analogous with those described for analogs of muscimol (**2**) and **5**,[35,39] and there are no conspicuous relationships between pK_a values, receptor affinity (Table 1), and agonist efficacy (Figure 3A,B) for these $GABA_A$ receptor ligands. On the basis of molecular-modeling analyses, we have previously proposed that **10** may adopt a somewhat bent conformation in order to fit into a $GABA_A$ agonist pharmacophore defined by the potent and specific agonist, **5**.[42] On the basis of this model, the unsaturated analogs of **10** and **11**, compounds **13** and **14**, respectively, were predicted to bind to $GABA_A$ receptor sites with lower affinity and to show pharmacological profiles different from those of **10** and **11**. Whereas **13** actually did show a lower affinity as well as a lower efficacy, as compared with **10** (Table 1 and Figure 3B), the SAR for the corresponding 3-isothiazolols, **11** and **14**, was strikingly different (Figures 3A and 4).

Since the 5-isoxazolol/2-isoxazolin-5-one nucleus has acylating properties,[43] compound **12** may have the capacity for interacting irreversibly with the $GABA_A$ recognition sites.

In conclusion, we have synthesized and pharmacologically characterized a series of partial $GABA_A$ agonists showing a range of relative efficacies. These compounds may be useful pharmacological tools for drug research projects in the fields of Alzheimer's disease, schizophrenia, and epilepsy. Whereas **10** does not seem to penetrate the BBB,[36] the relatively low I/U ratio for compound **11** (5000) (Table 1) suggests that this compound may be a useful model drug in animal behavioral studies. The SARs for the series of compounds described here are not straightforward and do not seem to fit into a previously described $GABA_A$ agonist pharmacophore model.[42] These aspects will be the subject of computational studies.

Experimental Section

Chemistry. General Procedures. Melting points were determined in capillary tubes and are uncorrected. ^1H NMR spectra were recorded on a Bruker AC-200 F (200 MHz) or a Varian EM 360L (60 MHz) instrument in $CDCl_3$ solutions using TMS as an internal standard or in D_2O solutions using 1,4-dioxane as an internal standard. Column chromatography (CC) and flash chromatography (FC) were performed on Merck silica gel 60 (0.06–0.200 mm) and Merck silica gel 60H (5–40 μM), respectively. 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 spraying reagent. Compounds containing the 3-isoxazolol or 3-isothiazolol units were visualized using a $FeCl_3$ spraying reagent. Elemental analyses were performed by Mr. G. Cornali, Microanalytical Laboratory, LEO Pharmaceutical Products, Denmark, or by Mr. P. Hansen, Department of General and Organic Chemistry, University of Copenhagen, and are within ±0.4% of the calculated values, unless otherwise stated. Evaporations were performed under vacuum on a rotary evaporator at 15 mmHg.

Determination of the Stoichiometric pK_a Values and Calculation of I/U Ratios. pK_a determinations were performed on an interconnected automatic titrator TitriLab system consisting of a burette station ABU 93 Triburette, a control unit VIT90 Video Titrator, and a sample station SAM90 from the Analytical Instruments Division of Radiometer A/S, Emdrupvej 72, DK - 2400 Copenhagen NV, Denmark, using the following Radiometer electrodes: glass electrode (pHG 201) and reference electrode (ref 201, Ag/AgCl). Titration curves are fitted by a weighted least squares method. The I/U ratio[12] for compound 11 (5000) was calculated on the basis of the pK_a values for 11 (6.9, 10.7) and the N-protected derivative, **22** (7.0).

3-[1-(Methoxycarbonyl)-4-piperidyl]-3-oxopropanamide (20). A mixture of **19**[25] (10.0 g, 39 mmol) and aqueous ammonia (100 mL, 23%) was stirred at 0 °C for 2 h and at room temperature for 3 days. The solution was evaporated and the residue subjected to CC (EtOAc/MeOH (9:1)). Recrystallization (EtOAc) gave **20** (5.9 g, 67%): mp 76–79 °C; ^1H NMR (200 MHz, $CDCl_3$) δ 6.93 (1H, br s), 5.95 (1H, br s), 4.15 (2H, br s), 3.69 (3H, s), 3.48 (2H, s), 2.86 (2H, br t, J = 11.7 Hz), 2.75–2.55 (1H, m), 1.96–1.78 (2H, m), 1.65–1.40 (2H, m). Anal. ($C_{10}H_{16}N_2O_4$) C, H, N.

3-[1-(Methoxycarbonyl)-4-piperidyl]-3-thioxopropanamide (21). To a saturated solution of HCl in EtOH (150 mL) was added H_2S for 20 min at −5 °C. A solution of **20** (5.8 g, 25.4 mmol) in EtOH (8 mL) was added slowly at −5 °C, followed by addition of excess H_2S in a vigrous flow for 3 h. The reaction mixture was kept at −18 °C for 16 h, and the precipitate was collected and washed with EtOAc to give crude **21** (3.3 g, 53%): mp 102–106 °C; ^1H NMR (200 MHz, $CDCl_3$) δ 8.85 (3H, br s), 6.56 (1H, s), 4.21 (2H, br s), 3.70 (3H, s), 3.14–2.84 (3H, m), 2.02 (2H, m), 1.60 (2H, m).

5-[1-(Methoxycarbonyl)-4-piperidyl]isothiazol-3-ol (22). K_2CO_3 (2.1 g, 15 mmol) was added to an ice-cooled solution of crude **21** (1.0 g, ca. 4.0 mmol) in EtOH (10 mL), followed by dropwise addition of a solution of iodine (950 mg, 4.0 mmol) in EtOH (10 mL). Stirring was continued at room temperature for 16 h. After evaporation, the residue was dissolved in H_2O (50 mL), acidified with H_2SO_4 (2 M), and extracted with Et_2O (3 × 75 mL). The combined extracts were dried and evaporated. CC (toluene/EtOAc (1:1) containing AcOH (1%)) of the crude product followed by recrystallization (toluene/light petroleum) gave **22** (567 mg, 59%) as light brown crystals: mp 167–168 °C; ^1H NMR (200 MHz, $CDCl_3$) δ 10.5 (1H, br s), 6.32 (1H, s), 4.24 (2H, br s), 3.70 (3H, s), 3.05–2.84 (3H, m), 2.00

(2H, br d, J = 12.7 Hz), 1.62 (2H, dq, J = 12.7 and 4.1 Hz). Anal. ($C_{10}H_{14}N_2O_3S$) C, H, N, S.

5-(4-Piperidyl)isothiazol-3-ol Hydrobromide (11). A solution of **22** (3.2 g, 13.2 mmol) in a solution of HBr in AcOH (33%, 150 mL) was stirred at room temperature for 16 h. The reaction mixture was evaporated, and the residue was recrystallized (MeOH/Et_2O) to give 11 (2.9 g, 82%): mp 215 °C dec; ^1H NMR (200 MHz, D_2O) δ 6.28 (1H, s), 3.48 (2H, br d, J = 13 Hz), 3.28–3.04 (3H, m), 2.23 (2H, br d, J = 13 Hz), 1.75–1.20 (2H, m). Anal. ($C_8H_{12}N_2OS$·HBr) C, H, Br, N, S.

3-Ethoxy-5-[1-(methoxycarbonyl)-4-piperidyl]isothiazole (23). To a solution of **22** (200 mg, 0.8 mmol) in acetone (5 mL) was added K_2CO_3 (250 mg, 2.5 mmol), and the mixture was stirred at 60 °C for 1 h. Ethyl bromide (130 μL, 1.7 mmol) was added to the mixture, and stirring was continued at 60 °C for 16 h. The reaction mixture was cooled, filtered, and evaporated, followed by CC (toluene/EtOAc (10:1)), to afford **23** (148 mg, 66%) as a light yellow oil: ^1H NMR (200 MHz, $CDCl_3$) δ 6.35 (1H, s), 4.35 (2H, q, J = 7.1 Hz), 4.20 (2H, br s), 3.70 (3H, s), 3.03–2.83 (3H, m), 1.97 (2H, br d), 1.58 (2H, dq, J = 12.2 and 4.2 Hz), 1.37 (3H, t, J = 7.1 Hz). Anal. ($C_{12}H_{18}N_2O_3S$) C, H, N, S.

5-[4-Bromo-1-(methoxycarbonyl)-4-piperidyl]-3-ethoxyisothiazole (24). A solution of **23** (229 mg, 0.85 mmol) in CCl_4 (15 mL) was treated under reflux with NBS (a total of 150 mg, 0.8 mmol) and benzoyl peroxide (a total of 15 mg, 0.06 mmol) over a period of 1.5 h. Each of the reagents was added in three equal portions every 30 min. Filtration and evaporation followed by CC (toluene/EtOAc (5:1)) gave **24** as a colorless oil (221 mg, 75%): ^1H NMR (200 MHz, $CDCl_3$) δ 6.61 (1H, s), 4.38 (2H, q, J = 7.1 Hz), 4.20 (2H, br d, J = 12.6 Hz), 3.71 (3H, s), 3.43–3.29 (2H, m), 2.37–2.30 (2H, m), 2.11–1.96 (2H, m), 1.39 (2H, t, J = 7.1 Hz). Anal. ($C_{12}H_{17}BrN_2O_3S$) C, H, Br, N, S.

5-(1,2,3,6-Tetrahydropyrid-4-yl)isothiazol-3-ol Hydrobromide (14). A solution of **24** (100 mg, 0.3 mmol) in HBr (48%, 5 mL) was refluxed for 4 h and evaporated. The residue was evaporated from toluene and recrystallized (MeOH/Et_2O) to give **14** (39 mg, 55%): mp 190 °C dec; ^1H NMR (200 MHz, D_2O) δ 6.43 (1H, s), 6.27 (1H, br s), 3.84 (2H, br s), 3.44 (2H, t, J = 6.1 Hz), 2.80–2.68 (2H, m). Anal. ($C_8H_{10}N_2OS$·HBr) C, H, N, S.

Ethyl [1-(Benzyloxycarbonyl)-4-piperidyl]-3-oxopropanoate (26). To a solution of *N,N'*-thionyldiimidazole[44] in dry THF (100 mL) was added dropwise a solution of **25**[45] (26.3 g, 100 mmol) in THF (50 mL). The reaction mixture was protected from light and stirred at room temperature for 20 h. This solution was added dropwise to a suspension of [3-ethoxy-3-hydroxyacrylato(2−)-O^1,O^3]magnesate(2+)[44] (300 mmol) in THF (300 mL). The mixture was stirred mechanically for 2 h and acidified with H_2SO_4 (4 M). Most of the THF was distilled off in vacuo, and the aqueous residue was extracted with Et_2O (3 × 150 mL). The combined extracts were washed with dilute $NaHCO_3$ and H_2O, dried, and evaporated. The crude product was purified by FC (toluene/EtOAc (9:1)) to give crude **26** (29.5 g, 88%) as an oil. Attempts to distill **26** led to decomposition: ^1H NMR (60 MHz, $CDCl_3$) δ 7.35 (5H, s), 7.20 (0.9H, s), 5.10 (2H, s), 4.20 (2H, q, J = 7 Hz), 4.3–3.9 (2H, m), 3.45 (1.1H, s), 3.2–2.4 (3H, m), 1.9–1.4 (4H, m), 1.25 (3H, t, J = 7 Hz).

5-[1-(Benzyloxycarbonyl)-4-piperidyl]isoxazol-3-ol (27) and 3-[1-(Benzyloxycarbonyl)-4-piperidyl]isoxazol-5-ol (28). A solution of hydroxylamine hydrochloride (1.3 g, 19 mmol) in NaOH (1 M, 20 mL) at 2 °C was adjusted to pH 10 with 1 M NaOH. The pH of the reaction mixture was kept at 10.0 ± 0.2 by using a pH-state (TTT80 combined with an ABU 80 autoburette, both from Radiometer, Copenhagen), while crude **26** (5.0 g, ca. 15 mmol) was added dropwise over a period of 1 h. The mixture was stirred at 2 °C for 30 min, and concentrated HCl (15 mL) was added in one portion. The mixture was kept at 5 °C for 20 h and extracted with CH_2Cl_2 (3 × 50 mL). The extracts were dried and evaporated, and the residue was submitted to FC (toluene/AcOH (99:1)). The first fractions contained **27** (2.19 g, 48%). A sample was recrystallized (EtOAc/light petroleum) to give **27**: mp 109–111 °C; ^1H NMR (60 MHz, $CDCl_3$) δ 7.20 (5H, s), 5.40 (1H, s), 4.95 (2H, s), 4.2–3.8 (2H, m), 3.1–2.5 (3H, m), 2.2–1.3 (4H, m). Anal. ($C_{16}H_{18}N_2O_4$) C, H, N. The later fractions contained **28** (1.19 g, 26%). Recrystallization (EtOAc/light petroleum) gave **28**: mp 124–126 °C; ^1H NMR (60 MHz, $CDCl_3$) δ 7.15 (5H, s), 5.10 (2H, s), 4.2 (1H, distorted t, J = 5 Hz), 3.95 (1H, distorted t, J = 5 Hz), 3.35 (2H, s), 3.2–2.4 (3H, m), 2.1–1.3 (4H, m). Anal. ($C_{16}H_{18}N_2O_4$) C, H, N.

3-(4-Piperidyl)isoxazol-5-ol (12). A solution of **28** (2.0 g, 6.6 mmol) in HBr in AcOH (33%, 9 mL) was stirred at room temperature for 3 min. Et_2O (50 mL) was added to precipitate the hydrobromide salt of **12**. A sample of the salt was recrystallized from MeOH/Et_2O to give **12**·HBr: mp 176–179 °C. Anal. ($C_8H_{12}N_2O_2$·HBr) C, H, Br, N. The salt darkened on standing, and zwitterionic **12** was prepared by ion exchange chromatography (IRA-400) using 1 M AcOH as an eluent. The fractions containing **12** were collected and evaporated. The residue was dissolved in H_2O and precipitated with EtOH to give **12** (644 mg, 54%): mp 255–260 °C dec; ^1H NMR (200 MHz, D_2O + DMSO-d_6) δ 3.5–3.35 (2H, m), 3.06 (2H, t), 2.9–2.7 (1H, m), 2.58 (1H, br s), 2.2–1.95 (2H, m), 1.9–1.65 (2H, m). Anal. ($C_8H_{12}N_2O_2$·$^2/_3$ H_2O) C, H, N.

(2*RS*,3*SR*)-Methyl 2,3-Dibromo-3-(4-pyridyl)propanoate Hydrobromide (30). To a solution of **29**[46] (9.79 g, 60 mmol) in AcOH (90 mL) was added a solution of HBr in AcOH (4.1 M, 14.6 mL, 60 mmol). Bromine (3.1 mL, 60 mmol) dissolved in AcOH (90 mL) was added dropwise, and the mixture was stirred at 20 °C for 30 min and then at 60 °C for 3 h. After the mixture cooled, the precipitate (21.9 g, 90%) was collected. A sample was recrystallized (MeOH/Et_2O) to give **30**: mp 165–168 °C; ^1H NMR (60 MHz, D_2O + DMSO-d_6) δ 8.85 (2H, d, J = 7 Hz), 8.10 (2H, d, J = 7 Hz), 5.70 (1H, d, J = 12 Hz), 5.30 (1H, d, J = 12 Hz), 3.85 (3H, s). Anal. ($C_9H_9Br_2NO_2$·HBr) C, H, Br, N.

2-Bromo-3-(4-pyridyl)propenohydroxamic Acid (31). Hydroxylamine hydrochloride (0.87 g, 12.5 mmol) was added to a solution of NaOH (1.2 g, 30 mmol) in H_2O (5 mL) and MeOH (5 mL). The mixture was stirred at 0 °C, and **30** (1.21 g, 3.0 mmol) was added in portions during a period of 30 min. After stirring at 0 °C for 1 h, the reaction mixture was neutralized (pH 6) with concentrated HBr, and the precipitate (0.53 g, 73%) was collected. Recrystallization (MeOH) gave **31**: mp 148–152 °C dec; ^1H NMR (60 MHz, DMSO-d_6) δ 8.60 (2H, dd, J = 2 and 7 Hz), 7.35 (2H, dd, J = 2 and 7 Hz), 7.20 (1H, s). Anal. ($C_8H_7BrN_2O_2$) C, H, Br, N.

5-(4-Pyridyl)isoxazol-3-ol (32). To an ice-cooled solution of NaOH (5.6 g, 140 mmol) in MeOH (100 mL) was added hydroxylamine hydrochloride (3.48 g, 50 mmol). The mixture was stirred at 0 °C for 10 min, and **30** (8.08 g, 20 mmol) was added in portions during a period of 1 h. The mixture was stirred at 0 °C for 1 h and then refluxed for 2 h. After evaporation, H_2O (50 mL) was added to the residue, and the pH of the solution was adjusted to 4 with concentrated HCl. The precipitate was collected, washed with H_2O, and recrystallized (aqueous MeOH) to give **32** (2.35 g, 72%): mp 236–240 °C dec; ^1H NMR (200 MHz, DMSO-d_6) δ 11.65 (1H, s), 8.72 (2H, d, J = 7 Hz), 7.76 (2H, d, J = 7 Hz), 6.85 (1H, s). Anal. ($C_8H_8N_2O_2$) C, H, N.

From 31: A mixture of **31** (73 mg, 0.30 mmol), NaOH (60 mg, 1.5 mmol), and MeOH (10 mL) was refluxed for 1 h. The reaction mixture was evaporated, and H_2O (2 mL) was added to the residue. Neutralization (pH 4) with HCl (4 M) precipitated **32** (42 mg, 86%), identical (IR, NMR, and TLC) with the compound prepared from **30**.

3-[(4-Methoxybenzyl)oxy]-5-(4-pyridyl)isoxazole (33). A mixture of **32** (1.04 g, 6.4 mmol) and K_2CO_3 (1.17 g, 8.5 mmol) in DMF (15 mL) was stirred at 60 °C for 1 h. 4-Methoxybenzyl chloride (1.00 mL, 7.3 mmol) was added, and the mixture was stirred at 60 °C for 20 h. After evaporation, H_2O (15 mL) was added to the residue, and the mixture was extracted with CH_2Cl_2 (3 × 20 mL). The combined extracts were dried and evaporated, and the crude product was purified by FC (toluene containing increasing amounts of EtOAc) to afford **33** (1.33 g, 74%). A sample was recrystallized (EtOH): mp 107–109 °C; ^1H NMR (60 MHz, $CDCl_3$) δ 8.70 (2H, d, J = 7 Hz), 7.55 (2H, d, J = 7 Hz), 7.35 (2H, d, J = 9 Hz), 6.85 (2H, d, J = 9 Hz), 6.25 (1H, s), 5.20 (2H, s), 3.75 (3H, s). Anal. ($C_{16}H_{14}N_2O_3$) C, H, N.

1-Methyl-4-[3-[(4-methoxybenzyl)oxy]isoxazol-5-yl]-

pyridinium Iodide (34). A mixture of **33** (1.13 g, 4.0 mmol) and methyl iodide (2.5 mL, 40 mmol) in EtOH (40 mL) was stirred at 40 °C for 24 h. Cooling afforded **34** (1.35 g, 80%): mp 163–165 °C; ^{1}H NMR (60 MHz, DMSO-d_6) δ 9.20 (2H, d, J = 8 Hz), 8.45 (2H, d, J = 8 Hz), 7.45 (1H, s), 7.30 (2H, d, J = 9 Hz), 6.90 (2H, d, J = 9 Hz), 5.20 (2H, s), 4.30 (3H, s), 3.70 (3H, s). Anal. ($C_{17}H_{17}IN_2O_3$) C, H, I, N.

3-[(4-Methoxybenzyl)oxy]-5-(1-methyl-1,2,3,6-tetrahydropyrid-4-yl)isoxazole Hydrochloride (35). Sodium borohydride (0.85 g, 22 mmol) was added portionwise at −5 °C to a suspension of **34** (1.59 g, 3.75 mmol) in MeOH (35 mL). The mixture was stirred at 0 °C for 1 h and then at room temperature overnight. After evaporation, H_2O (30 mL) was added, and the mixture was extracted with CH_2Cl_2 (3 × 50 mL). The organic extracts were dried and evaporated, and the crude product was purified by FC (EtOAc/light petroleum/Et_3N (5:5:1)) to afford **35** as the free base. The hydrochloride salt was precipitated from a solution of the free base in Et_2O by addition of excess HCl in EtOAc. Recrystallization from EtOH/MeCN/Et_2O gave **35** (0.80 g, 63%): mp 191–193 °C; ^{1}H NMR (60 MHz, D_2O + DMSO-d_6) δ 7.35 (2H, d, J = 9 Hz), 6.85 (2H, d, J = 9 Hz), 6.35 (1H, m), 6.15 (1H, s), 5.10 (2H, s), 3.9–3.6 (2H, m), 3.70 (3H, s), 3.6–3.2 (2H, m), 2.90 (3H, s), 2.9–2.5 (2H, m). Anal. ($C_{17}H_{20}N_2O_3$·HCl) C, H, Cl, N.

5-(1,2,3,6-Tetrahydropyrid-4-yl)isoxazol-3-ol Hydrochloride (13). A solution of **35** (0.27 g, 0.8 mmol), 1,2,2,6,6-pentamethylpiperidine (0.72 mL, 4.0 mmol), and 1-chloroethyl chloroformate (0.69 mL, 6.4 mmol) in 1,2-dichloroethane (10 mL) was refluxed for 1.5 h. After evaporation, the residue was extracted with Et_2O (3 × 15 mL), and the combined extracts were evaporated. The residue was purified by FC (toluene/EtOAc (9:1)) to give crude **36** (0.26 g, 83%) as a light brown oil: ^{1}H NMR (60 MHz, $CDCl_3$) δ 7.20 (2H, d, J = 9 Hz), 6.75 (2H, d, J = 9 Hz), 6.45 (1H, q, J = 7 Hz), 6.25 (1H, m), 5.60 (1H, s), 5.05 (2H, s), 4.05 (2H, m), 3.70 (3H, s), 3.55 (2H, t), 2.5–2.2 (2H, m), 1.75 (3H, d, J = 7 Hz). To a solution of **36** (0.25 g, 0.64 mmol) in MeOH (8 mL) was added HCl in EtOAc (2.5 M, 8 mL). The mixture was refluxed for 2 h and evaporated. The residue was recrystallized (MeOH/Et_2O) to give **13** (76 mg, 47%): mp >250 °C; ^{1}H NMR (200 MHz, D_2O) δ 6.51 (1H, m), 6.12 (1H, s), 3.93 (2H, m), 3.49 (2H, t), 2.72 (2H, m). Anal. ($C_8H_{10}N_2O_2$·HCl) C, H, Cl, N.

(*E*)-1-Methyl-4-[2-(methoxycarbonyl)ethen-1-yl]pyridinium Iodide (37). Methyl iodide (20 mL, 320 mmol) was added to a solution of **29**[46] (10.9 g, 66.6 mmol) in EtOH (150 mL), and the mixture was stirred at 40 °C for 20 h. After the mixture cooled, Et_2O (75 mL) was added to precipitate **37** (19.3 g, 95%). A sample was recrystallized (EtOH) to give **37**: mp 186–190 °C dec; ^{1}H NMR (200 MHz, D_2O) δ 8.70 (2H, d, J = 6 Hz), 8.07 (2H, d, J = 6 Hz), 7.72 (1H, d, J = 15 Hz), 6.96 (1H, d, J = 15 Hz), 4.30 (3H, s), 3.81 (3H, s). Anal. ($C_{10}H_{12}INO_2$) C, H, I, N.

(*E*)-Methyl 3-(1-Methyl-1,2,3,6-tetrahydropyrid-4-yl)propenoate Sesquifumarate (38). Sodium borohydride (1.67 g, 44 mmol) was added in small portions to a solution of **37** (10.1 g, 33 mmol) in MeOH (100 mL) at −5 °C. The mixture was stirred at 0 °C for 2 h and then at room temperature for 20 h. After evaporation, H_2O (100 mL) was added to the residue, and the mixture was extracted with Et_2O (3 × 125 mL). The organic extracts were dried and evaporated, and the crude product was purified by FC (toluene/EtOAc (1:1)) to give the free base of **38** (3.57 g, 60%) as an oil. The crude free base was dissolved in Et_2O (150 mL), and a warm solution of fumaric acid (2.49 g, 29 mmol) in 2-propanol (45 mL) was added to precipitate **38**. Recrystallisation (EtOH/MeCN) gave **38** (5.74 g, 49%): mp 133–135 °C; ^{1}H NMR (200 MHz, D_2O) δ 7.27 (1H, d, J = 15 Hz), 6.64 (3H, s), 6.08 (1H, m), 5.94 (1H, d, J = 15 Hz), 3.9–3.7 (2H, m), 3.66 (3H, s), 3.5 (1H, m), 3.2 (1H, m), 2.88 (3H, s), 2.55 (2H, m). Anal. ($C_{10}H_{15}NO_2$·1.5 x $C_4H_4O_4$) C, H, N.

(*E*)-Methyl 3-(1,2,3,6-Tetrahydropyrid-4-yl)propenoate Hydrochloride (40). An ice-cooled solution of **38** (4.76 g, 16 mmol) in NaOH (1.5 M, 85 mL) was rapidly extracted with CH_2Cl_2 (3 × 100 mL). The organic extracts were dried and evaporated. The residue was dissolved in 1,2-dichloroethane (125 mL), and a solution of 1-chloroethyl chloroformate (12.2 mL, 112 mmol) in 1,2-dichloroethane (20 mL) was added dropwise. The mixture was refluxed for 2 h and evaporated. Et_2O (100 mL) was added to the residue, the mixture filtered, and the filtrate evaporated to give **39** (3.3 g, 76%) as a yellow oil. A solution of **39** in MeOH (40 mL) was refluxed for 1 h and evaporated. The residue was recrystallized (EtOH/MeCN) to give **40** (1.11 g, 34%): mp 218–219 °C; ^{1}H NMR (200 MHz, D_2O) δ 7.38 (1H, d, J = 16 Hz), 6.24 (1H, m), 6.01 (1H, d, J = 16 Hz), 3.89 (2H, m), 3.78 (3H, s), 3.44 (2H, t, J = 7 Hz), 2.59 (2H, m). Anal. ($C_9H_{13}NO_2$·HCl) C, H, Cl, N.

(*E*)-3-(1,2,3,6-Tetrahydropyrid-4-yl)propenoic Acid Hydrochloride (15). A solution of **40** (0.57 g, 2.8 mmol) in HCl (1 M, 15 mL) was refluxed for 2 h and evaporated. The residue was recrystallized (aqueous MeOH) to give **15** (0.36 g, 68%): mp 261–263 °C; ^{1}H NMR (200 MHz, D_2O) δ 7.36 (1H, d, J = 16 Hz), 6.23 (1H, m), 5.99 (1H, d, J = 16 Hz), 3.89 (2H, m), 3.46 (2H, t, J = 7 Hz), 2.59 (2H, m). Anal. ($C_8H_{11}NO_2$·HCl) C, H, Cl, N.

Methyl 3-(4-Piperidyl)propanoate Hydrochloride (41). A mixture of **40** (0.50 g, 2.45 mmol) and 5% Pd on carbon (100 mg) in H_2O (15 mL) was hydrogenated at 300 kPa for 1 h. The mixture was filtered and evaporated, and the residue was recrystallized (MeOH/Et_2O) to give **41** (0.35 g, 69%): mp 124–127 °C; ^{1}H NMR (60 MHz, D_2O) δ 3.70 (3H, s), 3.65–3.3 (2H, m), 3.05–2.65 (2H, m), 2.50 (2H, t, J = 6 Hz), 2.1–1.4 (7H, m). Anal. ($C_9H_{17}NO_2$·HCl) C, H, Cl, N.

3-(4-Piperidyl)propanoic Acid Hydrochloride (18). A solution of **41** (0.25 g, 1.2 mmol) in HCl (4 M, 5 mL) was refluxed for 1 h and evaporated. The residue was recrystallized (EtOH/MeCN/Et_2O) to give **18**[48] (183 mg, 79%): mp 235–239 °C; ^{1}H NMR (60 MHz, D_2O) δ 3.6–3.1 (2H, m), 3.1–2.75 (2H, m), 2.45 (2H, t), 2.2–1.15 (7H, m). Anal. ($C_8H_{15}NO_2$·HCl) C, H, Cl, N.

1-Methyl-4-[2-(methoxycarbonyl)ethyl]pyridinium Iodide (43). Compound **43** was synthesized as described above for **37** by using **42**[47] (10.0 g, 60.5 mmol) in MeOH (200 mL) and methyl iodide (19 mL, 304 mmol). The reaction gave **43** (14.3 g, 77%): mp 89–90 °C; ^{1}H NMR (200 MHz, D_2O) δ 8.59 (2H, d, J = 7.1 Hz), 7.88 (2H, d, J = 7.1 Hz), 4.29 (3H, s), 3.62 (3H, s), 3.21 (2H, t, J = 7.0 Hz), 2.89 (2H, t, J = 7.0 Hz). Anal. ($C_{10}H_{14}INO_2$) C, H, I, N.

Methyl 3-(1-Methyl-1,2,3,6-tetrahydropyrid-4-yl)propanoate (44). Compound **44** was synthesized as described above for **38** by using **43** (6.5 g, 21.1 mmol) and sodium borohydride (1.1 g, 29 mmol) in MeOH (90 mL). The crude product was purified by CC (light petroleum/EtOAc/Et_3N (9:9:2)) to give **44** (2.6 g, 68%) as an oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.40 (2H, m), 3.66 (3H, s), 2.88 (2H, m), 2.51–2.31 (4H, m), 2.33 (3H, s), 2.12 (2H, m). Anal. ($C_{10}H_{17}NO_2$) C, H, N.

3-(1,2,3,6-Tetrahydropyrid-4-yl)propanoic Acid Hydrochloride (17). Compound **17** was synthesized as described for **15** by using **44** (470 mg, 2.6 mmol) in 1,2-dichloroethane (20 mL) and 1-chloroethyl chloroformate (2.0 mL, 18 mmol) in 1,2-dichloroethane (3 mL) to give the intermediate **45** (510 mg, 75%) as a yellow oil. A solution of crude **45** in MeOH (20 mL) was refluxed for 1 h and evaporated. The residue was refluxed in HCl (2 M, 10 mL) for 2 h and evaporated. Recrystallization (EtOH/Et_2O) of the residue gave **17** (90 mg, 18%): mp 217–219 °C; ^{1}H NMR (200 MHz, D_2O) δ 5.42 (1H, broad s), 3.60 (2H, broad s), 3.28 (2H, m), 2.28 (4H, m). Anal. ($C_8H_{13}NO_2$·HCl) C, H, Cl, N.

Ethyl (*RS*)-3-Hydroxy-3-[1-(methoxycarbonyl)-4-piperidyl]propanoate (46). A solution of **19**[25] (10.5 g, 40.8 mmol) in EtOH (100 mL) was hydrogenated (300 kPa) in a Parr hydrogenation apparatus using Ra nickel, W2 (prepared from 15 g of $NiAl_2$ alloy).[49] The reaction mixture was filtered and evaporated to dryness, and the resulting oil was subjected to CC (toluene containing EtOAc (25–33%)) to give TLC pure **46** (7.34 g, 66%). Ball–tube distillation (15 Pa; oven temperature, 250 °C) gave **46** (4.44 g, 61%): ^{1}H NMR (60 MHz, $CDCl_3$ + D_2O) δ 4.5–3.7 (3H, m), 4.18 (2H, q, J = 7 Hz), 3.7 (3H, s), 3.0–2.6 (2H, m), 2.6–2.3 (2H, m), 2.0–1.0 (5H, m), 1.25 (3H, t, J = 7 Hz). Anal. ($C_{12}H_{21}NO_5$) H, N; C: calcd, 55.59; found, 54.91.

Ethyl (*RS*)-3-Acetoxy-3-[1-(methoxycarbonyl)-4-piperidyl]propanoate (47). $(Ac)_2O$ (3.6 mL, 38.1 mmol) was added to an ice-cooled solution of **46** (3.0 g, 11.6 mmol) in

EtOAc (30 mL) and piperidine (30 mL). After stirring at 60 °C overnight, the reaction mixture was evaporated and re-evaporated twice from toluene. The residue was dissolved in CH_2Cl_2, and the organic phase was washed with NaOH (0.2 M), dried, and evaporated. The resulting oil was subjected to CC (toluene/EtOAc (2:1)) to give TLC pure **47** (3.4 g, 97%). Ball–tube distillation (25 Pa; oven temperature, 250 °C) of a sample gave analytically pure **47**: ^{1}H NMR (60 MHz, $CDCl_3$) δ 5.0 (1H, m), 4.0 (2H, q, J = 7 Hz), 4.3–3.7 (2H, m), 3.6 (3H, s), 2.9–2.3 (4H, m), 2.0 (3H, s), 1.8–0.8 (5H, m), 1.2 (3H, t, J = 7 Hz). Anal. ($C_{14}H_{23}NO_6$) C, H, N.

(*E*)-3-[1-(Methoxycarbonyl)-4-piperidyl]propenoic Acid (48). A solution of NaOH (0.2 M, 54 mL, 10.8 mmol) was added to a stirred solution of **47** (1.30 g, 5.39 mmol) in EtOH (30 mL). After stirring for 3 h at room temperature, the reaction mixture was concentrated, acidified with HCl (1 M) at 0 °C, and extracted three times with EtOAc. The combined organic phases were dried and evaporated to give **48** (1.05 g, 91%). A small sample was recrystallized (EtOAc/Et_2O/light petroleum): mp 116–117 °C; ^{1}H NMR (60 MHz, $CDCl_3$) δ 9.15 (1H, br s), 6.85 (1H, dd, J = 6 and 15 Hz), 5.65 (1H, dd, J = 2 and 15 Hz), 4.05 (2H, m), 3.60 (3H, s), 2.75 (2H, m), 2.5–2.0 (1H, m). Anal. ($C_{10}H_{15}NO_4$) C, H, N.

(*E*)-3-(4-Piperidyl)propenoic Acid Hydrochloride (16). A solution of NaOH (1.2 g, 30 mmol) in MeOH (23 mL) and H_2O (0.8 mL) was added to 48 (640 mg, 3.0 mmol). The reaction mixture was refluxed for 3 days, acidified with HCl (2 M), and evaporated. The residue was extracted three times with boiling EtOH, and the extracts were evaporated. Recrystallization (EtOH) gave **16** (200 mg, 35%). Further recrystallization (EtOH/H_2O/MeCN (1:1:4)) gave **16**: mp >250 °C; ^{1}H NMR (200 MHz, D_2O) δ 6.90 (1H, dd, J = 6.5 and 15.9 Hz), 5.88 (1H, d, J = 15.9 Hz), 3.41 (2H, m), 3.08 (2H, m), 2.55 (1H, m), 2.01 (2H, m), 1.60 (2H, m). Anal. ($C_8H_{13}NO_2$·HCl·0.1H_2O) C, H, Cl, N.

Receptor-Binding and Uptake Assays. $GABA_A$ and $GABA_B$ receptor-binding assays were performed using rat brain synaptic membranes from Sprague-Dawley rats, and tissue preparation was performed as described by Ransom and Stec.[50] On the day of the assay, the membrane preparation was thawed at room temperature for 45 min, suspended in 75 volumes (w/v) of 5 mM Tris-HCl buffer (pH 7.1) using an Ultra-Turrax homogenizer, and centrifuged at 48000g for 20 min at 4 °C (Sorvall, rotor SM 34). This step was repeated four times. The final pellet was resuspended in incubation buffer from the relevant binding assay. $GABA_A$ binding was studied using a modified version of the method described previously.[51] The assay was carried out in triplicate by incubation of synaptic membranes (0.4–0.5 mg of protein) in 0.75 mL of Tris-HCl buffer (50 mM, pH 7.1), 0.1 mL of 100 μM baclofen, 0.05 mL of 0.1 μM [^{3}H]GABA, and 0.1 mL of the test substance at various concentrations. Following incubation at 0 °C for 30 min, the samples were filtered through Whatman GF/B filters, which were washed with 2 × 5 mL of ice-cooled buffer. IC_{50} values were determined by measuring the inhibition of at least four different concentrations of test compound. Nonspecific binding in the presence of 0.1 mM THIP (**5**) was subtracted. $GABA_B$ binding was carried out in triplicate by incubation of membranes (0.4–0.5 mg of protein) in 0.75 mL of Tris-HCl buffer (50 mM + 2.5 mM of $CaCl_2$, pH 7.1), 0.1 mL of isoguvacine (8) (200 μM), 0.05 mL of [^{3}H]GABA (0.1 μM), and 0.1 mL of the test substances at various concentrations. Following incubation at 24 °C for 45 min, the bound ligand was isolated as described for $GABA_A$ receptor binding. Nonspecific binding in the presence of 0.1 mM baclofen was subtracted.

The effects on GABA uptake were studied using a crude synaptosomal preparation, prepared from rat brains as described elsewhere in detail.[52] The whole brains were homogenized in 10 volumes of ice-cold 0.32 M sucrose, and the homogenate was centrifuged at 600g at 4 °C for 10 min. The pellet was discarded and the supernatant centrifuged at 25000g at 4 °C for 55 min. The pellet fraction was resuspended in 40 volumes of oxygenated phosphate medium at 0 °C. The synaptosome suspensions (500 μL) were preincubated for 10 min at 25 °C with 1.9 mL of phosphate medium containing the inhibitor. Then [^{3}H]GABA (100 μL) was added to give a final GABA concentration of 50 nM, and the incubation was continued for a further 10 min. The synaptosomes were isolated by rapid filtration through Whatman GF/C glass fiber filters, and the filters were washed with ice-cold phosphate medium (10 mL).

Electrophysiology in Vitro. Cerebral cortical neurons were cultured essentially as described by Herts et al.[53] from 15-day-old mouse embryos. Whole-cell patch–clamp recordings were made from cerebral cortical neurons cultured for 7–9 days. The culture dish was placed on the stage of a Zeiss Axiovert 10 inverted phase contrast microscope (Zeiss, Germany), where the individual neurons were viewed at 200× magnification. The culture medium in the 35 mm petri dish was replaced with about 4 mL of artificial balanced salt solution (ABSS), which was continuously renewed by constant perfusion at 0.5 mL/min at room temperature (20–22 °C). The composition of ABSS was as follows (in mM): NaCl 140, KCl 3.5, Na_2HPO_4 1.25, $MgSO_4$ 2, $CaCl_2$ 2, glucose 10, and HEPES 10; pH was 7.35 at 22 °C.

Standard patch–clamp techniques[54] were used to record from the neurons in the whole-cell configuration using an EPC-9 patch–clamp amplifier (HEKA, Germany). The patch electrodes were pulled from 1.5 mm od glass (World Precision Instruments, United States) on a BB-CH-PC electrode puller (Mecanex, Switzerland) and had resistances of 2–6 MΩ. The medium in the patch electrodes had the following composition (in mM): KCl 140, $MgCl_2$ 1, $CaCl_2$ 1, EGTA 10, Mg-ATP 2, and HEPES 10; pH was 7.35 at 22 °C. A holding potential of −60 mV was usually used, but in some cells, the holding potential was varied in order to locate the reversal potential of the agonists under study. Current signals were recorded to disk on a computer and subsequently analyzed.

The drugs used were premixed at the required concentrations in ABSS. The solutions were applied in the vicinity (about 100 μM) of the recorded neuron from a multibarreled perfusion pipette, with the multiple barrels ending in a single glass cap with an opening of about 100 μm.[55] When applied, solutions emerged rapidly from the cap and surrounded the neuron completely. Between applications, bath solution was sucked into the cap and out through one of the barrels of the perfusion pipette in order to prevent any leakage of drug from the other barrels. Drugs were applied for 5 s, every 1 min. Within the 5 s of drug application, the responses always peaked or reached a stable maximum plateau. Responses were quantified by measuring the maximum current recorded during the application of drugs. Reversal potentials for the $GABA_A$ agonists were established by interpolation, using the currents induced at holding potentials of 0 and +10 mV.

Acknowledgment. This work was supported by grants from the Danish Technical Research Council and the Lundbeck Foundation. The technical assistance of Mrs. Lærke Andersen, Mrs. Ulla Geneser, Mrs. Tina Lindgreen, and Mr. Jørgen S. Johansen, the preparation of cell cultures by Mrs. Gunilla Steven, and the secretarial assistance of Mrs. Anne Nordly are gratefully acknowledged.

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JM950264+