Novel Class of Potent 4-Arylalkyl Substituted 3-Isoxazolol $GABA_A$ Antagonists: Synthesis, Pharmacology, and Molecular Modeling

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A number of analogues of the low-efficacy partial $GABA_A$ agonist 5-(4-piperidyl)-3-isoxazolol (4-PIOL, **5**), in which the 4-position of the 3-isoxazolol ring was substituted by different groups, were synthesized and tested as $GABA_A$ receptor ligands. Substituents of different size and structural flexibility such as alkyl, phenylalkyl, diphenylalkyl, and naphthylalkyl were explored. Pharmacological characterization of the synthesized compounds was carried out using receptor binding assays and by electrophysiological experiments using whole-cell patch-clamp techniques. Whereas none of these compounds significantly affected $GABA_B$ receptor sites or GABA uptake, they did show affinity for the $GABA_A$ receptor site. While alkyl or benzyl substitution, compounds **7a**−**h**, provided receptor affinities comparable with that of **5** (K_i = 9.1 μM), diphenylalkyl and naphthylalkyl substitution, as in compounds **7m**−**t**, resulted in a dramatic increase in affinity relative to **5**. The 3,3-diphenylpropyl and the 2-naphthylmethyl analogues, compounds **7s** and **7m**, respectively, showed the highest affinities of the series (K_i = 0.074 μM and K_i = 0.049 μM). In whole-cell patch-clamp recordings from cultured cerebral cortical neurons, all of the tested compounds were able to inhibit the effect of the specific $GABA_A$ agonist isoguvacine (**1**), compounds **7m** and **7s** showing antagonist potency (IC_{50} = 0.37 μM and IC_{50} = 0.02 μM) comparable with or markedly higher than that of the standard $GABA_A$ antagonist **4** (IC_{50} = 0.24 μM). Highly potent convulsant activity was demonstrated in mice with compounds **7m** (ED_{50} = 0.024 μmol/kg) and **7s** (ED_{50} = 0.21 μmol/kg) after intracerebroventricular administration, whereas no effects were found after subcutaneous administration. According to a previously proposed pharmacophore model for $GABA_A$ receptor agonists, a receptor cavity in the vicinity of the 4-position of the 3-isoxazolol ring in 4-PIOL exists. A molecular modeling study, based on compounds **7o**,**m**,**l**,**q**,**s**, was performed to explore the dimensions and other properties of the receptor cavity. This study demonstrates the importance of the arylalkyl substituents in **7m** and **7s** and the considerable dimensions of this proposed receptor cavity.

Introduction

4-Aminobutyric acid (GABA), the major inhibitory neurotransmitter in the central nervous system (CNS), operates through three different classes of receptors consisting of the ionotropic $GABA_A$ and $GABA_C$ receptors and the metabotropic $GABA_B$ receptors.[1,2] Besides playing an important role in central transmission processes, these receptors, especially the $GABA_A$ receptors, have been associated with certain neurological and psychiatric disorders and are therapeutic targets in certain diseases.[3,4] To pharmacologically characterize this receptor class, a number of $GABA_A$ ligands (Figure 1), such as the highly selective $GABA_A$ agonists isoguvacine (**1**), muscimol (**2**),[5] and 4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol (THIP, **3**),[5,6] have been developed. 2-(3′-(Carboxypropyl)-3-amino-6-(paramethoxyphenyl)pyridazinium bromide (SR 95531, **4**),[7] now used as a standard antagonist for the $GABA_A$ receptors, represents another structural class of ligands.

Hypoactivity of the GABA neuronal function seems to be associated with neurological disorders such as epilepsy,[8] Huntingtons chorea,[9] anxiety,[10] sleep disorders, and pain.[11] In contrast, GABA receptor mediated hyperactivity has been suggested to be an important component of schizophrenic symptoms.[12−15] Using full $GABA_A$ receptor agonists or antagonist may therapeutically lead to either desensitization of the receptors or severe side effects, respectively. This has focused interest on partial $GABA_A$ receptor agonists as potential therapeutics. In a previous study, we have described 5-(4-piperidyl)-3-isoxazolol (4-PIOL, **5**)[16,17] and, more recently, analogues of **5** as low-efficacy partial $GABA_A$ agonists showing dominating antagonist profiles.[18]

In a preliminary communication,[19] we proposed a binding mode of the bioactive conformations of **2** and **5**. In these binding modes of **2** and **5**, the 3-isoxazolol rings do not overlap. This means that the 4-position in the 3-isoxazolol ring in **2** does not correspond to the 4-position in the 3-isoxazolol ring of **5** in the receptor-bound conformations. To investigate this in further detail, we

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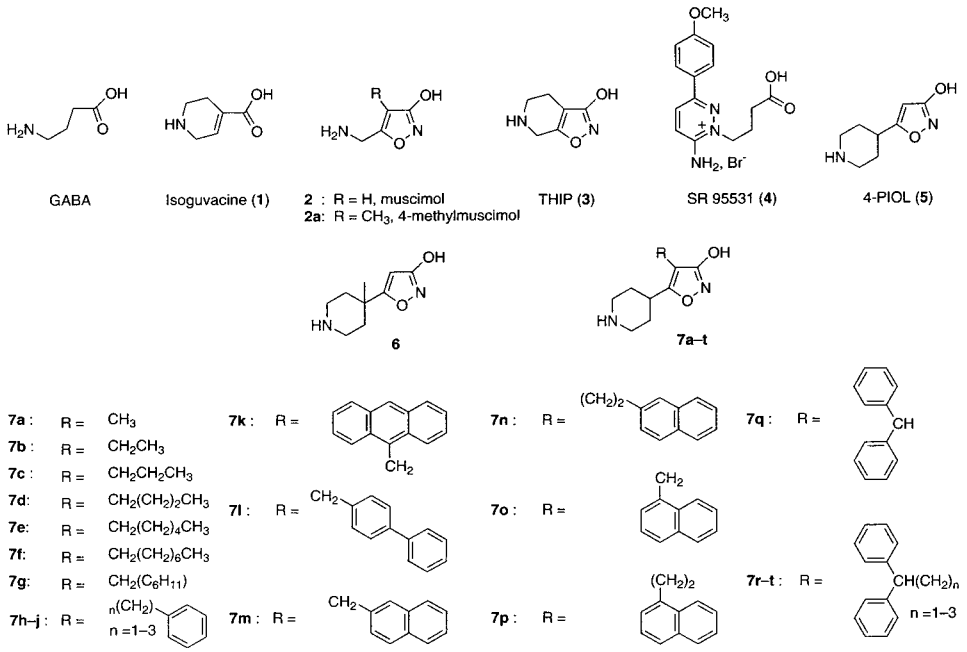


Figure 1. Structures of GABA, the GABA$_A$ agonists isoguvacine (**1**), muscimol (**2**), 4-methylmuscimol (**2a**), and THIP (**3**), the GABA$_A$ antagonist **4**, the low-efficacy partial GABA$_A$ agonist 4-PIOL (**5**), and the new isoxazolols (**6** and **7a**−**t**).

synthesized and pharmacologically tested a small series of 4-PIOL analogues substituted in the 4-position of the 3-isoxazolol ring of **5**. The results indicated that alkyl substituents in the 4-position of **5** are allowed, in contrast to what has been found for the corresponding position of **2**. Introduction of large aromatic substituents into the 4-position of the 3-isoxazolol ring of **5** markedly enhanced the affinity for the GABA$_A$ receptors. Furthermore, these structural modifications led to a change in the pharmacological profile of the compounds from low-efficacy partial GABA$_A$ agonist activity of **5** to potent GABA$_A$ antagonist effect. These results supported the hypothesis concerning the binding modes of muscimol (**2**) and **5**, indicating the existence of a large cavity at the 4-PIOL (**5**) recognition site of the GABA$_A$ receptor.

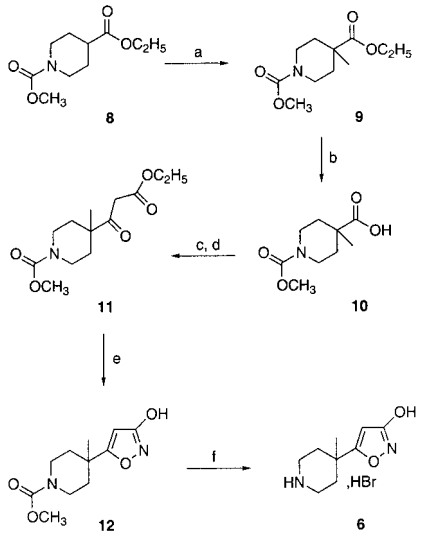
To explore this cavity in more detail, we here expand the structure−activity study to include a broader spectrum of 4-substituted analogues of **5**, with substituents that differ mainly in size and conformational flexibility. The synthesis and pharmacological characterization of this series of compounds are described, and the results of a molecular modeling study exploring the dimensions and other properties of the receptor cavity accommodating the 4-substituents of the analogues of **5** are reported.

Results and Discussion

Chemistry. The analogues of 4-PIOL were synthesized using two different strategies, introducing the substituent in the 4-position of the 3-isoxazolol ring at either an early stage or a later stage in the synthetic sequence. Compounds **6** and **7a**−**f**,**h**−**p**,**s**,**t** were all synthesized from the appropriate β-oxo esters, **11** or **15**, as outlined in Schemes 1 and 2. The preparation of both β-oxo esters was based on malonate. Compound **11** was synthesized via the imidazolide of carboxylic acid **10** using the complex formed from monoethyl malonate and magnesium in THF,[20] whereas compound **15** was prepared from acyl chloride **14** and potassium ethyl malonate in the presence of magnesium chloride and triethylamine, using the method described by Clay et al.[21] Alkylation of **15** was performed using the appropriate alkyl halide in the presence of sodium ethoxide to give compounds **16a**−**f**,**h**−**p**,**s**,**t**. The halides necessary for these reactions were either commercially available or prepared by bromination of the corresponding commercially available alcohols using either phosphorus tribromide or aqueous hydrogen bromide. In cases where the alcohol was not available, it was prepared by reduction of the corresponding carboxylic acid, using lithium aluminum hydride. Cyclization of the alkylated β-oxo esters with hydroxylamine at −30 °C followed by heating with concentrated hydrochloric acid at 80 °C gave the 3-isoxazolols **17a**−**f**,**h**−**p**,**s**,**t**, which were deprotected by treatment with hydrogen bromide in glacial acetic acid to give the target compounds **7a**−**f**,**h**−**p**,**s**,**t**.

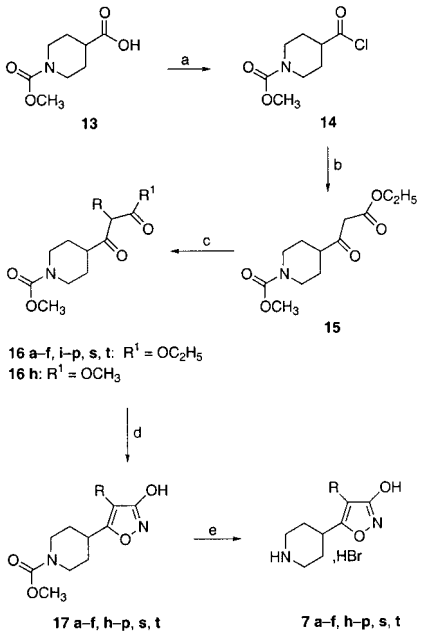
Cyclization and subsequent deprotection to give compound **6** was done in a similar manner. Alkylation of the β-oxo esters represents the limiting step of the above-mentioned route (Scheme 2) reflecting steric hindrance and risk of elimination of hydrogen halide from the alkylating agent under strongly basic conditions. An alternative approach was set up, as shown in Scheme 3, for the preparation of compounds **7g**,**q**,**r**, where the substituent was introduced in the 4-position of the 3-isoxazolol ring of **18** following cyclization of the β-oxo ester **15** (Scheme 3). Protection of the hydroxy group of **18** using isopropyl bromide in the presence of potassium carbonate selectively gave the *O*-protected compound **19** in high yield. The iodinated analogue of **19** underwent magnesium−iodine exchange using ethylmagnesium chloride followed by reaction with the appropriate aldehyde or ketone to give the hydroxy compounds **21g**,**q**,**r**. Attempts to remove the benzylic hydroxy group by catalytical hydrogenation failed. However, ionic hydrogenation using triethylsilane and trifluoroacetic acid in dichloromethane,[22] at 0 °C for the more activated hydroxy group in **21q** and at 50 °C for compounds **21g** and **21r**, gave compounds **22g**,**q**,**r** in

Scheme 1[a]



[a] Reagents. (a) LDA, THF, −70 °C; CH_3I. (b) 2 M NaOH, 100 °C; $ClCOOCH_3$, 0 °C, pH = 10. (c) *N*,*N*′-Thionyldiimidazole, THF. (d) [3-Ethoxy-3-hydroxyacrylato(2−)-O^1O^3]magnesate(2+), THF. (e) NH_2OH·HCl, NaOH, then concentrated HCl. (f) HBr in AcOH, room temperature.

Scheme 2[a]

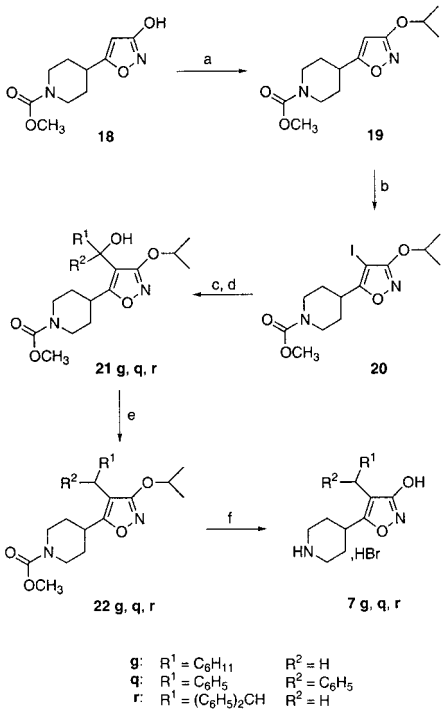


[a] Reagents: (a) $SOCl_2$, DMF, 85 °C; (b) potassium ethyl malonate, EtOAc, Et_3N, $MgCl_2$; (c) NaOEt, RBr or RI (R, see Figure 1), 80 °C; (d) NH_2OH·HCl, NaOH, then concentrated HCl; (e) HBr in AcOH, room temperature.

high yields. Deprotection to give the target compounds **7g**,**q**,**r** was accomplished by treatment with hydrogen bromide in acetic acid at 65 °C.

In Vitro Pharmacology. The compounds were characterized in receptor binding studies using rat brain membrane preparations and electrophysiologically using whole-cell patch-clamp recordings from cultured cerebral cortical neurons. The affinities of the compounds for $GABA_A$ and $GABA_B$ receptor sites or GABA uptake sites, using either [^{3}H]muscimol or [^{3}H]GABA, were determined using a modified version of the methods described previously.[18] At test concentrations of 100 μM

Scheme 3[a]



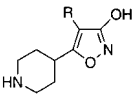
[a] Reagents: (a) isopropyl bromide, K_2CO_3, DMF, 60 °C; (b) ICl, AcOH, H_2O, 80 °C; (c) EtMgCl, THF, −30 °C; (d) R_1COR_2, THF, 0 °C; (e) CF_3COOH, Et_3SiH, CH_2Cl_2; (f) HBr, AcOH, 65 °C.

for **6** and **7a**−**h**,**j**,**r** and because of solubility problems 10 μM for **7i**,**k**−**q**,**s**, none of the compounds showed detectable affinity for $GABA_B$ receptors or GABA uptake sites. Like 4-PIOL (**5**), all of the tested compounds did show affinity for the $GABA_A$ receptor sites, and binding affinities (K_i values) of compounds **6** and **7a**−**t** for $GABA_A$ receptor sites are listed in Table 1.

Introduction of a methyl group in the 4-position of the piperidine ring of **5** to give compound **6** resulted in a significant reduction of affinity for the $GABA_A$ receptor sites compared to that of **5**. In contrast, unbranched alkyl groups as substituents in the 4-position of the 3-isoxazolol ring of **5** such as methyl, ethyl, propyl, butyl, hexyl, and octyl groups as well as a benzyl group are tolerated, compounds **7b**−**f**,**h** showing affinity for the $GABA_A$ receptor sites comparable to or slightly higher than that of **5**. Increasing the length of the alkyl chain joining the phenyl group and the 3-isoxazolol ring of compound **7h** gave compounds **7i** and **7j**, where the 2-phenylethyl compound, **7i**, was equipotent with **5**, while the 3-phenylpropyl compound, **7j**, showed enhanced affinity compared to **5**. Reduction of the phenyl group of **7h** to a cyclohexyl group in **7g** was tolerated, with only a slight decrease in affinity. Affinity was increased 10-fold relative to compound **7h** when a phenyl group was attached to the 4-position of the phenyl group to give the biphenyl-4-methyl analogue **7l**. Addition of another phenyl group to the terminal carbon of **7j**, as in the 3,3-diphenylpropyl compound **7s**, gave a 16-fold increase in affinity relative to the 3-phenylpropyl analogue **7j**. The diphenylmethyl, 2,2-diphenylethyl, and 4,4-diphenylbutyl analogues **7q**,**r**,**t** all showed reduced affinity compared to **7s**.

An even greater increase in affinity was observed when the more bulky 2-naphthylmethyl was introduced

Table 1. Receptor Binding and in Vitro Electrophysiological Data

compound	R	[^{3}H]muscimol binding,[a] K_i (μM)[d]	$GABA_B$ binding[a] and GABA uptake,[b] IC_{50} (μM)	electrophysiology,[c] IC_{50} (μM)[d]
4		0.074 (0.059; 0.094)	>10[e]	0.24 (0.22; 0.25)
6		37 (20; 70)	>100	nd
5 (4-PIOL)	H	9.1 (8.2; 10)	>100	110 (76; 170)
7a	methyl	10 (10; 11)	>100	26 (22; 31)
7b	ethyl	6.3 (5.1; 7.6)	>100	10.3 (7.7; 13)
7c	propyl	6.6 (5.9; 7.3)	>100	4.6 (4.3; 5.0)
7d	butyl	7.7 (6.6; 9.0)	>100	3.0 (2.6, 3.5)
7e	hexyl	4.5 (3.6; 5.5)	>100	1.1 (0.86; 1.3)
7f	octyl	1.8 (1.1; 1.3)	>100	0.44 (0.41; 0.48)
7g	cyclohexylmethyl	4.9 (4.4; 5.5)	>100	1.1 (0.97; 1.3)
7h	benzyl	3.8 (3.3; 4.5)	>100	4.0 (3.7; 4.3)
7i	2-phenylethyl	5.0 (4.7; 5.4)	>10	4.1 (3.8; 4.5)
7j	3-phenylpropyl	1.1 (0.7; 1.6)	>100	0.53 (0.48; 0.58)
7k	9-anthracylmethyl	5.9 (5.0; 7.0)	>10	1.3[f] (1.2; 1.5)
7l	4-biphenylmethyl	0.4 (0.31; 0.43)	>10	0.71 (0.63; 0.80)
7m	2-naphthylmethyl	0.049 (0.043; 0.057)	>10	0.37 (0.31; 0.44)
7n	2-naphthylethyl	0.49 (0.43; 0.54)	>10	0.89 (0.83; 0.95)
7o	1-naphthylmethyl	0.10 (0.095; 0.11)	>10	0.48 (0.45; 0.50)
7p	1-naphthylethyl	1.7 (1.5; 1.9)	>10	1.50 (1.40; 1.61)
7q	diphenylmethyl	0.96 (0.48; 1.9)	>10	0.78[f] (0.71; 0.85)
7r	2,2-diphenylethyl	0.36 (0.30; 0.43)	>100	0.81[f] (0.75; 0.86)
7s	3,3-diphenylpropyl	0.068 (0.061; 0.074)	>10	0.02[f] (0.02; 0.03)
7t	4,4-diphenylbutyl	0.70 (0.67; 0.74)	>100	0.05[f] (0.05; 0.06)

[a] Standard receptor binding on rat brain synaptic membranes, $n = 3$. [b] Inhibition of GABA uptake into synaptosomes. [c] Whole-cell patch-clamp recordings from cerebral cortical neurons cultered for 7−9 days, $n = 6-17$. [d] Mean and SEM were calculated assuming a normal distribution of the logarithm of the IC_{50} and K_i values. Hence, numbers in parentheses (min; max) indicate the antilog of the mean ± SEM of IC_{50} and K_i.[50] [e] Reference 7. [f] Because of the slow onset of antagonism, the parameters for **7k**,**q**−**t** were calculated using the response magnitude after 5 s of application.

in the same position of the 3-isoxazolol ring to afford **7m**. This led to a 78-fold increase in binding affinity compared to the benzyl analogue **7h**. The introduction of the isomeric 1-naphthylmethyl group to provide **7o** proved to be less favorable showing a decrease in binding affinity compared to compound **7m**. Extending the linker joining the 2-naphthyl or the 1-naphthyl groups and the 3-isoxazolol ring with a carbon atom to give compounds **7n** and **7p**, respectively, resulted in both cases in a 10-fold reduction in affinity for the $GABA_A$ receptor relative to the parent compound. Further reduction was observed for compound **7k**, where the extension of the aromatic system from a naphthyl group to an anthracyl group to give **7k** markedly decreased the affinity for the $GABA_A$ receptor, producing a binding affinity comparable to that of **5**.

The pharmacology of compounds **7a**−**t** was studied using whole-cell patch-clamp recordings from cultured cerebral cortical neurons, performed as described previously.[18] The compounds were tested in the absence or presence of the specific $GABA_A$ receptor agonist isoguvacine (**1**) (20 μM). In a previous study, using cerebral cortical neurons, we have characterized 4-PIOL (**5**) as a partial agonist at $GABA_A$ receptors.[18] In the present study, all of the compounds tested were capable of inhibiting the current induced by **1** in a dose-dependent manner, as exemplified in Figure 2 for compounds **4**, **5**, and **7a**,**h**,**m**,**s**. With only a few exceptions, the electrophysiological data showed a fairly good correlation to the obtained binding affinities (Table 1). The 2-naphthylmethyl analogue **7m** was shown to have the highest affinity of this series, showing an affinity comparable with that of the standard $GABA_A$ antagonist SR 95531

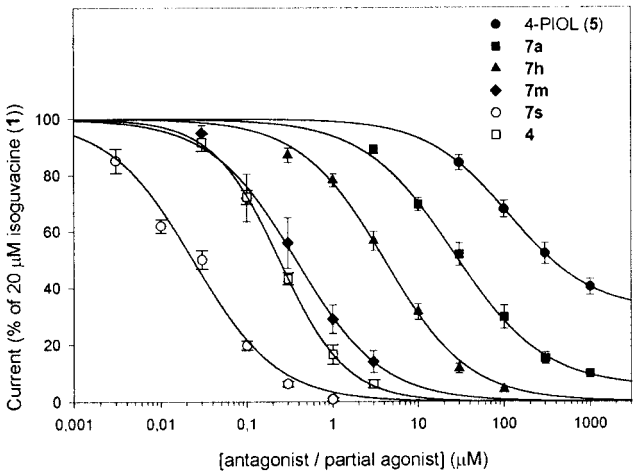


Figure 2. Effect of the partial agonists or antagonists on the response to 20 μM of the full $GABA_A$ agonist isoguvacine (**1**) using whole-cell patch-clamp recordings from cultured cerebral cortical neurons. A total of 20 μM **1** and varying concentrations of antagonists/partial agonist were applied simultaneously to the cells. The response of 20 μM **1** alone has been set as 100%, and the other responses are expressed as a fraction of this. The response to **1** is progressively reduced with increasing concentrations of the partial agonist or antagonist. The number of cells tested in this way with each compound varied from $n = 6$ to $n = 17$.

(**4**) in the $GABA_A$ receptor binding assay. Interestingly, results from the electrophysiological model showed the 3,3-diphenylpropyl and the 4,4-diphenylbutyl analogues **7s** and **7t** to be 19- and 7-fold, respectively, more potent than **7m** as $GABA_A$ antagonists. As in the $GABA_A$ receptor binding assay, **7m** showed antagonist potency

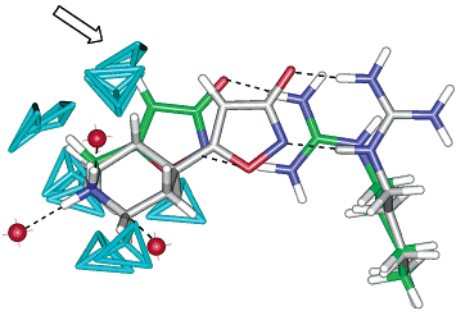


Figure 3. Pharmacophore model for $GABA_A$ receptor agonists showing the proposed binding modes of **2** (green bonds) and **5** (grey bonds) and their interactions with two different conformations of an arginine residue. The tetrahedrons indicate positions of methyl groups in $GABA_A$ agonists that cause strong steric repulsions with the receptor. The arrow points to the tetrahedron corresponding to the methyl group in the inactive compound 4-methylmuscimol (**2a**). The red spheres indicate sites to which the ammonium group in **2** interacts via hydrogen bonds.

comparable to that of **4**. As earlier described,[19] only compounds **7a** and **7b** retained some ability to induce currents themselves at concentrations of 1 mM (not shown) while compounds **7c**−**t** showed no detectable agonist effect at test concentrations of 100 μM (**7c**−**i**,**k**,**l**,**n**,**p**−**r**), 30 μM (**7j**,**m**,**o**), or 10 μM (**7s**,**t**).

In Vivo Pharmacology. Compounds **7m** and **7s** were tested for convulsant effects in mice after intracerebroventricular (icv) and subcutaneous (sc) administration following a previously described procedure.[23] After icv administration, compounds **7m** and **7s** showed potent convulsant activity with $ED_{50} = 0.024$ μmol/kg and $ED_{50} = 0.21$ μmol/kg, respectively. After sc administration, none of the compounds **7m** and **7s** showed significant convulsant effects, $ED_{50} > 51$ μmol /kg and $ED_{50} > 45$ μmol/kg, respectively. The lack of in vivo convulsant effects of the two compounds after sc administration may reflect poor blood−brain barrier permeability or rapid metabolism. These aspects will be the subject of further studies.

Molecular Modeling. We have previously reported a pharmacophore model for $GABA_A$ receptor agonists based on conformational analyses and superimposition studies of muscimol (**2**) and 4-PIOL (**5**).[19,24] In this model, as shown in Figure 3, the anionic parts of **2** and **5** (the deprotonated 3-isoxazolol ring) interact with two different conformations of an arginine residue. This makes it possible for the cationic part (the substituted ammonium ion) of the two compounds to overlap in space. When low or inactive methyl-substituted analogues of GABA, **2**, **3**, and **5** are fitted to the model, regions of repulsive steric interactions (receptor essential volumes) with the receptor could be identified.[24] These are shown as tetrahedrons in Figure 3. The corners of the tetrahedrons correspond to the positions of the carbon and the three hydrogen atoms of the methyl groups, causing a significant decrease in receptor affinity. Thus, the tetrahedrons indicate regions in space that are forbidden for high-affinity $GABA_A$ ligands. For instance, the methyl group in 4-methylmuscimol (**2a**) causes a very large decrease in the affinity ($IC_{50} > 100$ μM)[25] compared to that of **2** ($IC_{50} = 0.006$ μM).[26] An arrow in Figure 3 indicates the tetrahedron corresponding to the position of the methyl group in **2a** when fitted to the pharmacophore model.

In the proposed binding modes of **2** and **5** (Figure 3), the 3-isoxazolol rings do not overlap, and consequently, the 4-position in **5** does not correspond to the "forbidden" 4-position in **2**. In fact, as previously communicated[19] and further extended in the present work, high-affinity analogues of **5** could be obtained by the introduction of large substituents in the 4-position.

To explore the dimensions and properties of the part of the binding cavity accommodating the 4-substituents in the analogues of **5** included in Table 1, we have extended the pharmacophore model in the present work. Those compounds that display the highest affinity in [^{3}H]muscimol binding and at the same time have structural features that are useful for the exploration of the size of the binding cavity have been analyzed. The selected compounds are **7l**,**m**,**o**,**q**,**s**. In addition, **7h** and **7k** will be discussed as examples of lower affinity compounds.

The two isomeric naphthylmethyl analogues **7m** and **7o** were chosen as starting points for the molecular modeling study. These compounds display high affinities and contain only 2 degrees of torsional freedom, which facilitates the conformational analyses. For each compound, an exhaustive unconstrained conformational analysis for the aqueous solution was performed by using the Monte Carlo multiple minimum method (MCMM) and the MM3* force field in conjunction with the GB/SA hydration model as described in the Experimental Section. To identify possible bioactive conformations of the 4-substituents, conformational analyses of the 4-substituents were performed with the 4-PIOL (**5**) skeleton conformationally constrained to its proposed binding conformation (Figure 3).

The following criteria were applied in the search for a possible bioactive conformation of a 4-substituent: (i) no part of the substituent is allowed to come closer than 1 Å to any of the sterically "forbidden" regions shown as tetrahedrons in Figure 3; (ii) the calculated conformational energy penalties for binding, i.e., the conformational energy required for the molecule to adopt its bioactive conformation, should be low. Further details of the computational procedures are given in the Experimental Section. If more than one possible bioactive conformation was found by this procedure, the one with the lowest energy was selected as the most probable one.

The conformational analysis of **7o** resulted in only one possible bioactive conformation, fulfilling the criteria described above. This conformation with a calculated conformational energy penalty of 2.6 kcal/mol is shown in Figure 4a. The 1-naphthylmethyl substitutent in the proposed bioactive conformation of **7o** is close to orthogonal to the 3-isoxazolol ring and is located on the same side of the 3-isoxozolol ring as the piperidine nitrogen atom. The unsubstituted naphthyl ring is pointing away from the 3-isoxazolol ring.

For the highest affinity compound in the present work (the 2-naphthylmethyl analogue **7m**), two possible conformations fulfilling the criteria were found. The conformations display very low and similar calculated conformational energy penalties for binding (0.5 and 0.8 kcal/mol). The two conformations, shown in Figure 4b, differ only in the orientation of the distal aromatic ring.

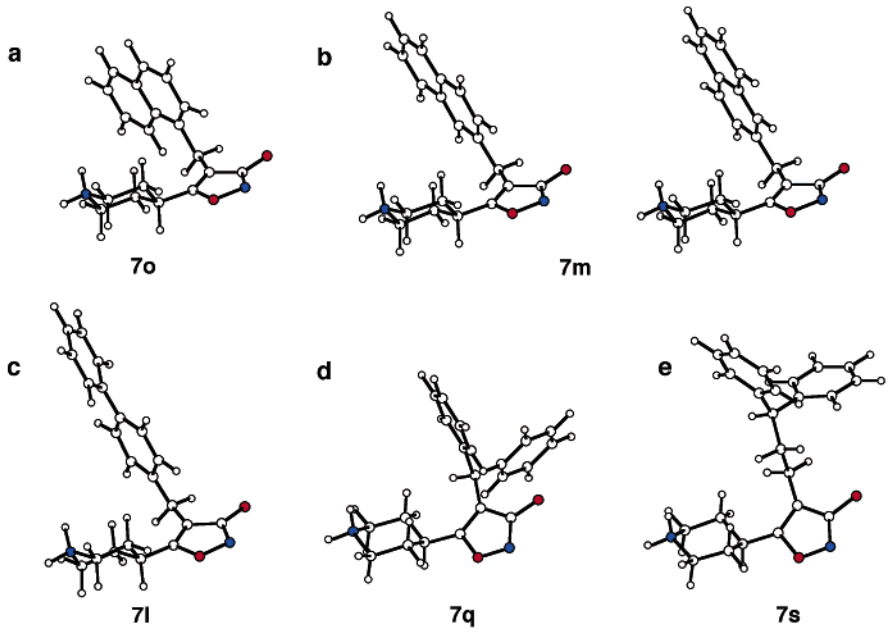


Figure 4. Proposed bioactive conformations for some high-affinity 4-substituted analogues of **5**. Compound **7m** is illustrated in two forms exhibiting different conformations of the 2-naphthylmethyl substituent.

As expected, the conformational analysis of the benzyl analogue **7h** yielded a bioactive conformation with a low-energy penalty (1.1 kcal/mol) in which the phenyl ring closely superimposes the substituted aromatic rings of **7m** and **7o**. The addition of a phenyl ring to the methyl-substituted compound **7a** to give **7h** leads to an increase in the affinity by a factor of less than 3 (Table 1). In contrast, addition of an aromatic ring to **7h** to give the naphthyl systems in **7m** and **7o** increases the affinity by factors of 78 and 38, respectively. The strong effect on the affinity of the unsubstituted aromatic rings in **7m** and **7o** is not due to an increase of the lipophilicity as expressed by log *P*. The increase of log *P* by replacing a methyl by a benzyl substituent is 1.43 log units, whereas the increase by substituting a naphthylmethyl for a benzyl substituent is smaller, 1.0 log unit, the log *P* values being calculated according to the method of Ghose and Crippen as implemented in the Spartan program.[27] This indicates that the distal aromatic rings in **7m** and **7o** have strong specific interactions with the binding cavity of the GABA$_A$ receptor and that the difference in affinity is not merely a consequence of a difference in solvation energy.

The dimensions of the cavity accommodating the 4-substituents may be extended beyond that of a 2-naphthylmethyl group as demonstrated by the high affinity of the biphenyl-4-methyl analogue **7l**. Conformational analysis resulted in two conformations fulfilling the criteria for a bioactive conformation. The one in Figure 4c has a calculated conformational energy penalty of 0.9 kcal/mol; the other one, which has an energy penalty of 1.1 kcal/mol, differs only by having the opposite sign of the inter-ring torsion in the biphenyl moiety.

Extending the 1-naphthyl ring system in **7o** to the 9-anthracyl system in **7k** decreases the affinity by a factor of 59 (Table 1). Conformational analysis displays a quite high conformational energy penalty for the most probable bioactive conformation of **7k** (6.9 kcal/mol). In addition, the added aromatic ring is in electrostatic conflict with the receptor binding site interacting with the axial piperidyl N^+H presumably via a hydrogen bond. Thus, this binding site should be negatively charged or at least have a high electron density. As shown in Figure 5, this putative binding site is located close to a line orthogonal to one of the aromatic rings in **7k** and passing through the centroid of this ring. The negative electrostatic potential of the 9-anthracyl system at this position in space leads to an electrostatic attraction with cations and consequently an electrostatic repulsion with anions or electron-rich functional groups.[28] This clearly shows that the aromatic ring system of the benzyl substituent in **7h** may be extended in a direction pointing away from the 3-isoxazolol ring, as demonstrated by the proposed bioactive conformation of **7o** (Figure 4a), but not in the opposite direction.

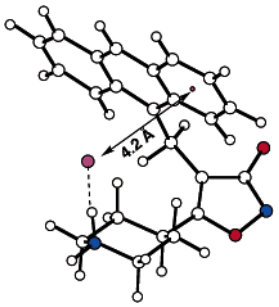


Figure 5. Proposed bioactive conformation of **7k** displaying electrostatic repulsion between one of the aromatic rings and a putative electron-rich binding site (magenta sphere).

The reasonably high affinity of the diphenylmethyl compound **7q** and the very high affinity of the 3,3-diphenylpropyl compound **7s** demonstrate that the binding cavity may be extended in a direction not exploited by any of the other substituents. The most probable bioactive conformation of the diphenylmethyl compound **7q**, according to the conformational analysis, is shown in Figure 4d (conformational energy penalty of 4.6 kcal/mol). One of its phenyl rings superimposes well on the aromatic rings in **7l**,**m**,**o** as shown by the

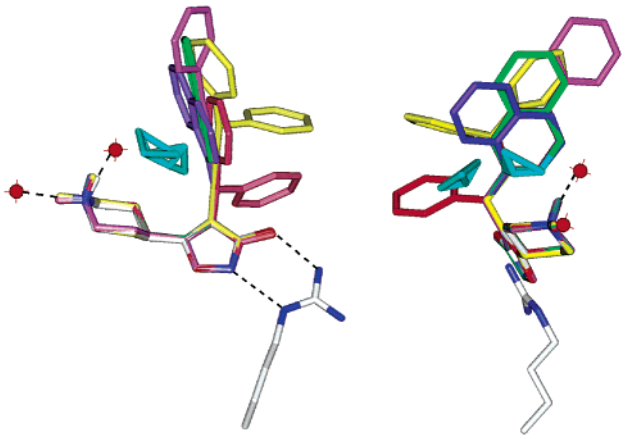


Figure 6. Superimposition (in two orientations) of compounds **7l** (magenta), **7m** (green), **7o** (blue), **7q** (red), and **7s** (yellow) in their proposed bioactive conformations illustrating the large space spanned by the 4-substituents. Hydrogen atoms except on nitrogen have been omitted for clarity. Only the sterically forbidden regions closest to the 4-substituents are shown.

superimposition in Figure 6, whereas the other phenyl ring extends in an almost orthogonal direction. This is also shown by the calculated bioactive conformation of **7s** displayed in Figure 4e and Figure 6. In **7s**, one of the phenyl ring overlaps well with the distal ring of the high-affinity 1-naphthylmethyl compound **7o** whereas the other phenyl ring occupies an unexplored region in space in the pharmacophore model. The observation that one of the phenyl rings in **7s** overlaps well with the distal aromatic ring in **7o** rationalizes the affinity difference between **7s** and **7q**. As concluded above, this position of an aromatic ring is highly favorable for the receptor affinity and it is not possible for any of the aromatic rings in **7q** to be positioned in this region in space.

The superimposition of the proposed bioactive conformations of **7l**,**m**,**o**,**q**,**s** shown in Figure 6 demonstrates the large space spanned by the high-affinity compounds in the present series of analogues of 4-PIOL (**5**) and that the receptor cavity in the vicinity of the 4-position in (**5**) is of considerable dimensions. The length of the biphenyl-4-methyl substituent in **7l** is 9.7 Å as measured from the methyl carbon to the para hydrogen atom of the distal ring, and one of the phenyl rings in **7s** extends 6.4 Å in a direction almost perpendicular to the rings in **7l**,**m**,**o**.

As described above, the compounds studied in the present work all display antagonistic properties. There is strong evidence that the GABA binding site in the $GABA_A$ receptor is located between an α- and a β-subunit.[29] It may be speculated that the large cavity accommodating the 4-substituents of the compounds studied in the present work is located in the space between these subunits, a space that may extend into the aqueous phase outside the receptor. $GABA_A$ receptors belong to the same superfamily as nicotinic acetylcholine receptors. On the basis of electron microscopic image analyses, it has been proposed that the mechanism for ligand-induced channel opening in nicotinic acetylcholine receptors involves rotations of the subunits in the ligand binding domain.[30,31] It is likely that other receptors in the same superfamily use a similar mechanism for channel opening. Large substituents, as the 4-substituents studied in the present work, may interfere with the mechanism for ion channel opening in $GABA_A$ receptors giving the compounds antagonistic properties.

Conclusion

We have here described a new series of competitive $GABA_A$ antagonists that are derived from the low-efficacy partial $GABA_A$ agonist 4-PIOL (**5**). With the aim of further investigating the previously described receptor cavity in the vicinity of the 4-position of the 3-isoxazolol ring in **5**, substituents of different size were incorporated. The effects of alkyl, phenylalkyl, diphenylalkyl, and naphthylalkyl substitution were investigated. The substitution of the 4-position with an alkyl or phenylalkyl group gave compounds with affinity and potency comparable with those of **5**, while introduction of more bulky groups such as diphenylalkyl and naphthylalkyl groups gave rise to a marked increase in both affinity and potency. The two most potent analogues of the series containing a 2-naphthylmethyl and a 3,3-diphenylpropyl substituent, compounds **7m** and **7s**, respectively, were evaluated in vivo and showed potent convulsant effects following icv administration. Molecular modeling studies of the compounds synthesized exposed a cavity of substantial size in the vicinity of the 4-position of **5**. On the basis of conformational analysis of the high-affinity compounds **7m** and **7s** containing a diphenyl ring system in the 4-position in the 3-isoxazolol ring, there seem to be additional sites for specific receptor interactions in the above-mentioned cavity.

This study has provided new information on the agonist and competitive antagonist binding site in the $GABA_A$ receptor and is going to be exploited in further development of the pharmacophore model. In addition, the compounds described could serve as useful tools for studies of the $GABA_A$ receptor mechanisms. Further studies in this area are in progress.

Experimental Section

Chemistry. General Procedures. Melting points were determined in capillary tubes and are uncorrected. ^{1}H NMR spectra were recorded on a Varian Gemini 2000 (300 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) was performed on Merck silica gel 60 (0.06−0.200 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 spraying reagent. Compounds containing the 3-isoxazolol unit 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.

Ethyl 1-Methoxycarbonyl-4-methyl-4-piperidinecarboxylate (9). To a solution of diisopropylamine (4.0 mL, 28 mmol) in anhydrous THF (100 mL) was added dropwise a solution of *n*-butyllithium in hexane (1.6 M, 18 mL, 28 mmol). The reaction mixture was stirred at 0 °C for 15 min. After it was cooled to −70 °C, a solution of ethyl 1-methoxycarbonyl-4-piperidinecarboxylate[16] (5.6 g, 26 mmol) in THF (40 mL) was added dropwise over 15 min, and the reaction mixture was then stirred at −70 °C for 1 h. A solution of methyl iodide (2.4 mL, 39 mmol) in THF (40 mL) was added dropwise over 15 min, and the reaction mixture was stirred for 4 h, during which

time the temperature was allowed to rise to room temperature. The reaction mixture was quenched with saturated aqueous ammonium chloride (60 mL) and extracted with EtOAc (3 × 100 mL). The combined extracts were evaporated, and CC (toluene/EtOAc (1:1)) gave the product as an oil (4.9 g, 82%). ^{1}H NMR (200 MHz, $CDCl_3$): δ 4.17 (2H, q, J = 7.1 Hz), 3.91−3.70 (2H, m), 3.68 (3H, s), 3.10−2.94 (2H, m), 2.14−2.00 (2H, m), 1.49−1.29 (2H, m), 1.27 (3H, t, J = 7.1 Hz), 1.20 (3H, s). Anal. ($C_{11}H_{19}NO_4$) C, H, N.

1-Methoxycarbonyl-4-methyl-4-piperidinecarboxylic Acid (10). A mixture of **9** (4.9 g, 21 mmol) in 2 M sodium hydroxide (20 mL) was stirred at 100 °C for 30 min. The reaction mixture was cooled to 0 °C, and 2 M HCl was added until pH 10 was attained. Methyl chloroformate (3.3 mL, 43 mmol) was added dropwise, and stirring was continued at 0 °C for 1 h followed by stirring at room temperature for 1 h. The mixture was extracted with CH_2Cl_2 (3 × 50 mL), and the combined extracts were dried, evaporated, and subjected to CC (toluene/EtOAc (4:1)) to give the product (3.18 g, 74%). ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.91−3.70 (2H, m), 3.69 (3H, s), 3.24−3.00 (2H, m), 2.18−1.98 (2H, m), 1.52−1.16 (2H, m), 1.29 (3H, s). Anal. ($C_9H_{15}NO_4$) C, H, N.

Ethyl 3-(1-Methoxycarbonyl-4-methyl-4-piperidyl)-3-oxopropionate (11). To a solution of *N*,*N*′-thionyldiimidazole[20] (21 mmol) in THF (60 mL) was added dropwise a solution of **10** (3.2 g, 16 mmol) in THF (8 mL). The reaction mixture was protected from light, and stirring was continued for 16 h at room temperature. The solution was added dropwise to a suspension of [3-ethoxy-3-hydroxyacrylato(2−)-O^1,O^3]magnesate-(2+)[16] (48 mmol) in THF (60 mL). The mixture was stirred for 2 h and acidified with HCl (4 M), and stirring was continued for 30 min. THF was distilled off in vacuo, and the aqueous residue was extracted with Et_2O (3 × 50 mL). The combined extracts were washed with saturated aqueous $NaHCO_3$ (3 × 50 mL) and then with H_2O (3 × 30 mL), dried, and evaporated to give the crude product as an oil (2.29 g, 53%). ^{1}H NMR (200 MHz, $CDCl_3$): δ 4.29−4.10 (3H, m), 3.69 (3H, s), 3.50 (2H, s), 3.39−3.95 (3H, m), 2.15−1.95 (2H, m), 1.49−1.16 (8H, m).

5-(1-Methoxycarbonyl-4-methyl-4-piperidyl)-3-isoxazolol (12). A solution of **11** (2.0 g, 7.4 mmol) in MeOH (0.5 mL) was added dropwise to a solution of NaOH (310 mg, 7.8 mmol) in MeOH (7 mL) and water (0.5 mL) at −30 °C. After the mixture was stirred for 10 min, a solution of hydroxylamine hydrochloride (1.0 g, 15 mmol) and NaOH (620 mg, 15 mmol) in MeOH/water (7 mL/0.5 mL) was added at −30 °C. Stirring was continued for 2 h at −30 °C, and the reaction mixture was poured into concentrated HCl (1.5 mL) at 80 °C and heated at 80 °C for 2 h. After the mixture was cooled, the organic solvent was evaporated and the residue was extracted with Et_2O (3 × 50 mL). The combined extracts were dried and evaporated. CC (toluene/EtOAc (1:1) containing AcOH (1%)) of the crude product gave the product as an oil (1.0 g, 57%). ^{1}H NMR (200 MHz, $CDCl_3$): δ 10.70 (1H, broad s), 5.70 (1H, s), 3.79−3.61 (2H, m), 3.70 (3H, s), 3.31−3.14 (2H, m), 2.11−1.97 (2H, m), 1.69−1.51 (2H, m), 1.31 (3H, s). Anal. ($C_{11}H_{16}N_2O_4$) C, H, N.

5-(4-Methyl-4-piperidyl)-3-isozaxolol Hydrobromide (6). A solution of **12** (360 mg, 1.6 mmol) in a solution of HBr in AcOH (33%, 15 mL) was stirred at room temperature for 16 h. The reaction mixture was evaporated, and the residue was recrystallized (MeOH/Et_2O) to give **6** (210 mg, 52%). ^{1}H NMR (200 MHz, D_2O): δ 5.93 (1H, s), 3.36−3.25 (2H, m), 3.11−2.97 (2H, m), 2.31−2.23 (2H, m), 1.92−1.77 (2H, m), 1.31 (3H, s). Anal. ($C_9H_{14}N_2O_2$·HBr·0.5H_2O) C, H, N.

1-Methoxycarbonyl-4-piperidinecarboxylic Acid Chloride (14). A mixture of **13**[16] (12 g, 64 mmol), thionyl chloride (14 mL, 190 mmol), and DMF (0.5 mL) was stirred at 85 °C for 20 min. The reaction mixture was evaporated, and ball-tube distillation (0.1 mmHg; oven temperature, 200 °C) of the residue gave **14** (11 g, 83%). ^{1}H NMR (200 MHz, $CDCl_3$): δ 4.19−4.00 (2H, m), 3.69 (3H, s), 3.02−2.82 (3H, m), 2.16−2.00 (2H, m), 1.90−1.61 (2H, m).

Ethyl 3-(1-Methoxycarbonyl-4-piperidyl)-3-oxopropionate (15). To a stirred mixture of potassium ethyl malonate (12.7 g, 75 mmol) in EtOAc (125 mL) cooled to 0−5 °C was added triethylamine (26 mL, 190 mmol) followed by $MgCl_2$ (8.6 g, 91 mmol). The mixture was slowly heated to 35 °C and then maintained at 35 °C for 6 h. The reaction mixture was cooled to 0 °C, and **14** (11 g, 54 mmol) was added dropwise over 30 min. Stirring was continued overnight at room temperature, and then the mixture was cooled to 0 °C and 4 M HCl (110 mL) was added slowly (T < 25 °C). The aquous phase was extracted with toluene (3 × 100 mL), and the combined organic phases were washed with 2% $NaHCO_3$ (2 × 40 mL) followed by water (2 × 25 mL). The organic phase was dried and evaporated. CC (toluene/EtOAc (4:1)) gave **15**[16] (8.9 g, 65%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.17 (2H, q, J = 7.2 Hz), 4.13 (2H, broad s), 3.66 (3H, s), 3.47 (2H, s), 2.88−2.98 (2H, m), 2.67−2.57 (1H, m), 1.90−1.79 (2H, m), 1.60−1.47 (2H, m), 1.25 (3H, t, J = 7.2 Hz).

Ethyl 3-(1-Methoxycarbonyl-4-piperidyl)-2-methyl-3-oxopropionate (16a). 15 (3.0 g, 12 mmol) was added to a solution of NaOEt (12 mmol) in EtOH (20 mL). Methyl iodide (1.2 mL, 13 mmol) was dropwise added to the reaction mixture at 80 °C, and stirring was continued at 80 °C for 16 h. Filtration and evaporation followed by CC (toluene/EtOAc (3:1)) gave **16a** as a colorless oil (1.7 g, 53%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.12−4.03 (4H, m), 3.71−3.61 (1H, m), 3.68 (3H, s), 2.92−2.62 (3H, m), 1.90−1.78 (2H, m), 1.76−1.40 (2H, m), 1.32 (3H, d, J = 7.4 Hz), 1.25 (3H, t, J = 7.2 Hz). Anal. ($C_{13}H_{21}NO_5$) C, H, N.

5-(1-Methoxycarbonyl-4-piperidyl)-4-methyl-3-isoxazolol (17a). 17a was synthesized as described for **12** by using **16a** (1.6 g, 5.9 mmol) and NaOH (250 mg, 6.3 mmol) in MeOH/water (5 mL/0.3 mL), hydroxylamine hydrochloride (820 mg, 11.8 mmol) and NaOH (472 mg, 11.8 mmol) in MeOH/water (6 mL/6 mL), and concentrated HCl (2.6 mL). The crude product was purified by CC (toluene/EtOAc (4:1) containing AcOH (1%)) and gave **17a** (810 mg, 57%). A sample was recrystallized (toluene/light petroleum): mp 145−147 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.70 (1H, broad s), 4.21 (2H, broad s), 3.70 (3H, s), 2.91−2.81 (3H, m), 1.87 (3H, s), 1.81−1.73 (4H, m). Anal. ($C_{11}H_{16}N_2O_4$) C, H, N.

4-Methyl-5-(4-piperidyl)-3-isoxazolol Hydrobromide (7a). A solution of **17a** (1.0 g, 4.4 mmol) in a solution of HBr in AcOH (33%, 50 mL) was stirred at room temperature for 16 h. The reaction mixture was evaporated, and the residue was recrystallized (MeOH/Et_2O) to give **7a** (950 mg, 82%): mp >250 °C. ^{1}H NMR (200 MHz, D_2O): δ 3.46−3.38 (2H, m), 3.21−2.98 (3H, m), 2.00−1.80 (4H, m), 1.74 (3H, s). Anal. ($C_9H_{14}N_2O_2$·HBr) H, Br, N; C, calcd, 54.97, found, 55.58.

Ethyl 2-Ethyl-3-(1-methoxycarbonyl-4-piperidyl)-3-oxopropionate (16b). 16b was synthesized as described above for **16a** by using **9** (3.0 g, 11.7 mmol), NaOEt (11.7 mmol) in EtOH (20 mL), and ethyl bromide (0.96 mL, 12.8 mmol). The crude product was purified by CC (toluene/EtOAc (4:1)) to give the title compund as an oil (1.9 g, 58%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.22−4.03 (4H, m), 3.70 (3H, s), 3.58 (1H, t, J = 7.3 Hz), 2.79−2.61 (3H, m), 1.98−1.78 (4H, m), 1.77−1.46 (2H, m), 1.25 (3H, t, J = 7.3 Hz), 0.98 (3H, t, J = 7.3 Hz). Anal. ($C_{14}H_{23}NO_5$) C, H, N.

4-Ethyl-5-(1-methoxycarbonyl-4-piperidyl)-3-isoxazolol (17b). 17b was synthesized as described for **12** by using **16b** (1.8 g, 6.3 mmol) and NaOH (270 mg, 6.7 mmol) in MeOH/water (6 mL/0.3 mL), hydroxylamine hydrochloride (870 mg, 13 mmol) and NaOH (500 mg, 13 mmol) in MeOH/water (8 mL/8 mL), and concentrated HCl (2.8 mL). The crude product was purified by CC (toluene/EtOAc (4:1) containing AcOH (1%)) and gave **17b** (900 mg, 56%): mp 129−132 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.36 (1H, broad s), 4.24 (2H, broad s), 3.71 (3H, s), 2.93−2.81 (3H, m), 2.34 (2H, q, J = 7.5 Hz), 1.83−1.77 (4H, m), 1.15, (3H, J = 7.5 Hz). Anal. ($C_{12}H_{18}N_2O_4$) C, H, N.

4-Ethyl-5-(4-piperidyl)-3-isoxazolol (7b). 7b was synthesized as described for **7a** by using **17b** (1.6 g, 6.3 mmol) and HBr in AcOH (33%, 80 mL). Recrystallization (MeOH/Et_2O) gave the title compound (712 mg, 41%): mp > 250 °C. ^{1}H NMR (300 MHz, D_2O): δ 3.50−3.38 (2H, m), 3.23−2.90 (3H,

m), 1.99 (2H, q, J = 7.6 Hz), 2.10−1.79 (4H, m), 0.95 (3H, t, J = 7.6 Hz). Anal. ($C_{10}H_{16}N_2O_2$·HBr·0.25H_2O) C, H, Br, N.

Ethyl 3-(1-Methoxycarbonyl-4-piperidyl)-2-propyl-3-oxopropionate (16c). 16c was synthesized as described above for **16a** by using **15** (3.0 g, 12 mmol), NaOEt (12 mmol) in EtOH (20 mL), and propyl bromide (1.2 mL, 12 mmol). The crude product was purified by CC (toluene/EtOAc (6:1)) to give the title compound as an oil (2.64 g, 75%). ^{1}H NMR (200 MHz, $CDCl_3$): δ 4.19 (2H, q, J = 7.2 Hz), 4.23−4.07 (2H, broad s), 3.69 (3H, s), 3.60 (1H, t, J = 7.2 Hz), 2.99−2.58 (3H, m), 1.98−1.40 (6H, m), 1.28 (5H, m), 1.25 (3H, t, J = 7.2 Hz), 0.98 (3H, t, J = 7.2 Hz).

5-(1-Methoxycarbonyl-4-piperidyl)-4-propyl-3-isoxazolol (17c). 17c was synthesized as described for **12** by using **16c** (1.5 g, 5.1 mmol) and NaOH (210 mg, 5.3 mmol) in MeOH/water (6 mL/0.3 mL), hydroxylamine hydrochloride (700 mg, 10 mmol) and NaOH in MeOH/water (7 mL/7 mL), and concentrated HCl (2.0 mL). The crude product was purified by CC (toluene/EtOAc (4:1) containing AcOH (1%)) and gave **17c** (560 mg, 40%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.52 (1H, broad s), 4.22 (2H, broad s), 3.70 (3H, s), 2.90−2.70 (3H, m), 2.28 (2H, q, J = 7.5 Hz), 1.85−1.68 (4H, m), 1.56 (2H, q, J = 7.3 Hz), 1.15−0.70, (3H, m). Anal. ($C_{13}H_{20}N_2O_4$) C, H, N.

5-(4-Piperidyl)-4-propyl-3-isoxazolol Hydrobromide (7c). 7c was synthesized as described for **7a** by using **17c** (0.6 g, 2.2 mmol) and HBr in AcOH (33%, 20 mL). Recrystallization (MeOH/Et_2O) gave the title compound (400 mg, 61%): mp > 200 °C. ^{1}H NMR (200 MHz, D_2O): δ 3.48−3.38 (2H, m), 3.10−2.90 (3H, m), 2.28−1.88 (6H, m), 1.51 (2H, sex, J = 7.4 Hz), 0.91 (3H, t, J = 7.4 Hz). Anal: ($C_{11}H_{18}N_2O_2$·HBr·0.5H_2O) C, H, N.

Ethyl 2-Butyl-3-(1-methoxycarbonyl-4-piperidyl)-3-oxopropionate (16d). 16d was synthesized as described for **16a** using **15** (3.0 g, 12 mmol), NaOEt (12 mmol) in EtOH (20 mL), and butyl bromide (1.4 mL, 13 mmol). The crude product was purified by CC (toluene/EtOAc (8:1)) to give the title compound as an oil (2.4 g, 65%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.18 (2H, q, J = 7.2 Hz), 4.21−4.13 (2H, m), 3.69 (3H, s), 3.58 (1H, t, J = 7.2 Hz), 2.91−2.79 (2H, m), 2.74−2.61 (1H, m), 1.92−1.75 (4H, m), 1.70−1.43 (2H, m), 1.34−1.18 (4H, m), 1.26 (3H, t, J = 6.9 Hz), 0.89 (3H, t J = 7.2 Hz). Anal. ($C_{16}H_{27}NO_5$) C, H, N.

4-Butyl-5-(1-methoxycarbonyl-4-piperidyl)-3-isoxazolol (17d). 17d was synthesized as described for **10** by using **16a** (1.8 g, 5.7 mmol), NaOH (240 mg, 6.1 mmol) in MeOH/water (6 mL/0.3 mL), hydroxylamine hydrochloride (790 mg, 11 mmol) and NaOH (460 mg, 11 mmol) in MeOH/water (9 mL/9 mL), and concentrated HCl (2.5 mL). The crude product was purified by CC (toluene/EtOAc (4:1) containing AcOH (1%)) and gave the pure product (640 mg, 40%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.41 (1H, broad s), 4.19 (2H, broad s), 3.68 (3H, s), 2.88−2.79 (3H, m), 2.27 (2H, t, J = 7.2 Hz), 1.80−1.74 (4H, m), 1.48 (2H, q, J = 7.5 Hz), 1.31 (2H, sex, J = 7.2 Hz), 0.89 (3H, J = 7.5 Hz). Anal. ($C_{14}H_{22}N_2O_4$) C, H, N.

4-Butyl-5-(4-piperidyl)-3-isoxazolol Hydrobromide (7d). 7d was synthesized as described above for **7a** by using **17d** (440 mg, 1.6 mmol) in HBr in AcOH (33%, 22 mL). Recrystallization (MeOH/Et_2O) gave the title compound (220 mg, 73%): mp 218−221 °C. ^{1}H NMR (300 MHz, D_2O): δ 3.45−3.39 (2H, m), 3.16−2.98 (3H, m), 2.21 (2H, t, J = 7.2 Hz), 2.01−1.89 (4H, m), 1.35 (2H, q, J = 7,2 Hz), 1.18 (2H, sex, J = 7.2 Hz), 0.77 (3H, t, J = 7.2 Hz). Anal. ($C_{12}H_{20}N_2O_2$·HBr) C, H, Br, N.

Ethyl 2-Hexyl-3-(1-methoxycarbonyl-4-piperidyl)-3-oxopropionate (16e). 16e was synthesized as described above for **16a** using **15** (3.0 g, 11.7 mmol), NaOEt (11.7 mmol) in EtOH (20 mL), and 1-bromohexane (1.8 mL, 12.8 mmol). The crude product was purified by CC (toluene/EtOAc (10:1)) to give the title compund as an oil (2.3 g, 60%). ^{1}H NMR (300 MHz, $CDCl_3$): 4.20−4.10 (4H, m), 3.68 (3H, s), 3.57 (1H, t, J = 7.2 Hz), 2.87−2.78 (2H, m), 2.70−2.62 (1H, m), 1.84−1.77 (4H, m), 1.66−1.47 (2H, m), 1.27−1.24 (11H, m), 0.85 (3H, t, J = 7.2 Hz). Anal. ($C_{18}H_{31}NO_5$) C, H, N.

4-Hexyl-5-(1-methoxycarbonyl-4-piperidyl)-3-isoxazolol (17e). 17e was synthesized as described for **10** by using **16e** (2.2 g, 6.6 mmol), NaOH (280 mg, 7.0 mmol) in MeOH/water (7 mL/0.35 mL), hydroxylamine hydrochloride (920 mg, 13 mmol) and NaOH (530 mg, 13.2 mmol) in MeOH/water (10 mL/10 mL), and concentrated HCl (3.0 mL). The crude product was purified by CC (toluene/EtOAc (4:1) containing AcOH (1%)) and gave the pure product (690 mg, 30%): mp 95−97 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.41 (1H, broad s), 4.21 (2H, broad s), 3.71 (3H, s), 2.92−2.79 (3H, m), 2.28 (2H, t, J = 7.2 Hz), 1.81−1.74 (4H, m), 1.52−1.46 (2H, m), 1.32−1.25 (6H, m), 0.87 (3H, J = 6.9 Hz). Anal. ($C_{16}H_{26}N_2O_4$) C, H, N.

4-Hexyl-5-(4-piperidyl)-3-isoxazolol Hydrobromide (7e). 7e was synthesized as described above for **7a** by using **17e** (620 mg, 2.0 mmol) in HBr in AcOH (33%, 30 mL). Recrystallization (MeOH/Et_2O) gave the title compound (290 mg, 43%): mp 228−232 °C. ^{1}H NMR (300 MHz, D_2O): δ 3.4−3.35 (2H, m), 3.10−2.94 (3H, m), 2.17 (2H, t, J = 7.2 Hz), 1.93−1.85 (4H, m), 1.34−1.29 (2H, m), 1.1−1.08 (6H, m), 0.67 (3H, t, J = 7.2 Hz). Anal. ($C_{14}H_{24}N_2O_2$·HBr) C, H, Br, N.

Ethyl 3-(1-Methoxycarbonyl-4-piperidyl)-2-octyl-3-oxopropionate (16f). 16f was synthesized as described above for **16a** using **15** (3.0 g, 11.7 mmol), NaOEt (11.7 mmol) in EtOH (20 mL), and 1-bromooctane (2.2 mL, 12.8 mmol). The crude product was purified by CC (toluene/EtOAc (8:1)) to give the title compund as an oil (2.5 g, 58%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.17 (2H, q, J = 7.2 Hz), 4.21−4.09 (2H, m), 3.68 (3H, s), 3.58 (1H, t, J = 7.2 Hz), 2.89−2.78 (2H, m), 2.68 (1H, dt, J =11.1 and 3.6 Hz), 1.84−1.78 (4H, m), 1.70−1.48 (3H, m), 1.4−1.25 (14H, m), 0.87 (3H, t, J = 7.2 Hz).

5-(1-Methoxycarbonyl-4-piperidyl)-4-octyl-3-isoxazolol (17f). 17f was synthesized as described for **12** by using **16f** (2.5 g, 6.8 mmol) and NaOH (300 mg, 7.5 mmol) in MeOH/water (12 mL/0.7 mL), hydroxylamine hydrochloride (945 mg, 13.6 mmol) and NaOH (544 mg, 13.6 mmol) in MeOH/water (15 mL/5 mL), and concentrated HCl (3.0 mL). The crude product was purified by CC (toluene/EtOAc (8:1) containing AcOH (1%)) and gave the pure product (1.0 g, 44%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.94 (1H, broad s), 4.15 (2H, broad s), 3.65 (3H, s), 2.83−2.73 (3H, m), 2.22 (2H, t, J = 7.2 Hz), 1.76−1.68 (4H, m), 1.50−1.38 (2H, m), 1.26−1.08 (10H, m), 0.80 (3H, t, J = 7.2 Hz).

5-(4-Piperidyl)-4-octyl-3-isoxazolol Hydrobromide (7f). 7f was synthesized as described for **7a** by using **17f** (500 mg, 1.4 mmol) in HBr in AcOH (33%, 15 mL). Recrystallization (MeOH/Et_2O) gave the title compound (225 mg, 42%): mp 226−234 °C. ^{1}H NMR (300 MHz, CD_3OD): δ 3.49−3.45 (2H, m), 3.19−3.10 (3H, m), 2.32 (2H, t, J = 7.2 Hz), 2.07−2.04 (4H, m), 1.60−1.42 (2H, m), 1.39−1.22 (10H, m), 0.89 (3H, t, J = 6.6 Hz) Anal. ($C_{16}H_{28}N_2O_2$·HBr) C, H, Br, N.

Methyl 2-Benzyl-3-(1-methoxycarbonyl-4-piperidyl)-3-oxopropionate (16h). 16h was synthesized as described above for **16a** using **15** (3.0 g, 11.7 mmol), NaOMe (11.7 mmol) in MeOH (20 mL), and benzyl bromide (1.5 mL, 12.8 mmol). The crude product was purified by CC (toluene/EtOAc (3:1)) to give the title compund as an oil (2.4 g, 60%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.32−7.02 (5H, m), 4.03 (2H, broad s), 3.98 (2H, t, J = 7.2 Hz), 3.70 (3H, s), 3.68 (3H, s), 3.19 (2H, d, J = 7.2 Hz), 2.82−2.60 (2H, m), 2.50−2.41 (1H, m), 1.78−1.63 (1H, m), 1.60−1.41 (2H, m), 1.24−1.50 (1H, m). Anal. ($C_{18}H_{23}NO_5$) C, H, N.

4-Benzyl-5-(1-methoxycarbonyl-4-piperidyl)-3-isoxazolol (17h). 17h was synthesized as described for **12** by using **16h** (2.4 g, 7.1 mmol) and NaOH (300 mg, 7.5 mmol) in MeOH/water (7 mL/0.35 mL), hydroxylamine hydrochloride (990 mg, 14.2 mmol) and NaOH (570 mg, 14.2 mmol) in MeOH/water (10 mL/10 mL), and concentrated HCl (3.0 mL). The crude product was purified by CC (toluene/EtOAc (4:1) containing AcOH (1%)) and gave the pure product (970 mg, 43%): mp 195−196 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.41 (1H, broad s), 7.34−7.10 (5H, m), 4.20−4.00 (2H, m), 3.68 (5H, s), 2.93−2.69 (3H, m), 1.93−1.40 (4H, m). Anal. ($C_{17}H_{20}N_2O_4$) C, H, N.

4-Benzyl-5-(4-piperidyl)-3-isoxazolol Hydrobromide (7h). 7h was synthesized as described above for **7a** by using **17h** (600 mg, 1.9 mmol) in HBr in AcOH (33%, 30 mL). Recrystallization (MeOH/Et_2O) gave the title compound (340

mg, 53%): mp 226−234 °C. ^{1}H NMR (300 MHz, D_2O): δ 7.27−7.13 (5H, m), 3.61 (2H, s), 3.38−3.31 (2H, m), 3.04−2.87 (3H, m), 1.89−1.82 (4H, m). Anal. ($C_{15}H_{18}N_2O_2$·HBr) C, H, Br, N.

Ethyl 3-(1-Methoxycarbonyl-4-piperidyl)-3-oxo-2-(2-phenylethyl)propionate (16i). 16i was synthesized as described for **16a** using **15** (3.0 g, 12 mmol), NaOEt (12 mmol) in EtOH (20 mL), and 2-phenylethyl bromide (1.7 mL, 13 mmol). The crude product was purified by CC (toluene/EtOAc (4:1)) to give the title compund as an oil (2.7 g, 64%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.28−7.15 (5H, m), 4.20−4.00 (4H, m), 3.68 (3H, s), 3.60 (1H, t, J = 7.2 Hz), 2.94−2.72 (2H, m), 2.64−2.56 (2H, m), 2.24−2.09 (2H, m), 1.92−1.40 (5H, m), 1.25 (3H, t, J = 7.2 Hz).

5-(1-Methoxycarbonyl-4-piperidyl)-4-(2-phenylethyl)-3-isoxazolol (17i). 17i was synthesized as described for **12** using **16i** (2.3 g, 6.4 mmol) and NaOH (270 mg, 6.7 mmol) in MeOH/water (7 mL/0.35 mL), hydroxylamine hydrochloride (890 mg, 13 mmol) and NaOH (510 mg, 13 mmol) in MeOH/water (9 mL/9 mL), and concentrated HCl (3.0 mL). The crude product was purified by CC (toluene/EtOAc (2:1) containing AcOH (1%)) and gave the pure product (1.0 g, 49%): mp 159−161 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.79 (1H, broad s), 7.29−7.10 (5H, m), 4.08 (2H, broad s), 3.68 (3H, s), 2.88−2.68 (2H, m), 2.64−2.60 (4H, m), 2.38−2.30 (1H, m), 1.60−1.47 (2H, m), 1.30−1.26 (2H, m). Anal. ($C_{18}H_{22}N_2O_4$) C, H, N.

4-(2-Phenylethyl)-5-(4-piperidyl)-3-isoxazolol Hydrobromide (7i). 7i was synthesized as described for **7a** using **17i** (400 mg, 1.2 mmol) in HBr in AcOH (33%, 20 mL). Recrystallization (MeOH/Et_2O) gave the title compound (250 mg, 59%): mp 246−249 °C. ^{1}H NMR (300 MHz, D_2O): δ 7.29−7.10 (5H, m), 3.30−3.26 (2H, m), 2.85−2.76 (4H, m), 2.64−2.59 (2H, m), 2.51−2.44 (1H, m), 1.70−1.56 (2H, m), 1.31−1.27 (2H, m). Anal. ($C_{16}H_{20}N_2O_2$·HBr) C, H, Br, N

Ethyl 3-(1-Methoxycarbonyl-4-piperidyl)-3-oxo-2-(3-phenylpropyl)propionate (16j). 16j was synthesized as described for **16a** using **15** (3.0 g, 12 mmol), NaOEt (12 mmol) in EtOH (20 mL), and 3-phenylpropyl bromide (1.9 mL, 13 mmol). The crude product was purified by CC (toluene/EtOAc (5:1)) to give the title compund as an oil (3.0 g, 68%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.29−7.13 (5H, m), 4.19−4.00 (4H, m), 3.68 (3H, s), 3.58 (1H, t, J = 7.2 Hz), 2.86−2.76 (2H, m), 2.66−2.59 (3H, m), 1.92−1.44 (8H, m), 1.25 (3H, t, J = 7.2 Hz).

5-(1-Methoxycarbonyl-4-piperidyl)-4-(3-phenylpropyl)-3-isoxazolol (17j). 17j was synthesized as described for **12** by using **16j** (2.5 g, 6.7 mmol) and NaOH (290 mg, 7.2 mmol) in MeOH/water (10 mL/0.7 mL), hydroxylamine hydrochloride (931 mg, 13.4 mmol) and NaOH (540 mg, 13 mmol) in MeOH/water (9 mL/9 mL), and concentrated HCl (4.0 mL). The crude product was purified by CC (toluene/EtOAc (5:1) containing AcOH (1%)) and gave the pure product (1.3 g, 49%): mp 127−128 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.88 (1H, broad s), 7.32−7.16 (5H, m), 4.19 (2H, broad s), 3.71 (3H, s), 2.86−2.63 (5H, m), 2.37−2.31 (2H, m), 1.93−1.70 (6H, m). Anal. ($C_{19}H_{24}N_2O_4$) C, H, N.

4-(3-Phenylpropyl)-5-(4-piperidyl)-3-isoxazolol Hydrobromide (7j). 7j was synthesized as described for **7a** by using **17j** (500 mg, 1.5 mmol) in HBr in AcOH (33%, 30 mL). Recrystallization (MeOH/Et_2O) gave the title compound (360 mg, 68%): mp 222−223 °C. ^{1}H NMR (300 MHz, D_2O): δ 7.38−7.25 (5H, m), 3.50−3.44 (2H, m), 3.09−2.98 (3H, m), 2.64 (2H, t, J = 7.5 Hz), 2.35 (2H, t, J = 7.5 Hz), 2.02−1.92 (4H, m), 1.84 (2H, pent, J = 7.5 Hz). Anal. ($C_{17}H_{22}N_2O_2$·HBr) C, H, Br, N.

Ethyl 2-(9-Anthracylmethyl)-3-(1-methoxycarbonyl-4-piperidyl)-3-oxopropionate (16k). 16k was synthesized as described above for **16a** using **15** (3.0 g, 12 mmol), NaOEt (12 mmol) in EtOH (20 mL), and 9-chloromethylanthracene (2.9 g, 13 mmol). The crude product was purified by CC (toluene/EtOAc (6:1)) to give the title compound as an oil (2.3 g, 43%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.38 (1H, s), 8.18 (2H, d, J = 8.4 Hz), 8.00 (2H, d, J = 8.4 Hz), 7.54−7.46 (4H m), 4.16−3.81 (5H, m), 3.68 (2H, d, J = 9 Hz), 3.60 (3H, s), 2.92−2.70 (2H, m), 2.62−2.50 (1H, m), 1.72−1.48 (2H, m), 1.42−1.21 (2H, m), 1.10 (3H, t, J = 7.2 Hz).

4-(9-Anthracylmethyl)-5-(1-methoxycarbonyl-4-piperidyl)-3-isoxazolol (17k). 17k was synthesized as described for **12** by using **16k** (1.8 g, 4.0 mmol) and NaOH (160 mg, 4.0 mmol) in MeOH/water (6 mL/0.3 mL), hydroxylamine hydrochloride (550 mg, 7.9 mmol) and NaOH (320 mg, 7.9 mmol) in MeOH/water (7 mL/7 mL), and concentrated HCl (2.5 mL). The crude product was purified by CC (toluene/EtOAc (4:1) containing AcOH (1%)) and gave the pure product (250 mg, 15%): mp 213-215 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.43 (1H, s), 8.24−8.21 (2H, m), 8.03−8.00 (2H, m), 7.53−7.44 (4H, m), 4.66 (2H, s), 3.70−3.55 (2H, m), 3.54 (3H, s), 1.60−1.41 (2H, m), 1.24−1.02 (3H, m), 0.60−0.31 (2H, m).

4-(9-Anthracylmethyl)-5-(4-piperidyl)-3-isoxazolol Hydrobromide (7k). 7k was synthesized as described for **7a** by using **17k** (100 mg, 0.24 mmol) in HBr in AcOH (33%, 10 mL). Recrystallization (MeOH/Et_2O) gave the title compound (61 mg, 58%): mp >250 °C. ^{1}H NMR (300 MHz, D_2O): δ 8.44 (1H, s), 8.22 (2H, d, J = 8.4 Hz), 8.05 (2H, d, J = 8.4 Hz), 4.52 (2H, s), 2.96−2.82 (2H, m), 2.01−1.76 (3H, m), 1.47−1.28 (2H, m), 0.8−0.75 (2H, m). Anal. ($C_{23}H_{22}N_2O_2$·HBr·1H_2O) C, H, N.

Ethyl 3-(1-Methoxycarbonyl-4-piperidyl)-3-oxo-2-(4-phenylbenzyl)propionate (16l). 16l was synthesized as described for **16a** using **15** (3.0 g, 12 mmol), NaOEt (12 mmol) in EtOH (20 mL), and 4-phenylbenzyl bromide[32] (3.2 g, 13 mmol). The crude product was purified by CC (toluene/EtOAc (10:1)) to give the title compund as an oil (2.95 g, 60%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.56 (2H, d, J = 7.2 Hz), 7.50 (2H, d, J = 7.2 Hz), 7.42 (2H, t, J = 8.1 Hz), 7.34 (1H, d, J = 7.2 Hz), 7.24 (2H, d, J = 8.4 Hz), 4.16 (2H, q, J = 7.5 Hz), 4.09 (2H, broad s), 3.97 (1H, t, J = 7.5 Hz), 3.65 (3H, s), 3.20 (2H, d, J = 7.2 Hz), 2.95−2.63 (2H, m), 2.58−2.41 (1H, m), 1.94−1.45 (3H, m), 1.35−1.10 (1H, m), 1.21 (3H, t, J = 7.2 Hz).

5-(1-Methoxycarbonyl-4-piperidyl)-4-(4-phenylbenzyl)-3-isoxazolol (17l). 17l was synthesized as described for **12** by using **16l** (2.6 g, 6.0 mmol) and NaOH (260 mg, 6.4 mmol) in MeOH/water (8 mL/0.8 mL), hydroxylamine hydrochloride (830 mg, 12 mmol) and NaOH (480 mg, 12 mmol) in MeOH/water (9 mL/9 mL), and concentrated HCl (2.8 mL). The crude product was purified by CC (toluene/EtOAc (4:1) containing AcOH (1%)) and gave the product (1.3 g, 55%). An analytical sample was recrystallized (toluene/light petroleum): mp 169−170 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.58−7.26 (9H, m), 4.17 (2H, broad s), 3.73 (2H, s), 3.69 (3H, s), 2.85−2.75 (3H, m), 1.79−1.71 (4H, m). Anal. ($C_{23}H_{24}N_2O_4$) C, H, N.

4-(4-Phenylbenzyl)-5-(4-piperidyl)-3-isoxazolol Hydrobromide (7l). 7l was synthesized as described for **7a** by using **17l** (600 mg, 1.5 mmol) in HBr in AcOH (33%, 30 mL). Recrystallization (MeOH/Et_2O) gave the title compound (400 mg, 63%): mp >240 °C. ^{1}H NMR (300 MHz, D_2O): δ 7.37−7.29 (4H, m), 7.17−7.00 (5H, m), 3.49 (2H, s), 2.66 (2H, d, J = 12.3 Hz), 2.41−2.23 (1H, m), 2.06 (2H, t, J = 12.3 Hz), 1.44−1.29 (4H, m). Anal. ($C_{21}H_{22}N_2O_2$·HBr) C, H, Br, N.

Ethyl 3-(1-Methoxycarbonyl-4-piperidyl)-2-(2-naphthylmethyl)-3-oxopropionate (16m). 16m was synthesized as described above for **16a** using **15** (15 g, 59 mmol), NaOEt (59 mmol) in EtOH (100 mL), and 2-(bromomethyl)naphthalene (14 g, 64 mmol). The crude product was purified by CC (toluene/EtOAc (8:1)) to give the title compound as an oil (15 g, 65%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.81−7.74 (3H m), 7.61 (1H, s), 7.46−7.41 (2H, m), 7.29 (1H, d, J = 8.4 Hz), 4.21−3.82 (5H, m), 3.63 (3H, s), 3.36−3.32 (2H, m), 2.81−2.59 (2H, m), 2.57−2.42 (1H, m), 1.80−1.64 (1H, m), 1.62−1.42 (2H, m), 1.30−1.18 (1H, m), 1.19 (3H, t, J = 7.2 Hz). Anal. ($C_{23}H_{27}NO_5$) C, H, N.

5-(1-Methoxycarbonyl-4-piperidyl)-4-(2-naphthylmethyl)-3-isoxazolol (17m). 17m was synthesized as described for **12** by using **16m** (2.4 g, 5.9 mmol) and NaOH (250 mg, 6.3 mmol) in MeOH/water (7 mL/0.36 mL), hydroxylamine hydrochloride (820 mg, 12 mmol) and NaOH (470 mg, 12 mmol) in MeOH/water (9 mL/9 mL), and concentrated HCl (2.5 mL). The crude product was purified by CC (toluene/EtOAc (8:1) containing AcOH (1%)) and gave the pure product (850 mg, 39%): mp 152−153 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.05 (1H, broad s), 7.80−7.73 (3H, m), 7.59 (1H, s), 7.43−7.41 (2H,

m), 7.33 (1H, d, J = 8.4 Hz), 4.10 (2H, broad s), 3.84 (2H, s), 3.66 (3H, s), 2.80−2.61 (3H, m), 1.79−1.58 (4H, m). Anal. ($C_{21}H_{22}N_2O_4$) C, H, N.

4-(2-Naphthylmethyl)-5-(4-piperidyl)-3-isoxazolol Hydrobromide (7m). 7m was synthesized as described for **7a** by using **17m** (510 mg, 1.4 mmol) in HBr in AcOH (33%, 20 mL). Recrystallization (MeOH/Et_2O) gave the title compound (370 mg, 68%): mp 223−224 °C. ^{1}H NMR (300 MHz, D_2O): δ 7.78−7.62 (3H, m), 7.47 (1H, s), 7.39−7.30 (2H, m), 7.21 (1H, d, J = 8.4 Hz), 3.67 (2H, s), 3.28−3.17 (2H, m), 2.98−2.85 (1H, m), 2.81−2.69 (2H, m), 1.87−1.68 (4H, m). Anal. ($C_{19}H_{20}N_2O_2$·HBr) C, H, Br, N.

Ethyl 3-(1-Methoxycarbonyl-4-piperidyl)-2-[2-(2-naphthyl)ethyl]-3-oxopropionate (16n). 16n was synthesized as described above for **16a** using **15** (3.0 g, 12 mmol), NaOEt (12 mmol) in EtOH (20 mL), and 2-(2-bromoethyl)naphthalene[33,34] (3.0 g, 13 mmol). The crude product was purified by CC (toluene/EtOAc (5:1)) to give the title compund as an oil (2.3 g, 48%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.80−7.74 (3H m), 7.58 (1H, s), 7.47−7.38 (2H, m), 7.30 (1H, dd, J = 8.4 and 1.8 Hz), 4.16 (2H, q, J = 7.2 Hz), 4.11 (2H, broad s), 3.67 (3H, s), 3.61 (1H, t, J = 7.2 Hz), 2.81−2.63 (4H, m), 2.61−2.54 (1H, m), 2.33−2.20 (2H, m), 1.80−1.42 (4H, m), 1.20 (3H, t, J = 7.2 Hz).

5-(1-Methoxycarbonyl-4-piperidyl)-4-[2-(2-naphthyl)-ethyl]-3-isoxazolol (17n). 17n was synthesized as described for **12** by using **16n** (2.0 g, 4.9 mmol) and NaOH (210 mg, 5.2 mmol) in MeOH/water (8 mL/0.6 mL), hydroxylamine hydrochloride (820 mg, 12 mmol) and NaOH (472 mg, 12 mmol) in MeOH/water (7 mL/7 mL), and concentrated HCl (3.0 mL). The crude product was purified by CC (toluene/EtOAc (5:1) containing AcOH (1%)) and gave the product (1.4 g, 52%). A sample was recrystallized (toluene/light petroleum) to give the product: mp 143−145 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.05 (1H, broad s), 7.81−7.74 (3H, m), 7.56 (1H, s), 7.48−7.41 (2H, m), 7.33 (1H, d, J = 8.4 Hz), 3.94 (2H, broad s), 3.65 (3H, s), 3.02 (2H, t, J = 6.9 Hz), 2.71 (2H, t, J = 6.9 Hz), 2.43−2.36 (2H, m), 2.21−2.14 (1H, m), 1.55−1.38 (2H, m), 1.14−1.09 (2H, m). Anal. ($C_{22}H_{24}N_2O_4$) C, H, N.

5-(4-Piperidyl)-4-[2-(2-naphthyl)ethyl]-3-isoxazolol Hydrobromide (7n). 7n was synthesized as described above for **7a** by using **17n** (400 mg, 1.1 mmol) in HBr in AcOH (33%, 30 mL). Recrystallization (MeOH/Et_2O) gave the title compound (340 mg, 80%): mp >255 °C. ^{1}H NMR (300 MHz, D_2O): δ 7.68−7.60 (3H, m), 7.39 (1H, s), 7.34−7.24 (2H, m), 7.17 (1H, dd, J = 8.4 Hz, J = 1.5 Hz), 2.77−2.72 (2H, m), 2.44−2.34 (4H, m), 1.78−1.60 (3H, m), 1.02−0.86 (2H, m), 0.53−0.49 (2H, m). Anal. ($C_{20}H_{22}N_2O_2$·HBr·1.75H_2O) C, H, N.

Ethyl 3-(1-Methoxycarbonyl-4-piperidyl)-2-(1-naphthylmethyl)-3-oxopropionate (16o). 16o was synthesized as described for **16a** using **15** (3.0 g, 12 mmol), NaOEt (12 mmol) in EtOH (20 mL), and 1-(bromomethyl)naphthalene[35] (2.8 g, 13 mmol). The crude product was purified by CC (toluene/EtOAc (4:1)) to give the title compund as an oil (3.2 g, 70%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.98 (1H, d, J = 8.4 Hz), 7.86 (1H, d, J = 8.1 Hz), 7.71 (1H, d, J = 8.1 Hz), 7.57−7.46 (2H, m), 7.34 (1H, t, J = 6.9 Hz), 7.25 (1H, d, J = 6.0 Hz), 4.21−4.13 (3H, m), 4.00 (1H, broad s), 3.81 (1H, broad s), 3.71−3.64 (2H, m), 3.62 (3H, s), 2.74−2.52 (3H, m), 2.38−2.23 (1H, m), 1.70−1.64 (1H, m), 1.57−1.43 (1H, m), 1.19 (3H, t, J = 7.2 Hz), 1.27−1.17 (1H, m).

5-(1-Methoxycarbonyl-4-piperidyl)-4-(1-naphthylmethyl)-3-isoxazolol (17o). 17o was synthesized as described for **12** by using **16o** (3.0 g, 7.5 mmol) and NaOH (320 mg, 8.0 mmol) in MeOH/water (9 mL/0.6 mL), hydroxylamine hydrochloride (1.0 g, 15 mmol) and NaOH (600 mg, 15 mmol) in MeOH/water (10 mL/10 mL), and concentrated HCl (3.0 mL). The crude product was purified by CC (toluene/EtOAc (5:1) containing AcOH (1%)) and recrystallized (toluene/light petroleum) to give the product (560 mg, 31%): mp 166−167 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.75 (1H, broad s), 8.07 (1H, d, J = 8.1 Hz), 7.89 (1H, d, J = 7.5 Hz), 7.78 (1H, d, J = 8.4 Hz), 7.58−7.42 (2H, m), 7.40 (1H, t, J = 7.5 Hz), 7.28 (1H, d, J = 8.4 Hz), 4.16 (2H, s), 4.30 (2H, broad s), 3.67 (3H, s), 2.54−2.36 (3H, m), 1.64−1.53 (2H, m), 1.39−1.34 (2H, m). Anal. ($C_{21}H_{22}N_2O_4$) C, H, N.

5-(4-Piperidyl)-4-(1-naphthylmethyl)-3-isoxazolol Hydrobromide (7o). 7o was synthesized as described for **7a** by using **17o** (630 mg, 1.8 mmol) in HBr in AcOH (33%, 30 mL). Recrystallization (MeOH/Et_2O) gave the title compound (560 mg, 80%): mp 253−255 °C. ^{1}H NMR (300 MHz, D_2O): δ 8.00−7.97 (1H, m), 7.90−7.87 (1H, m), 7.79 (1H, d, J = 8.1 Hz), 7.54−7.46 (2H, m), 7.38 (1H, t, J = 6.9 Hz), 7.26−7.24 (1H, m), 4.02 (2H, s), 3.23−3.19 (2H, m), 2.70−2.58 (3H, m), 1.78−1.64 (2H, m), 1.53−1.50 (2H, m). Anal. ($C_{19}H_{20}N_2O_2$·HBr) C, H, N.

Ethyl 3-(1-Methoxycarbonyl-4-piperidyl)-2-[2-(1-naphthyl)ethyl]-3-oxopropionate (16p). 16p was synthesized as described for **16a** using **15** (3.0 g, 12 mmol), NaOEt (12 mmol) in EtOH (20 mL), and 1-(2-bromoethyl)naphthalene[36,37] (2.9 g, 12 mmol). The crude product was purified by CC (toluene/EtOAc (4:1)) to give the title compound as an oil (2.5 g, 52%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.08 (1H, d, J = 8.4 Hz), 7.86 (1H, d, J = 7.5 Hz), 7.74 (1H, d, J = 8.1 Hz), 7.58−7.32 (2H, m), 7.27 (1H, t, J = 8.1 Hz), 7.18 (1H, d, J = 8.1 Hz), 4.21 (2H, q, J = 7.5 Hz), 4.14 (2H, broad s), 3.69 (3H, s), 3.72−3.13 (1H, m), 3.11−3.02 (2H, m), 2.8−2.75 (2H, m), 2.67−2.60 (1H, m), 2.33−2.25 (2H, m), 1.8−1.73 (2H, m), 1.63−1.39 (2H, m), 1.27 (3H, t, J = 6.9 Hz).

5-(1-Methoxycarbonyl-4-piperidyl)-4-[2-(1-naphthyl)-ethyl]-3-isoxazolol (17p). 17p was synthesized as described for **12** by using **16p** (2.1 g, 5.1 mmol) and NaOH (200 mg, 5.1 mmol) in MeOH/water (16 mL/1.6 mL), hydroxylamine hydrochloride (700 mg, 10 mmol) and NaOH (400 mg, 10 mmol) in MeOH/water (13 mL/1.3 mL), and concentrated HCl (3.0 mL). The crude product was purified by CC (toluene/EtOAc (4:1) containing AcOH (1%)) and gave the product (730 mg, 38%). An analytical sample was recrystallized (toluene/light petroleum): mp 160−163 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.09 (1H, d, J = 8.1 Hz), 7.85 (1H, d, J = 8.6 Hz), 7.70 (1H, d, J = 8.1 Hz), 7.57−7.46 (2H, m), 7.33 (1H, t, J = 7.5 Hz), 7.19 (1H, d, J = 6.0 Hz), 3.96 (2H, broad s), 3.67 (3H, s), 3.39−3.33 (2H, m), 2.87−2.75 (2H, m), 2.35−2.19 (2H, m), 1.96−1.82 (1H, m), 1.47−1.29 (2H, m), 1.04−0.90 (2H, m). Anal. ($C_{22}H_{24}N_2O_4$) C, H, N.

4-[2-(1-Naphthyl)ethyl]-5-(4-piperidyl)-3-isoxazolol Hydrobromide (7p). 7p was synthesized as described for **7a** by using **17p** (250 mg, 0.65 mmol) in HBr in AcOH (33%, 15 mL). Recrystallization (MeOH/Et_2O) gave the title compound (194 mg, 75%): mp >250 °C. ^{1}H NMR (300 MHz, D_2O): δ 8.06 (1H, d, J = 8.1 Hz), 7.89 (1H, d, J = 7.8 Hz), 7.74 (1H, d, J = 8.1 Hz), 7.57−7.48 (2H, m), 7.35 (1H, t, J = 8.1 Hz), 7.17 (1H, d, J = 6.9 Hz), 3.29−3.25 (2H, m), 3.06 (2H, m), 2.80−2.75 (2H, m), 2.30 (2H, m), 1.89−1.79 (1H, m), 1.34 (2H, m), 0.84−0.78 (2H, m). Anal. ($C_{20}H_{22}N_2O_2$·HBr·H_2O) C, H, Br, N.

Ethyl 2-(3,3-Diphenylpropyl)-3-(1-methoxycarbonyl-4-piperidyl)-3-oxopropionate (16s). 16s was synthesized as described for **16a** using **15** (15.7 g, 61.0 mmol), NaOEt (61.0 mmol) in EtOH (100 mL), and 3,3-diphenylpropyl bromide[38] (18.4 g, 67.0 mmol). The crude product was purified by CC (toluene/EtOAc (4:1)) to give the title compound as an oil (13.4 g, 49%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.30−7.15 (10H, m), 4.14 (2H, q, J = 7.2 Hz), 4.11 (2H, broad s), 3.89 (1H, t, J = 8.1 Hz), 3.67 (3H, s), 3.58 (1H, t, J = 7.2 Hz), 2.85−2.70 (2H, m), 2.62−2.50 (1H, m), 2.10−2.90 (2H, m), 1.90−1.62 (4H, m), 1.61−1.39 (2H, m), 1.22 (3H, t, J = 7.2 Hz).

4-(3,3-Diphenylpropyl)-5-(1-methoxycarbonyl-4-piperidyl)-3-isoxazolol (17s). 17s was synthesized as described for **12** by using **16s** (1.4 g, 3.1 mmol) and NaOH (133 mg, 3.3 mmol) in MeOH/water (6 mL/0.4 mL), hydroxylamine hydrochloride (430 mg, 6.2 mmol) and NaOH (248 mg, 6.2 mmol) in MeOH/water (5 mL/5 mL), and concentrated HCl (2.0 mL). The crude product was purified by CC (toluene/EtOAc (5:1) containing AcOH (1%)) and gave the product (600 mg, 46%). An analytical sample was recrystallized (toluene/light petroleum): mp 150−151 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.55 (1H, broad s), 7.32−7.15 (10H, m), 4.14 (2H, broad s), 3.92−

3.88 (1H, m), 3.71 (3H, s), 3.74−2.66 (2H, m), 2.50−2.42 (1H, m), 2.34 (4H, s), 1.70−1.63 (4H, m). Anal. ($C_{25}H_{28}N_2O_4$) C, H, N.

4-(3,3-Diphenylpropyl)-5-(4-piperidyl)-3-isoxazolol Hydrobromide (7s). 7s was synthesized as described for **7a** by using **17s** (150 mg, 0.36 mmol) in HBr in AcOH (33%, 10 mL). Recrystallization (MeOH/Et_2O) gave the title compound (110 mg, 69%): mp >250 °C. ^{1}H NMR (300 MHz, D_2O): δ 7.50−7.25 (10H, m), 4.14−4.10 (1H, m), 3.62−3.58 (2H, m), 3.50−3.05 (2H, m), 3.01−2.86 (1H, m), 2.56 (4H, s), 2.20−2.00 (4H, m). Anal. ($C_{23}H_{26}N_2O_2$·HBr) C, H, N.

Ethyl 2-(4,4-Diphenylbutyl)-3-(1-methoxycarbonyl-4-piperidyl)-3-oxopropionate (16t). 16t was synthesized as described for **16a** using **15** (3.0 g, 11.7 mmol), NaOEt (11.7 mmol) in EtOH (20 mL), and 4,4-diphenylbutyl bromide[39] (3.7 g, 12.8 mmol). The crude product was purified by CC (toluene/EtOAc (4:1)) to give the title compound as an oil (3.3 g, 61%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.26−7.15 (10H, m), 4.11 (2H, q, J = 7.2 Hz), 4.10 (2H, broad s), 3.88 (1H, t, J = 7.2 Hz), 3.67 (3H, s), 3.51 (1H, t, J = 7.2 Hz), 2.83−2.72 (2H, m), 2.62−2.52 (1H, m), 2.05 (2H, q, J = 7.5 Hz), 1.92−1.80 (2H, m), 1.78−1.34 (4H, m), 1.23−1.15 (2H, m), 1.18 (3H, t, J = 7.2 Hz).

4-(4,4-Diphenylbutyl)-5-(1-methoxycarbonyl-4-piperidyl)-3-isoxazolol (17t). 17t was synthesized as described for **12** by using **16t** (3.3 g, 7.1 mmol) and NaOH (303 mg, 7.6 mmol) in MeOH/water (10 mL/0.6 mL), hydroxylamine hydrochloride (985 mg, 14.2 mmol) and NaOH (568 mg, 14.2 mmol) in MeOH/water (12 mL/12 mL), and concentrated HCl (3.0 mL). The crude product was purified by CC (toluene/EtOAc (4:1) containing AcOH (1%)) and gave the product (750 mg, 24%). An analytical sample was recrystallized (toluene/light petroleum): mp 139−140 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.55 (1H, broad s), 7.30−7.14 (10H, m), 4.17 (2H, broad s), 3.92 (1H, t, J = 7.8 Hz), 3.73 (3H, s), 2.82−2.65 (3H, m), 2.32 (2H, t, J = 7.8 Hz), 2.12−2.04 (2H, m), 1.76−1.61 (4H, m), 1.54−1.44 (2H, m). Anal. ($C_{26}H_{30}N_2O_4$) C, H, N.

4-(4,4-Diphenylbutyl)-5-(4-piperidyl)-3-isoxazolol Hydrobromide (7t). 7t was synthesized as described for **7a** by using **17t** (400 mg, 0.9 mmol) in HBr in AcOH (33%, 20 mL). Recrystallization (MeOH/Et_2O) gave the title compound (330 mg, 78%): mp >225 °C. ^{1}H NMR (300 MHz, D_2O): δ 6.81−6.58 (10H, m), 3.55−3.50 (1H, m), 2.47−2.44 (2H, m), 2.02−1.70 (7H, m), 1.23−1.05 (6H, m). Anal. ($C_{24}H_{28}N_2O$·HBr) C, H, N.

3-Isopropoxy-5-(1-methoxycarbonyl-4-piperidyl)isoxazole (19). A mixture of 5-(1-methoxycarbonyl-4-piperidyl)-3-isoxazolol (**18**)[16] (3.8 g, 17 mmol) and potassium carbonate (3.2 g, 24 mmol) in DMF (38 mL) was stirred at 60 °C for 30 min. Isopropyl bromide (4.4 mL, 50 mmol) was added dropwise to the reaction mixture, and stirring was continued for 2 days at 60 °C. After the mixture was cooled, water was added and the aqueous phase was extracted with light petroleum (5 × 25 mL). The combined extracts were washed with water (2 × 30 mL), dried, and evaporated. CC (toluene/EtOAc (3:1)) of the crude product gave the product as an oil (3.36 g, 82%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.57 (1H, s), 4.87 (1H, hep, J = 4.8 Hz), 4.17 (2H, broad s), 3.73 (3H, s), 2.97−2.78 (3H, m), 2.07−1.80 (2H, m), 1.68−1.54 (2H, m), 1.37 (6H, d, J = 4.8 Hz).

4-Iodo-3-isopropoxy-5-(1-methoxycarbonyl-4-piperidyl)-isoxazole (20). To a solution of **19** (7.4 g, 28 mmol) in AcOH (50 mL), a solution of iodomonochloride (5.4 g, 33 mmol) in AcOH (40 mL) was added followed by water (125 mL). The reaction mixture was stirred for 18 h at 85 °C. After the mixture was cooled, sodium thiosulfate was added and the mixture was evaporated. Water (100 mL) was added to the residue, and the mixture was extracted with Et_2O (3 × 200 mL). The organic extracts were dried and evaporated. CC (toluene/EtOAc (5:1)) gave the product (9.8 g, 90%): mp 118−120 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.89 (1H, hep, J = 6.0 Hz), 4.22 (2H, broad s), 3.70 (3H, s), 3.00−2.81 (3H, m), 1.90−1.77 (4H, m), 1.39 (6H, d, J = 6.0 Hz). Anal. ($C_{13}H_{19}IN_2O_4$) C, H, N.

4-(Cyclohexylhydroxymethyl)-3-isopropoxy-5-(1-methoxycarbonyl-4-piperidyl)isoxazole (21g). A solution of **20** (700 mg, 1.8 mmol) in THF (1 mL) was added dropwise to a solution of EtMgCl in THF (2.8 M, 640 mM, 1.78 mmol) at −30 °C. The reaction mixture was allowed to warm to 0 °C, and stirring was continued for 1 h. A solution of cyclohexanecarboxaldehyde (210 mg, 1.8 mmol) in THF (1 mL) was added at 0 °C, and stirring was continued for 18 h at room temperature. The reaction mixture was quenched with saturated ammonium chloride (2 mL), and Et_2O (10 mL) was added. The aqueous phase was extracted with Et_2O (2 × 10 mL), and then the combined extracts were dried and evaporated. CC (toluene/EtOAc (2:1)) of the residue gave the product as a viscous oil (510 mg, 76%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.91 (1H, hep, J = 6.0 Hz), 4.20 (2H, broad s), 4.18 (1H, d, J = 8.4 Hz), 3.70 (3H, s), 3.02−2.80 (3H, m), 2.10−2.04 (1H, m), 1.86−1.64 (8H, m), 1.46−0.86 (7H, m), 1.37 (6H, d, J = 6.0 Hz).

4-(Cyclohexylmethyl)-3-isopropoxy-5-(1-methoxycarbonyl-4-piperidyl)isoxazole (22g). To a solution of **21g** (200 mg, 0.5 mmol) and triethylsilane (150 mL, 0.9 mmol) in CH_2Cl_2 (1 mL) was added dropwise trifluoroacetic acid (1.1 mL) at room temperature, and the mixture was then stirred at 50 °C for 2 h. The mixture was cooled, water (2 mL) was added, and the organic phase was extracted with Et_2O (3 × 10 mL). The combined organic extracts were dried and evaporated. CC (toluene/EtOAc (2:1)) afforded the title compound (150 mg, 78%) as a viscous oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.88 (1H, hep, J = 6.0 Hz), 4.20 (2H, broad s), 4.18 (1H, d, J = 8.4 Hz), 3.72 (3H, s), 2.98−2.73 (3H, m), 2.11 (1H, m), 1.87−1.63 (9 H, m), 1.48−1.40 (1H, m), 1.36 (6H, d, J = 6.0 Hz), 1.23−1.09 (3H, m), 0.96−0.81 (2H, m).

4-(Cyclohexylmethyl)-5-(4-piperidyl)-3-isoxazolol Hydrobromide (7g). A solution of **22g** (150 mg, 0.5 mmol) in a solution of HBr in AcOH (33%, 4 mL) was stirred at 65 °C for 18 h. The reaction mixture was evaporated, and the residue was recrystallized (MeOH/Et_2O) to give the title compound (79 mg, 49%): mp >220 °C. ^{1}H NMR (300 MHz, D_2O): δ 3.55−3.48 (2H, m), 3.27−3.05 (3H, m), 2.20 (2H, d, J = 7.2 Hz), 2.11−2.00 (4H, m), 1.70−1.58 (5H, m), 1.50−1.39 (1H, m), 1.25−1.10 (3H, m), 0.90−0.83 (2H, m). Anal. ($C_{15}H_{24}N_2O_2$·HBr·H_2O) C, H, N.

4-(Diphenylhydroxymethyl)-3-isopropoxy-5-(1-methoxycarbonyl-4-piperidyl)isoxazole (21q). 21q was synthesized as described for **21g** using **20** (700 mg, 1.8 mmol) in THF (1 mL), EtMgCl in THF (2.8 M, 640 μL, 1.8 mmol), and benzophenone (320 mg, 1.8 mmol) in THF (1 mL). CC (toluene/EtOAc (6:1)) gave the product (500 mg, 63%): mp 135−137 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.31 (10H, s), 4.86 (1H, hep, J = 6.0 Hz), 4.05 (3H, m), 3.63 (3H, s), 2.23−2.10 (2H, m), 2.70−1.56 (2H, m), 1.40−1.32 (2 H, m), 1.27−1.21 (1H, m), 1.17 (6H, d, J = 6.0 Hz). Anal. ($C_{26}H_{30}N_2O_5$) C, H, N.

4-(Diphenylmethyl)-3-isopropoxy-5-(1-methoxycarbonyl-4-piperidyl)isoxazole (22q). 22q was synthesized as described above for **21g** using **21q** (250 mg, 0.6 mmol), triethylsilane (150 mL, 0.9 mmol) in CH_2Cl_2 (1 mL), and trifluoroacetic acid (1.1 mL) at 0 °C. CC (toluene/EtOAc (8:1)) afforded the title compound (187 mg, 78%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.32−7.21 (6H, m), 7.14−7.10 (4H, m), 5.30 (1H, s), 4.80 (1H, hep, J = 6.0 Hz), 4.09 (2H, broad s), 3.67 (3H, s), 2.54−2.40 (2H, m), 2.26−2.17 (1H, m), 1.79−1.61 (2 H, m), 1.47−1.42 (2H, m), 1.19 (6H, d, J = 6.0 Hz).

4-(Diphenylmethyl)-5-(4-piperidyl)-3-isoxazolol Hydrobromide (7q). 7q was synthesized as described above for **7g** using **22q** (190 mg, 0.4 mmol) in HBr in AcOH (33%, 3 mL). Recrystallization (EtOH/Et_2O) gave the title compound (44 mg, 24%): mp >220 °C. ^{1}H NMR (300 MHz, D_2O): δ 7.43−7.32 (6H, m), 7.25−7.21 (4H, m), 5.48 (1H, m), 3.42−3.32 (2H, m), 2.76−2.63 (2H, m), 2.42−2.30 (1H, m), 1.98−1.82 (2H, m), 1.78−1.68 (2H, m). Anal. ($C_{21}H_{22}N_2O_2$·HBr·0.25H_2O) C, H, N.

4-(2,2-Diphenyl-1-hydroxyethyl)-3-isopropoxy-5-(1-methoxycarbonyl-4-piperidyl)isoxazole (21r). 21r was synthesized as described for **21g** using **20** (420 mg, 1.1 mmol) in THF (1 mL), EtMgCl in THF (2.8 M, 380 mL, 1.8 mmol), and diphenylacetaldehyde (190 mL, 1.1 mmol) in THF (1 mL). CC (toluene/EtOAc (4:1)) gave the product (360 mg, 73%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.44 (2H, d, J = 7.2 Hz),

7.36 (2H, $J = 7.2$ Hz), 7.28−7.08 (6H, m), 5.22 (1H, d, $J = 9.6$ Hz), 4.93 (1H, hep, $J = 6.3$ Hz), 4.41 (1H, d, $J = 9.6$ Hz), 4.06 (3H, broad s), 3.67 (3H, s), 2.79−2.56 (3H, m), 2.46 (1H, broad s), 1.72−1.52 (2H, m), 1.51−1.41 (1 H, m), 1.44 (3H, d, $J = 6.0$ Hz), 1.36 (3H, d, $J = 6.0$ Hz), 0.96−0.91 (1H, m).

4-(2,2-Diphenylethyl)-3-isopropoxy-5-(1-methoxycarbonyl-4-piperidyl)isoxazole (22r). 22r was synthesized as described above for **21g** using **21r** (270 mg, 0.6 mmol), triethylsilane (150 mL, 0.9 mmol) in CH_2Cl_2 (1 mL), and trifluoroacetic acid (1.1 mL). CC (toluene/EtOAc (4:1)) afforded the title compound (245 mg, 93%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.50−7.09 (10H, m), 4.94 (1H, hep, $J = 6.0$ Hz), 4.16 (1H, t, $J = 7.8$ Hz), 4.08 (2H, broad s), 3.68 (3H, s), 2.99 (2H, $J = 7.8$ Hz), 2.92−2.57 (3H, m), 2.32−1.98 (1H, m), 1.56−1.46 (2 H, m), 1.40 (6H, d, $J = 6.0$ Hz), 1.28−1.02 (1H, m).

4-(2,2-Diphenylethyl)-5-(4-piperidyl)-3-isoxazolol Hydrobromide (7r). 7r was synthesized as described above for **7g** using **22r** (240 mg, 0.54 mmol) in HBr in AcOH (33%, 3 mL). Recrystallization (MeOH/Et_2O) gave the title compound (90 mg, 39%): mp >220 °C. ^{1}H NMR (300 MHz, CD_3OD): δ 7.31−7.10 (10H, m), 4.20 (1H, t, $J = 7.8$ Hz), 3.31−3.29 (2H, m), 3.09 (2H, d, $J = 7.8$ Hz), 2.91 (2H, m), 2.73−2.64 (1H, m), 1.76−1.60 (2H, m), 1.26−1.20 (2H, m). Anal. ($C_{22}H_{24}N_2O_2$·HBr·1.5 H_2O) C, H, N.

Receptor Binding and Uptake Assays. $GABA_A$ and $GABA_B$ receptor binding assays were performed using rat brain synaptic membranes from male Sprague Dawley rats, and tissue preparation was performed as described by Ransom and Stec.[40] On the day of the assay, the membrane preparation was quickly thawed, suspended in 50 volumes (w/v) of 50 mM Tris-HCl buffer (pH 7.4) using an Ultra-Turrax homogenizer, and centrifuged at 48000*g* for 10 min at 4 °C. This step was repeated four times. The final pellet was resuspended in incubation buffer for the relevant binding assay.

$GABA_A$ binding was studied using a modified version of the method described by Ebert et al.[41] The assay was carried out in triplicate and in total volumes of 1 mL by incubation of synaptic membranes (15−20 mg of original tissue per aliqout) in 0.8 mL of Tris-HCl buffer (50 mM, pH 7.4), 0.1 mL of [^{3}H]-muscimol (final concentration 3−5 nM), and 0.1 mL of test substance in various concentrations. Following incubation at 0 °C for 45 min, the samples were filtered through Whatman GF/B filters and filters were washed with 3 × 3 mL of ice-cooled buffer. Nonspecific binding in the presence of 0.1 mM GABA was subtracted.

$GABA_B$ binding was carried out in triplicate by incubation of membranes (15−20 mg of original tissue per aliqout) in 0.75 mL of Tris-HCl buffer (50 mM + 2.5 mM $CaCl_2$, pH 7.4), 0.1 mL isoguvacine (200 μM), 0.05 mL of [^{3}H]GABA (final concentration 3−5 nM), and 0.1 mL of the test substances in various concentrations. Following incubation at 25 °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 compounds' effects on GABA uptake were studied using a crude synaptosomal preparation, prepared from rat brains as described elsewhere in detail.[42] The whole brains were homogenized in 10 volumes of ice-cold 0.32 M sucrose, and the homogenate was centrifuged at 600*g* at 4 °C for 10 min. The pellet was discarded, and the supernatant was centrifuged at 25000*g* at 4 °C for 55 min. The pellet fraction was resuspended in 40 volumes of oxygenated phosphate medium at 0 °C. The synaptosome suspensions (2000 μL) were preincubated for 10 min at 25 °C with 0.4 mL of phosphate medium containing the inhibitor. Then [^{3}H]GABA (100 μL) was added to give a final GABA concentration of 50 nM (isotope dilution). Samples were vortexed, and the incubation was continued for an additional 10 min. The synaptosomes were isolated by rapid filtration through Whatman GF/C glass fiber filters, and the filters were washed with 3 × 2 mL of buffer.

IC_{50} values were estimated by measuring the inhibition of at least five different test concentrations and were estimated from the function

$$\text{bound} = B_{\max}\frac{[\text{inhibitor}]}{IC_{50} + [\text{inhibitor}]}$$

using the nonlinear curve-fitting program Grafit (Leatherbarrow, 1992). K_i values were calculated from the Cheng−Prusoff equation

$$K_i = \frac{IC_{50}}{1 + \frac{[\text{radioligand}]}{K_D}}$$

Electrophysiology in Vitro. Cerebral cortical neurons were cultured essentially as described by Herts et al.[43] 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. 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[44] 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 o.d. glass (World Precision Instruments) on a BB-CH-PC electrode puller (Mecanex, Switzerland) and had resistances of 2−5 MΩ. The medium in the patch electrodes had the following composition (in mM): KCl 140, $MgCl_2$ 1, $CaCl_2$ 1, EGTA 10, MgATP 2, and HEPES 10; pH was 7.35 at 22 °C. A holding potential of −60 mV was used. Current signals were recorded to disk on a computer and analyzed subsequently.

The compounds used were premixed at the required concentrations in ABSS. When necessary, the compounds were initially dissolved in DMSO and then diluted with ABSS to final concentrations of DMSO of less than 0.2%. This concentration of DMSO was in itself without effect on membrane currents. The solutions were applied in the vicinity (about 100 μm) of the recorded neuron from a multibarreled perfusion pipet, with the multiple barrels ending in a single cap with an opening of about 100 μm.[45] When applied, solutions emerged rapidly from the cap and surrounded the neuron completely. Drugs were applied for 5 s every 1 min. Within 5 s of drug application, the responses always peaked or reached a stable maximum plateau. Between ligand applications, ligand-free ABSS was applied from one of the barrels in order to quickly remove the drug from the cell. For all drugs except **7k**,**q**−**t**, current responses were quantified by measuring the maximum currents recorded during application of drugs. For **7k**,**q**−**t**, the current responses were quantified using the response magnitude after 5 s of application.

For the antagonists, an equation

$$I = \frac{I_0}{1 + \text{antilog}\{(\log [\text{B}] - \log IC_{50})n_H\}}$$

was fitted to the experimental concentration response data. I is the current, I_0 is the current induced by 20 mM isoguvacine (**1**) alone, [B] is the concentration of antagonist, IC_{50} is the concentration of antagonist that reduces the peak current to 50% of I_0, and n_H is the Hill coefficient. For the partial agonists, another equation

$$I = I_\infty + \frac{I_0 - I_\infty}{1 + \text{antilog}\{(\log [\text{B}] - \log IC_{50})n_H\}}$$

was used. Here, I_∞ is the current in the presence of 20 mM **1** and an infinitely high concentration of partial agonist, which

should theoretically be equal to the peak current in the presence of an infinitely high concentration of partial agonist alone, and IC_{50} is the concentration of partial agonist that reduces the peak current to $I_\infty + 0.5(I_0 - I_\infty)$, i.e., the concentration, which gives rise to 50% of the maximum inhibition.

Conformational Analysis. All calculations were performed with the MM3* force field as implemented in MacroModel, version 6.5.[46] To obtain an accurate geometry for the 3-isoxazolol moiety, some force-field parameters were added and others were modified.[24]

The truncated Newton−Raphson conjugate gradient (TNCG) algorithm was used for all energy minimizations. The low-energy conformations were searched by the Monte Carlo multiple minimum method (MCMM)[47] as implemented in MacroModel, version 6.5, with an energy cut-off of 6 kcal/mol (25 kJ/mol) above the global energy minimum. The conformational searches were continued until all low-energy minima had been found multiple times. All non-hydrogen atoms were superimposed in the test for duplicate structures.

Two conformational searches were performed for each molecule, both for aqueous solution using the generalized Born/solvent accessible surface area (GB/SA) dielectric continuum solvation model.[48] One analysis was performed to search the ensemble of conformations for the free ligand in water. In this search, no constraints were employed. The other conformational search was performed in order to identify possible receptor-bound conformations of the 4-substituents. To ensure that the conformation of the 4-PIOL moiety in this conformational search was in agreement with the $GABA_A$ pharmacophore model, the bond between the piperidine ring and the 3-isoxazolol ring (dihedral angle $Csp^2-Csp^2-Csp^3-Csp^3$) was constrained to 9.6° with a force constant of 9999 kJ mol^{-1} rad^{-1}.

All calculations were performed on the N-protonated form of the compounds but with a hydroxy group instead of a oxyanion in the 3-isoxazolol moiety. This was done in order to avoid force field problems with calculations on multiply charged compounds due to unrealistically strong electrostatic attractions between oppositely charged parts of the molecule.[49] In addition, the oxyanion of the 3-isoxazolol moiety most probably is strongly hydrogen-bonded to an arginine residue in the receptor.[24] Thus, electrostatic interactions between the 4-substituent and an oxyanion may, for the bioactive conformation, be better modeled by the hydroxy form of the 3-isoxazolol moiety than by the oxyanion form.

Identification of Possible Bioactive Conformations. The evaluation of the conformations obtained by the conformational search was based on two criteria: the energy penalty of the conformation and the structural fit to the $GABA_A$ pharmacophore model. The conformational energy penalties were calculated by subtracting the internal energy of the global energy minimum conformation in water from the internal energy of the constrained conformations.[49] The conformations were fitted to the $GABA_A$ pharmacophore model using the 3-isoxazolol nitrogen, C4 of the 3-isoxazolol moiety, C4 of the piperidine ring, and the piperidine nitrogen as fitting points.

Acceptable bioactive conformations must exhibit a distance above 1 Å between all parts of the 4-substituent and the sterical hindrances indicated by tetrahedrons in Figure 3. This minimum distance was defined by the shortest distance between the methyl group in **7a** and the corners of the tetrahedrons. In addition, a bioactive conformation should have low conformational energy penalties, preferably below 3 kcal/mol.[49] If no conformation was found to fulfill both criteria, torsional modifications were performed manually and the conformational energy penalty was recalculated. The conformation was then considered a possible bioactive conformation, if no distance between the 4-substituent and the sterical hindrances was less than 1 Å and the energy penalty was sufficiently low.

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References

(1) Chebib, M.; Johnston, G. A. R. GABA-activated ligand gated ion channels: medicinal chemistry and molecular biology. *J. Med. Chem.* **2000**, *43*, 1427−1447.

(2) Enna, S. J., Bowery, N. G., Eds. *The GABA Receptors*; The Humana Press: Totowa, NJ, 1997.

(3) Thomsen, C.; Ebert, B. Modulators of the GABA receptor. Novel therapeutic prospects. In *Glutamate and GABA Receptors and Transporters. Structure, Function and Pharmacology*; Egebjerg, J., Schousboe, A., Krogsgaard-Larsen, P., Eds.; Taylor & Francis: New York, 2002; pp 407−427.

(4) Mehta, A. K.; Ticku, M. K. An update on $GABA_A$ receptors. *Brain Res. Rev.* **1999**, *29*, 196−217.

(5) Krogsgaard-Larsen, P.; Johnston, G. A. R.; Curtis, D. R.; Game, C. J. A.; McCulloch, R. M. Structure and biological activity of a series of conformationally restricted analogs of GABA. *J. Neurochem.* **1975**, *25*, 803−809.

(6) Krogsgaard-Larsen, P.; Johnston, G. A. R.; Lodge, D.; Curtis, D. R. A new class of GABA agonist. *Nature* **1977**, *268*, 53−55.

(7) Wermuth, C. G.; Bourguignon, J.-J.; Schlewer, G.; Gies, J.-P.; Schoenfelder, A.; Melikian, A.; Bouchet, M.-J.; Chantreux, D.; Molimard, J.-C.; Heaulme, M.; Chambon, J.-P.; Biziere, K. Synthesis and structure−activity relationships of a series of aminopyridazine derivatives of γ-aminobutyric acid acting as selective GABA-A antagonists. *J. Med. Chem.* **1987**, *30*, 239−249.

(8) Sherwin, A. L. Neuroactive amino acids in focally epileptic human brain: a review. *Neurochem. Res.* **1999**, *24*, 1387−1395.

(9) Holthoff, V. A.; Koeppe, R. A.; Frey, K. A.; Penney, J. B.; Markel, D. S.; Kuhl, D. E.; Young, A. B. Positron emission tomography measures of benzodiazepine receptors in Huntington's disease. *Ann. Neurol.* **1993**, *34*, 76−81.

(10) Petty, F. GABA and mood disorders: A brief review and hypothesis. *J. Affective Disord.* **1995**, *34*, 275−281.

(11) Sawynok, J. GABAergic mechanisms of analgesia: An update. *Pharmacol. Biochem. Behav.* **1987**, *26*, 463−474.

(12) Benes, F. M.; Vincent, S. L.; Alsterberg, G.; Bird, E. D.; SanGiovanni, J. P. Increased GABA-A receptor binding in superficial layers of cingulate cortex in schizophrenics. *J. Neurosci.* **1992**, *12*, 924−929.

(13) Tamminga, C. A.; Gao, X.-M.; Lahti, A. C. Amino acids: Evidence for GABAergic and glutamatergic transmission abnormalities in schizophrenia. In *Schizophrenia−An Integrated View*; Fog, R., Gerlach, J., Hemmingsen, R., Eds.; Munksgaard: Copenhagen, 1995; pp 96−111.

(14) Dean, B.; Hussain, T.; Hayes, W.; Scarr, E.; Kitsoulis, S.; Hill, C.; Opeskin, K.; Copolov, D. L. Changes in $serotonin_{2A}$ and $GABA_A$ receptors in schizophrenia: Studies on the human dorsolateral prefrontal cortex. *J. Neurochem.* **1999**, *72*, 1593−1599.

(15) Hanada, S.; Mita, S.; Nishino, N.; Tanaka, C. [^{3}H]Muscimol binding sites increased in autopsied brains of chronic schizophrenics. *Life Sci.* **1987**, *40*, 259−266.

(16) Byberg, J. R.; Labouta, I. M.; Falch, E.; Hjeds, H.; Krogsgaard-Larsen, P.; Curtis, D. R.; Gynther, B. D. Synthesis and biological activity of a GABA-A agonist which has no effect on benzodiazepine binding and structurally related glycine antagonists. *Drug Des. Delivery* **1987**, *1*, 261−274.

(17) Kristiansen, U.; Lambert, J. D. C.; Falch, E.; Krogsgaard-Larsen, P. Electrophysiological studies of the $GABA_A$ receptor ligand, 4-PIOL, on cultured hippocampal neurones. *Br. J. Pharmacol.* **1991**, *104*, 85−90.

(18) Frølund, B.; Kristiansen, U.; Brehm, L.; Hansen, A. B.; Krogsgaard-Larsen, P.; Falch, E. Partial $GABA_A$ receptor agonists. Synthesis and in vitro pharmacology of a series of nonannulated analogs of 4,5,6,7-tetrahydroisoxazolo[4,5-c]pyridin-3-ol (THIP). *J. Med. Chem.* **1995**, *38*, 3287−3296.

(19) Frølund, B.; Tagmose, L.; Liljefors, T.; Stensbøl, T. B.; Engblom, C.; Kristiansen, U, Krogsgaard-Larsen, P. A novel class of potent 3-isoxazolol $GABA_A$ antagonists: Design, synthesis, and pharmacology. *J. Med. Chem.* **2000**, *43*, 4930−4933.

(20) Bram, G.; Vilkas, M. Nouvelle Synthése de β-cétoesters du type $RCOCH_2CO_2Et$ á partir du malonate acide d'éthyle (New synthesis of $RCOCH_2CO_2Et$ β-ketoesters starting from ethyl malonate). *Bull. Soc. Chim. Fr.* **1964**, 945−951.

(21) Clay, R. J.; Collom, T. A.; Karrick, G. L.; Wemple, J. A safe, economical method for the preparation of β-oxo esters. *Synthesis* **1993**, 290−292.
(22) Kursanov, D. N.; Parnes, Z. N.; Loim, N. M. Applications of ionic hydrogenation to organic synthesis. *Synthesis* **1974**, 633−651.
(23) Arnt, J.; Sánchez, C.; Lenz, S. M.; Madsen, U.; Krogsgaard-Larsen, P. Differentiation of in vivo effects of AMPA and NMDA receptor ligands using drug discrimination methods and convulsant anticonvulsant activity. *Eur. J. Pharmacol.* **1995**, *285*, 289−297.
(24) Tagmose, L. A Pharmacophore Model for $GABA_A$ Receptor Agonists. Ph.D. Thesis, Royal Danish School of Pharmacy, Copenhagen, 2000.
(25) Krogsgaard-Larsen, P.; Johnston, G. A. R. Structure−activity studies on the inhibition of GABA binding to rat brain membranes by muscimol and related compounds. *J. Neurochem.* **1978**, *30*, 1377−1382.
(26) Krogsgaard-Larsen, P.; Hjeds, H.; Curtis, D. R.; Lodge, D.; Johnston, G. A. R. Dihydromuscimol, thiomuscimol and related heterocyclic compounds as GABA analogues. *J. Neurochem.* **1979**, *32*, 1717−1724.
(27) *Spartan SGI*, version 5.1.1; Wavefunction, Inc., 18401 von Karman, suite 370, Irvine, CA 92612.
(28) Dougherty, D. A. Cation−π interactions in chemistry and biology: a new view of benzene, Phe, Tyr, and Trp. *Science* **1996**, *271*, 163−168.
(29) Smith, G. B.; Olsen, R. W. Functional domains of $GABA_A$ receptors. *Trends Pharmacol. Sci.* **1995**, *16*, 162−168.
(30) Unwin, N. Acetylcholine receptor channel imaged in the open state. *Nature* **1995**, *373*, 37−43.
(31) Unwin, N. Nicotinic acetylcholine receptor at 9 Å resolution. *J. Mol. Biol.* **1993**, *229*, 1101−1124.
(32) Kelley, J. L.; Linn, J. A.; Selway, J. W. Synthesis and antirhinovirus activity of 6-(dimethylamino)-2-(trifluoromethyl)-9-(substitutedbenzyl)-9H-purines. *J. Med. Chem.* **1989**, *32*, 1757−1763.
(33) Novâk, L.; Roháley, J.; Poppe, L.; Hornyánszky, G.; Kolonits, P.; Zelei, I.; Fehér, I.; Fekete, J.; Szabó, É.; Záhorsky, U.; Jávor, A.; Szantay, C. Naphthalene analogs of mevinolin. *Liebigs Ann. Chem.* **1992**, *2*, 145−158.
(34) Campaigne, E.; Heaton, B. G. Sulfur heterocycles from the ring closure of bisarylalkyl disulfides. *J. Org. Chem.* **1964**, *29*, 2372−2378.
(35) Dixon, E. A.; Fisher, A.; Robinson, F. P. Preparation of a series of substituted fluoromethylnaphthalenes. *Can. J. Chem.* **1981**, *59*, 2629−2641.
(36) Mitra, A.; Ghoshe, S. Synthesis of substituted chrysenes and phenanthrenes. *Indian J. Chem., Sect. B* **1996**, *35*, 785−789.
(37) Nevill, S. M.; Pincock, J. A. The design of radical clocks to probe the reactivity of the intermediates in arylmethyl ester photochemistry. *Can. J. Chem.* **1997**, *75*, 232−247.
(38) Caldirola, P. M.; Goot, H. v. d.; Timmerman, H. New prenylamine analogues: Synthesis and Ca^{2+}-entry blocking activity. *Eur. J. Med. Chem.* **1992**, *27*, 571−579.
(39) N'Goka, V.; Schlewer, G.; Linget, J. M.; Chambon, J. P.; Wermuth, C. G. GABA-uptake inhibitors: Construction of a general pharmacophore model and successful prediction of a new representative. *J. Med. Chem.* **1991**, *34*, 2547−2557.
(40) Ransom, R. W.; Stec, N. L. Cooperative modulation of [^{3}H]MK-801 binding to the *N*-methyl-D-aspartate receptor ion channel complex by L-glutamate, glycine and polyamines. *J. Neurochem.* **1988**, *51*, 830−836.
(41) Ebert, B.; Frølund, B.; Diemer, N. H.; Krogsgaard-Larsen, P. Equilibrium binding characteristics of [^{3}H]thiomuscimol. *Neurochem. Int.* **1999**, *34*, 427−434.
(42) Fjalland, B. Inhibition by neuroleptics of uptake of [^{3}H]GABA into rat brain synaptosomes. *Acta Pharmacol. Toxicol.* **1978**, *42*, 73−76.
(43) Herts, E.; Yu, A. C. H.; Hertz, L.; Juurlink, B. H. J.; Schousboe, A. Preparation of primary cultures of mouse cortical neurons. *A Dissection and Tissue Culture Manual of the Nervous System*; Alan R. Liss Inc.: New York, 1989.
(44) Hamill, O. P.; Marty, A.; Neher, E.; Sakmann, B.; Sigworth, F. J. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pfluegers Arch.* **1981**, *391*, 85−100.
(45) Carbone, E.; Lux, H. D. Kinetics and selectivity of a low-voltage-activated calcium current in chick and rat sensory neurones. *J. Physiol.* **1987**, *386*, 547−570.
(46) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. MacroModel (version 6.5). An integrated software system for modeling organic and bioorganic molecules using molecular mechanics. *J. Comput. Chem.* **1990**, *11*, 440−467.
(47) Chang, G.; Guida, W. C.; Still, W. C. An internal coordinate Monte Carlo method for searching conformational space. *J. Am. Chem. Soc.* **1989**, *111*, 4379−4386.
(48) Still, W. C.; Tempczyk, A.; Hawley, R. C.; Hendrickson, T. Semianalytical treatment of solvation for molecular mechanics and dynamics. *J. Am. Chem. Soc.* **1990**, *112*, 6127−6129.
(49) Boström, J.; Norrby, P.-O.; Liljefors, T. Conformational energy penalties of protein-bound ligands. *J. Comput.-Aided Mol. Des.* **1998**, *12*, 383−396.
(50) Christopoulos, A. Assessing the distribution of parameters in models of ligand−receptor interaction: to log or not to log. *Trends Pharmacol. Sci.* **1998**, *19*, 351−357.

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