Potent 4-Aryl- or 4-Arylalkyl-Substituted 3-Isoxazolol $GABA_A$ Antagonists: Synthesis, Pharmacology, and Molecular Modeling

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We have previously described a series of competitive $GABA_A$ antagonists derived from the low-efficacy partial agonist 5-(4-piperidyl)-3-isoxazolol (4-PIOL, **4**). The 2-naphthylmethyl analogue, 4-(2-naphthylmethyl)-5-(4-piperidyl)-3-isoxazolol (**5**), provided affinity for the $GABA_A$ receptor site higher than that of the standard $GABA_A$ receptor antagonist, SR 95531 (**3**). Molecular modeling studies of these compounds exposed a cavity at the receptor recognition site capable of accommodating aromatic groups of substantial size in the 4-position in the 3-isoxazolol ring. Here we present a series of analogues of **5**, with various substituents in different positions in the naphthyl ring system (**6a**−**k**), and compounds with aromatic substituents directly attached to the 4-position of the 3-isoxazolol ring (**7l**−**n**). The compounds have been pharmacologically characterized using receptor-binding assays and electrophysiological whole-cell patch-clamp techniques. All of the tested compounds show affinity for the $GABA_A$ receptor site. While the 5-, 7-, and 8-bromo analogues, **6b**−**d**, showed receptor affinities (K_i = 45, 109, and 80 nM, respectively) comparable with that of **5** (K_i = 49 nM), the 1-bromo analogue, **6a**, provided the highest receptor affinity of the series (K_i = 10 nM). Introduction of a series of different substituents in the 1-position in the 2-naphthyl ring system led to compounds, **6e**−**k**, with retained high affinity for the $GABA_A$ receptor (K_i = 16−250 nM). Introduction of a phenyl ring directly into the 4-position on the 3-isoxazolol ring gave a 41-fold increase in affinity relative to that of 4-PIOL. 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, **6a** showing antagonist potency (IC_{50} = 42 nM) markedly higher than that of **3** (IC_{50} = 240 nM). Molecular modeling studies, based on the compounds described, emphasized the importance of the distal ring in **5** for receptor affinity and the considerable dimensions of the proposed receptor cavity. Furthermore, the phenyl rings in **7l** and in **6k** were shown to represent highly favorable positions for an aromatic ring in previously unexplored receptor regions in terms of a pharmacophore model.

Introduction

The main inhibitory neurotransmitter γ-aminobutyric acid (GABA) exerts its effects in the central nervous system through two distinct classes of receptors, the ionotropic $GABA_A$ and $GABA_C$ receptors and the metabotropic $GABA_B$ receptors.[1,2] The $GABA_A$ receptors are involved in many different physiological functions and have considerable interest as therapeutic targets in the treatment of pain and a number of neurodegenerative and psychiatric disorders.[3,4]

The $GABA_A$ receptor is a receptor complex consisting of a GABA recognition site and numerous allosteric and modulatory binding sites for clinically active drugs such as benzodiazepines, barbiturates, and steroids. The GABA binding site has been pharmacologically identified by a number of $GABA_A$ ligands such as the $GABA_A$ agonists muscimol (**1**)[5] and 4,5,6,7-tetrahydroisoxazolo-[5,4-*c*]pyridin-3-ol (THIP, **2**).[5,6] 2-(3′-(Carboxypropyl)-3-amino-6-(paramethoxyphenyl)pyridazinium bromide (SR 95531, **3**)[7] is now used as a standard antagonist for the $GABA_A$ receptors (Figure 1).

The $GABA_A$ receptors are heteromeric assemblies of subunits forming chloride-gated ion channels. To date, 16 homologous mammalian $GABA_A$ subunits have been identified (α_{1-6}, β_{1-3}, γ_{1-3}, δ, ϵ, π, and θ), which have been subdivided based on sequence identity.[8] Although a high number of different subunit compositions are possible, only a limited number have been identified, the $\alpha_1\beta_2\gamma_2$ apparently being the most abundant in the human central nervous system.[9]

There is currently no experimentally determined three-dimensional (3D) structure of the $GABA_A$ receptor complex. To obtain knowledge on the three-dimensional structure of the ligand binding site in the $GABA_A$ receptor, we have previously developed a 3D-pharmacophore model based on a hypothesis of the different binding modes of the bioactive conformations of **1** and the partial $GABA_A$ agonist 4-PIOL (**4**) and on pharmacological data for a series of $GABA_A$ ligands.[10,11] According to this model, a receptor cavity exists in the

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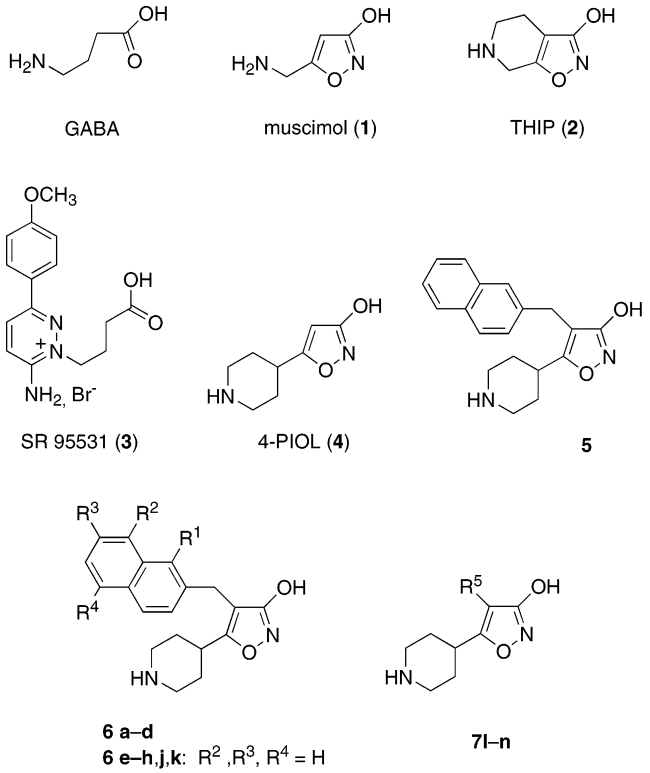


6a: R^1 = Br, R^2 = R^3 = R^4 = H
6b: R^2 = Br, R^1 = R^3 = R^4 = H
6c: R^3 = Br, R^1 = R^2 = R^4 = H
6d: R^4 = Br, R^1 = R^2 = R^3 = H
6e: R^1 = F
6f: R^1 = Cl
6g: R^1 = SCH_3
6h: R^1 = SPh
6j: R^1 = CN
6k: R^1 = phenyl
7l: R^5 = phenyl
7m: R^5 = 1-naphthyl
7n: R^5 = 2-naphthyl

Figure 1. Structures of GABA, the $GABA_A$ agonists muscimol (**1**), THIP (**2**), the $GABA_A$ antagonists **3** and **5**, the low-efficacy partial $GABA_A$ agonist 4-PIOL (**4**), and the new isoxazolols (**6a**−**h**,**j**,**k** and **7l**−**m**).

vicinity of the 4-position of the 3-isoxazolol ring of **4** but not the corresponding position in **1** (Figure 2). This proposed receptor cavity has been shown to be of considerable dimensions containing specific sites for receptor interactions based on structure−activity relationship studies of a number of potent $GABA_A$ ligands as exemplified by compound **5**, which displays the highest affinity of the previously studied series of compounds.[12] Furthermore, introduction of large substituents in the 4-position of the 3-isoxazolol ring of **4** led to a change of pharmacological profile of the compounds from low-efficacy partial $GABA_A$ agonist action of **4** to potent $GABA_A$ antagonist effect.[12]

The results of these studies prompted us to further explore the receptor cavity accommodating the 4-substituent of the 4-PIOL analogues. This paper describes the synthesis, pharmacological characterization, and molecular modeling of a series of analogues of **5** shown in Figure 1 and compounds **6a**−**h**,**j**,**k** and **7l**−**n**.

Results and Discussion

Chemistry. The synthetic approaches to the target compounds **6a**−**h**,**j**,**k** and **7l**−**n** are outlined in Schemes 1-4. The key step in this strategy was the introduction of the substituent in the 4-position of the O-alkylated 3-isoxazolol ring of **8** (Scheme 1).

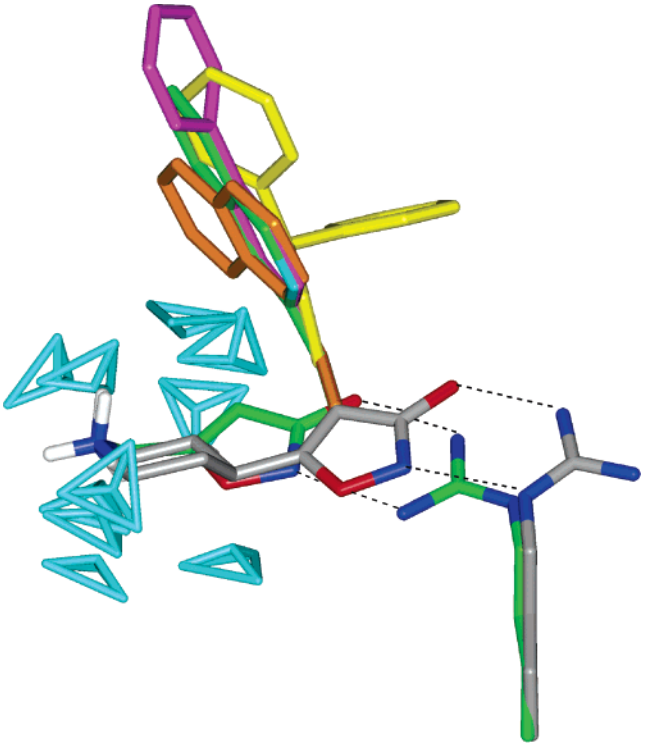


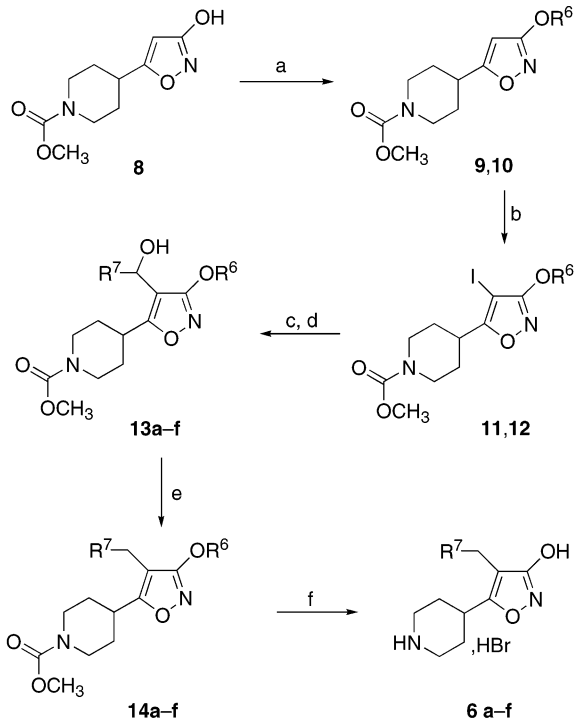
Figure 2. A pharmacophore model for $GABA_A$ receptor ligands[10,11] displaying the proposed binding modes of **1** (green carbon atoms), **4** (grey carbon atoms), and a series of 4-substituted 4-PIOL compounds previously studied[12] illustrating the large space spanned by the 4-substituents. The tetrahedrons indicate postions of methyl groups in $GABA_A$ agonists causing strong steric repulsions with the receptor.

The iodinated analogues **11** and **12** underwent iodine/magnesium exchange using either ethylmagnesium chloride or bromide followed by reaction with the appropriate aldehyde to give the hydroxy compounds **13a**−**f**. The benzylic hydroxy group was subsequently removed by ionic hydrogenation using trifluoroacetic acid and triethylsilane in dichloromethane to give the compounds **14a**−**f**. Deprotection to give the target compounds **6a**−**f** in Scheme 1 was accomplished by treatment with hydrogen bromide in acetic acid.

The aldehydes required for these reactions were synthesized as illustrated in Scheme 2, partly using literature procedures. The starting bromomethylnaphthalenes, **15a**−**d**, were either commercially available or synthesized as described in the literature. The fluoromethylnaphthalene, **15e**, was prepared starting from the bromomethylnaphthalene, **15a**, using lithium−bromine exchange followed by electrophilic halogenation using *N*-fluorobis(phenylsulfonyl)amine. Benzylic bromination followed by Hass−Bender oxidation using 2-nitropropane gave the aldehydes **17a**−**e**. Quantitative introduction of chloride for the synthesis of **17f** was accomplished using the 1,3-dioxolane protected aldehyde **18** and lithium−bromine exchange followed by treatment with hexachloroethane as the electrophile to give **19**. Attemps to synthesize the corresponding 1-iodo analogue of **16e** resulted, in the deprotection step, in substantial decomposition.

Different substituents were introduced in the 1-position of the naphthalene ring using **14a** as the starting compound (Scheme 3). Replacement of the bromide atom in compound **14a** was performed by lithium−bromine

Scheme 1[a]



a: R^7 = 1-Bromo-2-naphthyl
b: R^7 = 8-Bromo-2-naphthyl
c: R^7 = 7-Bromo-2-naphthyl
d: R^7 = 5-Bromo-2-naphthyl
e: R^7 = 1-Fluoro-2-naphthyl
f: R^7 = 1-Chloro-2-naphthyl

9, **11**, **13a**,**e**,**14a**,**e**: R^6 = benzyl
10, **12**, **13b–d**,**f**, **14b–d**,**f**: R^6 = isopropyl

[a] Reagents: (a) isopropyl bromide or benzyl bromide, K_2CO_3, acetone, 70 °C; (b) ICl, AcOH, H_2O, 80 °C; (c) EtMgCl, THF, −30 °C; (d) **17a**−**f**, THF, 0 °C; (e) TFA, Et_3SiH, CH_2Cl_2; (f) 33% HBr, AcOH, 65 °C or 48% HBr, H_2O, 100°C.

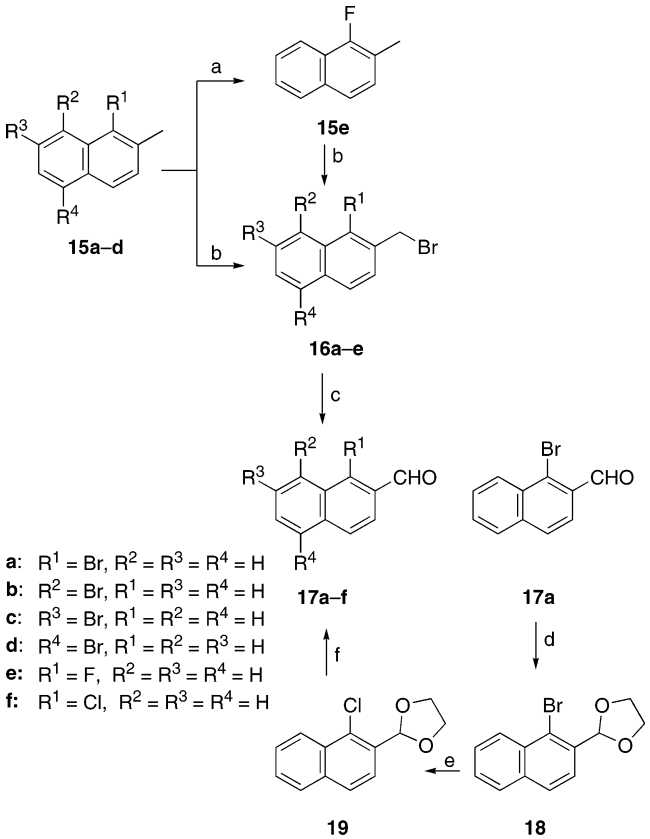
exchange followed by treatment with an appropriate electrophile such as dimethyl disulfide, diphenyl disulfide, or dimethylformamide. The nitrile **20j** was achieved by direct conversion[13] of the corresponding aldehyde **20i** by treatment with iodine in aqueous ammonia at room temperature.

As illustrated in Scheme 4, coupling under Suzuki conditions using **14a** or **11**, $Pd(PPh_3)_2Cl_2$, and the appropriate arylboronic acid gave the 4-aryl-substituted compounds **21** and **22l**−**n** in high yields. Deprotection using hydrogen bromide in acetic acid as described above gave the target compounds **6k** and **7l**−**n**.

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 for $GABA_A$ and $GABA_B$ receptor sites, using [^{3}H]muscimol and [^{3}H]-GABA, respectively, were determined using methods described previously.[12] Like 4-PIOL and compound **5**, all of the tested compounds show affinity for the $GABA_A$ receptor sites (Table 1).

Introduction of a bromo substituent in the 1-position of the naphthyl ring system of compound **5** affording compound **6a** resulted in a 5-fold enhancement in affinity for the $GABA_A$ receptor sites as compared to that of **5**. Compound **6b** with a bromo substituent in

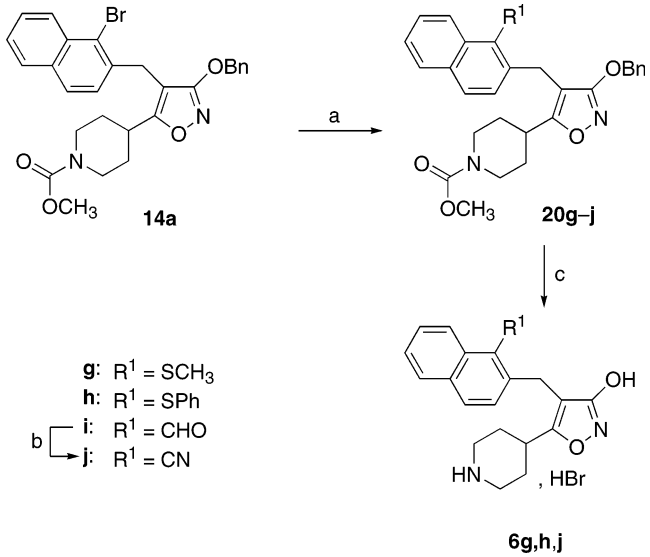
Scheme 2[a]



a: R^1 = Br, R^2 = R^3 = R^4 = H
b: R^2 = Br, R^1 = R^3 = R^4 = H
c: R^3 = Br, R^1 = R^2 = R^4 = H
d: R^4 = Br, R^1 = R^2 = R^3 = H
e: R^1 = F, R^2 = R^3 = R^4 = H
f: R^1 = Cl, R^2 = R^3 = R^4 = H

[a] Reagents: (a) *n*-BuLi, THF, −78 °C, then *N*-fluorobis(phenylsulfonyl)amine; (b) NBS, benzoylperoxide, CCl_4; (c) 2-nitropropane, NaOEt in EtOH; (d) ethylenglycole, *p*-TsOH, toluene; (e) *n*-BuLi, THF, −78 °C, then hexachloroethane; (f) *p*-TsOH, acetone.

Scheme 3[a]



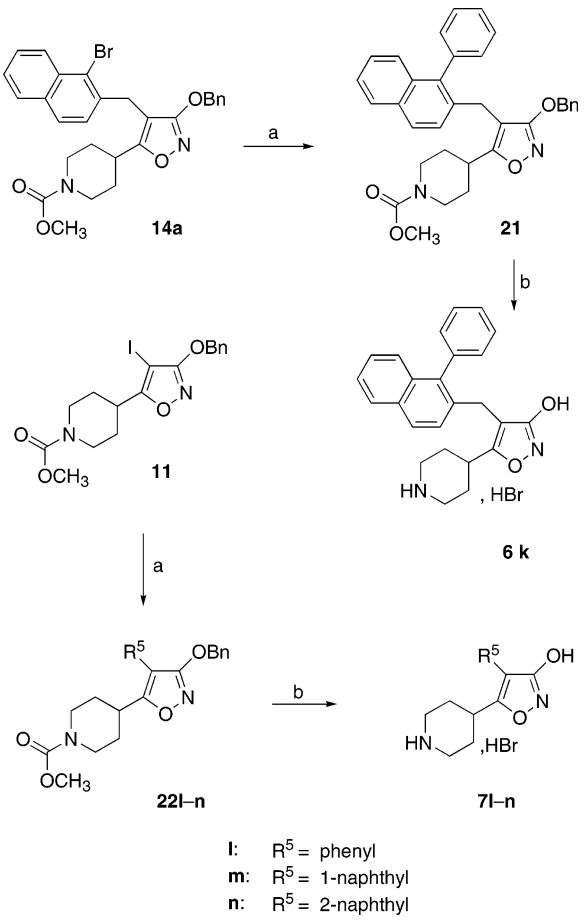
g: R^1 = SCH_3
h: R^1 = SPh
i: R^1 = CHO
j: R^1 = CN

6g,**h**,**j**

[a] Reagents: (a) *n*-BuLi, THF, −78 °C, then $S_2(CH_3)_2$, $S_2(Ph)_2$ or DMF; (b) NH_3(aq. 25%), I_2; (c) 33% HBr, AcOH, 65 °C.

the 8-position of the naphthyl ring system showed affinity comparable to that of **5**. In contrast, introduction of a bromo substituent in the 5- or 7-position of the naphthyl ring system to give compounds **6d** and **6c**, respectively, reduced receptor affinity. On the basis of these observations, the influence of different substituents in the 1-position of the naphthyl ring system on binding to the $GABA_A$ receptor was further investigated.

Scheme 4[a]



[a] Reagents: (a) $Pd(PPh_3)_2Cl_2$, $NaHCO_3$, $R^5B(OH)_2$, DMF; (b) HBr, AcOH, 65 °C.

Replacement of the bromide by fluoride or a chloride resulted in minor decrease in affinity. Also, the methylthio analogue **6g** and the cyano analogue **6j** retained high affinity for the $GABA_A$ receptor showing some 3-fold lower affinity relative to **6a**. Whereas the larger phenylthio analogue, compound **6h**, showed a markedly lower affinity, the phenyl group of compound **6k** was well-tolerated showing affinity for the $GABA_A$ receptor comparable to those of **6e**−**g**,**j**. The phenyl analogue of 4-PIOL, **7l**, provided a 40-fold increase in affinity for the $GABA_A$ receptor relative to 4-PIOL. Expanding the aromatic system in **7l** with an additional phenyl ring affording the 1- and 2-naphthyl analogues **7m** and **7n**, respectively, affected the affinity in opposite directions. The 1-naphthyl isomer, **7m**, showed a marked decrease in affinity, whereas the 2-naphthyl isomer, **7n**, showed an enhanced affinity relative to the phenyl analogue, **7l**.

The pharmacological profile of the compounds was studied using whole-cell patch-clamp recordings from cultured cerebral cortical neurons, performed as described previously.[12] The compounds were tested in the presence or absence of isoguvacine (20 μM), a specific $GABA_A$ receptor agonist. In previous studies, 4-PIOL and the 4-methyl and 4-ethyl analogues have been characterized as low-efficacy partial agonists at $GABA_A$ receptors,[14] whereas a series of 4-substituted 4-PIOL analogues with larger substituents, including **5**, were shown to be competitive $GABA_A$ antagonists without detectable agonist effect even at high concentrations.[12,14] As shown for **5**, all of the compounds tested in the present study were capable of inhibiting the current induced by isoguvacine in a dose-dependent manner, as exemplified in Figure 3 for compounds **6a**,**c**,**g** and **7n**. The electrophysiological data showed a fairly good correlation to the obtained binding affinities (Table 1). The 1-bromo and the methylthio-substituted analogues, **6a** and **6g**, showed antagonist potencies considerably higher than that of the standard $GABA_A$ antagonist SR 95531 (**3**) and of **5**.

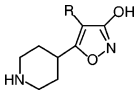
Molecular Modeling. As mentioned in the Introduction, our previously reported studies on the affinities of 4-substituted 4-PIOL analogues for the $GABA_A$ receptor and the interpretation of the affinities in terms of a pharmacophore model have revealed the presence of a large receptor cavity in the vicinity of the 4-position of the 3-isoxazolol ring in 4-PIOL (**4**) as illustrated in Figure 2.[11,12,14] The 2-naphthylmethyl substituted compound **5** displays the highest affinity of the previously studied series of compounds (Table 1).

Bromosubstituted 2-Naphthylmethyl Compounds (6a−d). Our previous analysis of compound **5** showed that the distal ring of the naphthyl ring system is the determinant for the high affinity of this compound. The distal aromatic ring in **5** increases the affinity by a factor of 78 compared to the 4-benzyl-substituted compound, whereas the 4-benzyl compound only displays an affinity increase by a factor of less than 3 compared to the 4-methyl compound.[12] This indicates that the distal ring of the naphthyl substituent in **5** has strong specific interactions with the receptor.

The data in Table 1 show that bromo substitution in the 1-position of the naphthyl ring (**6a**) increases the affinity by a factor of 5. In contrast, the 7-bromo compound (**6c**) displays a decrease in affinity by a factor of 11 compared to that of **6a**. The 5- and 8-bromo-substituted compounds **6d** and **6b**, respectively, display intermediate and similar affinities. To investigate if the observed different affinities due to bromo substitution in different positions in the 2-naphthylmethyl ring system may be due to electrostatic effects, the molecular electrostatic potentials (MEP) at the center of the distal naphthyl ring in compounds **6a**−**d** were calculated by using the semiempirical AM1 method as implemented in Spartan version 5.1.3.[15] The MEPs for **6a**, **6b**, **6d**, and **6c** were calculated to be −17.7, −16.6, −16.5, and −15.8 kcal/mol, respectively, nicely correlating with the order of affinities (Table 1): 1-Br (**6a**) > 8-Br (**6b**) > 5-Br (**6d**) > 7-Br (**6c**). This indicates that the variation of the affinities due to substitution at different positions in the naphthyl system may be due to electrostatic interactions between the distal 2-naphthyl ring and the receptor, most probably of the $\pi-\pi$ or cation$-\pi$ type. However, because the corresponding MEP of the unsubstituted naphthyl group in **5** is calculated to be −21.3 kcal/mol, the 5 times higher affinity of the 1-bromo-substituted compound **6a** compared to **5** must largely be due to the high hydrophobicity of the bromo substituent.

1-Substituted 2-Naphthylmethyl Compounds. In our previous analysis of compound **5** in terms of conformational energy penalties for adopting the bioactive conformation, it was not possible to conclude which of the two 2-naphthyl rotamers **A** and **B** shown in Figure

Table 1. Receptor Binding and in Vitro Electrophysiological Data

compound	R	[^{3}H]muscimol binding[a] K_i (nM)[c]	electrophysiology[b] IC_{50} (nM)[c]
3		74 (59; 94)	240 (220; 250)
4 (4-PIOL)	H	9100 (8200; 1000)	11000 (7600; 17 000)
5	2-naphthylmethyl	49 (43; 57)	370 (310; 440)
6a	1-bromo-2-naphthylmethyl	10 (9; 12)	42 (39; 46)
6b	8-bromo-2-naphthylmethyl	45 (37; 55)	226 (190; 269)
6c	7-bromo-2-naphthylmethyl	109 (96; 123)	384[d] (336; 439)
6d	5-bromo-2-naphthylmethyl	80 (76; 85)	215 (197; 235)
6e	1-fluoro-2-naphthylmethyl	19 (17; 22)	242 (214; 273)
6f	1-chloro-2-naphthylmethyl	16 (15; 17)	113 (92; 138)
6g	1-methylthio-2-naphthylmethyl	28 (24; 33)	61[d] (57; 65)
6h	1-phenylthio-2-naphthylmethyl	250 (220; 300)	126[d] (111; 143)
6j	1-cyano-2-naphthylmethyl	28 (24; 33)	140 (124; 158)
6k	1-phenyl-2-naphthylmethyl	21 (21; 22)	198[d] (174; 225)
7l	phenyl	220 (210; 230)	159 (131; 192)
7m	1-naphthyl	820 (650; 1000)	334[d] (272; 411)
7n	2-naphthyl	36 (31; 42)	141 (131; 153)

[a] Standard receptor binding on rat brain synaptic membranes, $n = 3$. [b] Whole-cell patch clamp recordings from cerebral cortical neurones cultered for 7−9 days, $n = 6-17$. [c] Mean and SEM were calculated assuming a normal distribution of the logarithm of the IC_{50} and K_i values. Hence, numbers in parentheses indicate antilog of mean ±SEM of IC_{50} and K_i.[30] [d] Because of the slow onset of antagonism, the parameters for **6c**,**g**,**h**,**k** and **7m** were calculated using the response magnitude after 5 s of application.

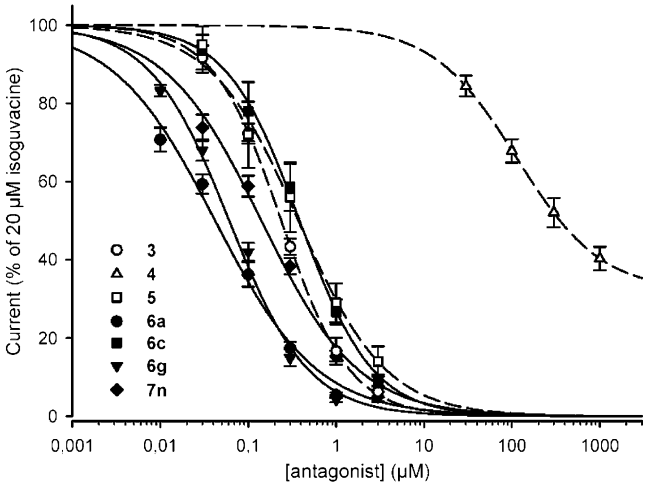


Figure 3. Effect of the antagonists on the response to 20 μM of the full $GABA_A$ agonist isoguvacine using whole-cell patch-clamp recordings from cultured cerebral cortical neurons. A total of 20 μM isoguvacine and varying concentrations of antagonists were applied simultaneously to the cells. The response of 20 μM isoguvacine alone has been set as 100%, and the other responses are expressed as a fraction of this. The response to isoguvacine is progressively reduced with increasing concentrations of the antagonist. The number of cells tested in this way with each compound varied from $n = 6-17$.

4 is the more probable bioactive one.[12] However, the high affinity of the 1-phenyl-2-naphthylmethyl compound **6k** (Table 1) makes it highly unlikely that rotamer **B** is the bioactive rotamer for this compound. A phenyl substituent in the 1-position of the 2-naphthyl ring rotamer **B** will have severe steric repulsive interactions with the 4-PIOL system. Thus, the most probable bioactive rotamer of the naphthyl moiety in **6k** is rotamer **A** in Figure 4. This implies that the receptor cavity beyond the 2-naphthyl group in the direction of the C1−H1 bond in rotamer **A** can accommodate substituents at least as large as a phenyl group. This region represents a previously unexplored receptor region in

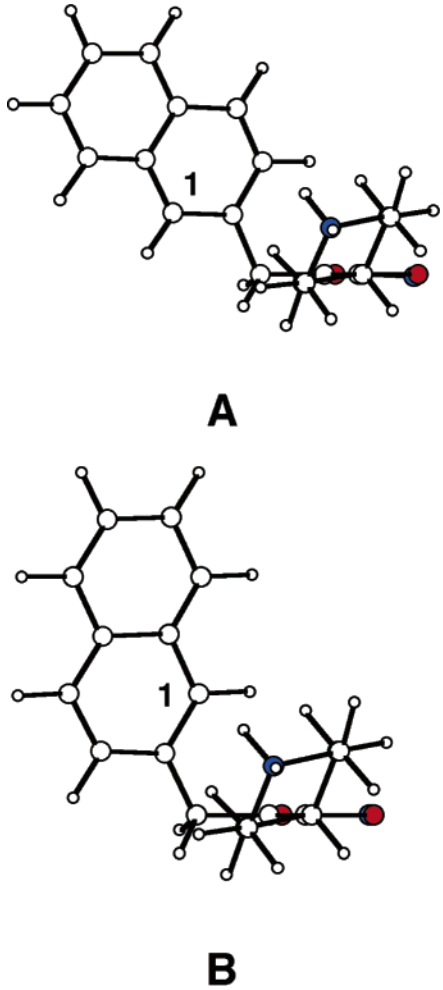


Figure 4. Alternative bioactive conformations of compound **5** according to our previous analysis.[12] The 1-position of the naphthyl ring is indicated.

terms of the pharmacophore model. The substituents in the naphthyl rings in **6a** and **6e**−**k** are presumably all occupying this receptor region. The data in Table 1

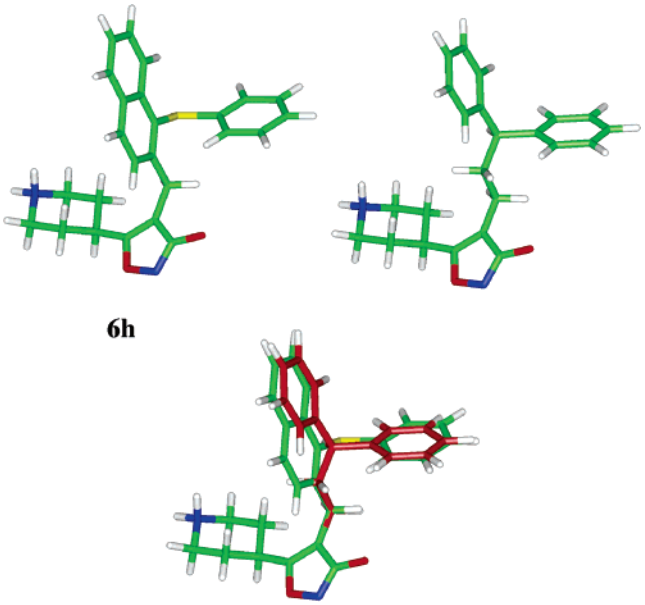


Figure 5. Superposition of compound **6h** and the 3,3-diphenylpropyl-substituted 4-PIOL compound previously studied[12] (colored red in the superposition).

show that, with the exception of the phenylthio substituents in **6h**, all of the investigated substitutents in the 1-position of the 2-naphthyl ring show virtually identical effects on the affinity with an affinity increase compared to **5** by only a factor of less than 5. This indicates that these substituents are located in a water exposed receptor area and are only partially desolvated in the receptor cavity.

The previously reported 3,3-diphenylpropyl compound (Figure 5) displays an affinity of 68 nM.[12] The affinity of the corresponding 3-phenylpropyl compound is lower by a factor of 16, showing that the added phenyl ring in the 3,3-diphenylpropyl compound is occupying a position in the receptor cavity, which is favorable for the affinity.[12] The 1-phenylthio compound **6h** may adopt a conformation in which the distal naphthyl ring and the phenyl ring of the phenylthio groups are overlapping the corresponding rings in the 3,3-diphenylpropyl compound (Figure 5). The calculated conformational energy penalty of **6h** in the conformation shown in Figure 5 is 4.8 kcal/mol but may be as low as 2.1 kcal/mol if coplanarity of the phenyl ring of the phenylthio group and the corresponding phenyl ring in 3,3-diphenylpropyl compound is not required. The calculated increase in conformational energy penalty going from **5** (conformational energy penalty 0.8 kcal/mol) to **6h** is 1.3−4.0 kcal/mol, depending on the degree of coplanarity. The corresponding energy difference for the 3,3-diphenylpropyl versus 3-phenylpropyl compounds is calculated to be 1.7 kcal/mol. Compound **6h** displays an affinity that is lower than that of **5** by a factor of 5 (Table 1). Thus, the affinity increase by a factor of 16 observed going from the 3-phenylpropyl to the 3,3-diphenylpropyl compound is not observed for **6h** versus **5**. Considering the calculated conformational energy penalties above, we may conclude that a coplanar or close to coplanar orientation of the phenyl rings as shown in Figure 5 is required for high-affinity binding and that this conformation is energetically too unfavorable for **6h** in order to realize the potentially favorable receptor interaction for the phenyl ring of the phenylthio substituent.

Compounds 7l−7n. The conformational energy penalty for the bioactive conformation of **7l** according to the pharmacophore model is calculated to be 1.8 kcal/mol. In this conformation, the phenyl ring has a torsional angle of 75°. The corresponding energy for the 4-benzyl-substituted 4-PIOL previously studied is 1.1 kcal/mol.[12] A superimposition of the two compounds is shown in Figure 6a. Despite the slightly more favorable conformational energy for the 4-benzyl compound, the affinity of **7l** is higher by a factor of 17. Furthermore, the affinity of **7l** is some 40 times higher than that of the parent compound **4** (Table 1), whereas the affinity of the 4-benzyl-4-PIOL compound is only 2.6 times higher than that of the 4-methyl-substituted compound.[12] Thus, the interaction between a phenyl ring directly attached to the isoxazolol ring and the receptor is significantly stronger than that displayed by a phenyl ring linked to the 4-PIOL system via a methylene group. The phenyl ring in **7l** represents a highly favorable position for an aromatic ring in a region, which has not previously been explored (Figure 6a). This region is clearly of great interest for further exploration.

The addition of an aromatic ring to **7l** to give the 1-naphthyl compound **7m** leads to a reduction of the affinity by a factor of 4 (Table 1). In terms of our pharmacophore model, the additional aromatic ring superimposes well with the phenyl ring of 4-benzyl-substituted compound as shown in Figure 6b. As mentioned above, the effect of the phenyl ring in the benzyl compound is an affinity increase by a factor of 2.6, indicating that the effect on the affinity of an aromatic ring in this position is largely a weak hydrophobic effect due to partial desolvation of the substituent.[12] The weak effect on the affinity of the distal ring in **7m** is consistent with the superimposition with the 4-benzyl compound displayed in Figure 6b.

In contrast to the 1-naphthyl substituent in **7m**, the 2-naphthyl substituent in **7n** increases the affinity by a factor of 6 as compared to that of phenyl-substituted compound **7l**. In terms of our pharmacophore model, the distal aromatic ring in **7n** in the deduced bioactive conformation is located in the same region as the distal ring in the previously studied 4-(1-naphthylmethyl)-PIOL compound[12] (Figure 6c). However, the superimposition of the distal rings in the two naphthyl moieties is not as good. As the conformational energy penalties for the two compounds are calculated to be similar (2.6 and 1.4 kcal/mol), the less optimal superimposition may explain why the distal ring in the naphthyl unit of **7n** only increases the affinity by a factor of 6 compared to the affinity increase by a factor of 38 for the 1-naphthylmethyl compound[12] compared to the 4-benzyl compound. Thus, the high affinity of **7n** is mainly due to the favorable position of the aromatic ring directly attached to the 3-isoxazolol ring.

Conclusions

With the aim of exploring the receptor cavity in the vicinity of the 4-position of the 3-isoxazolol ring in the low-efficacy partial $GABA_A$ agonist 4-PIOL (**4**), a series of analogues, based on the structure of the 2-naphthylmethyl analogue **5**, a previously reported potent $GABA_A$ antagonist, have been synthesized and pharmacologically characterized. Different substituents have

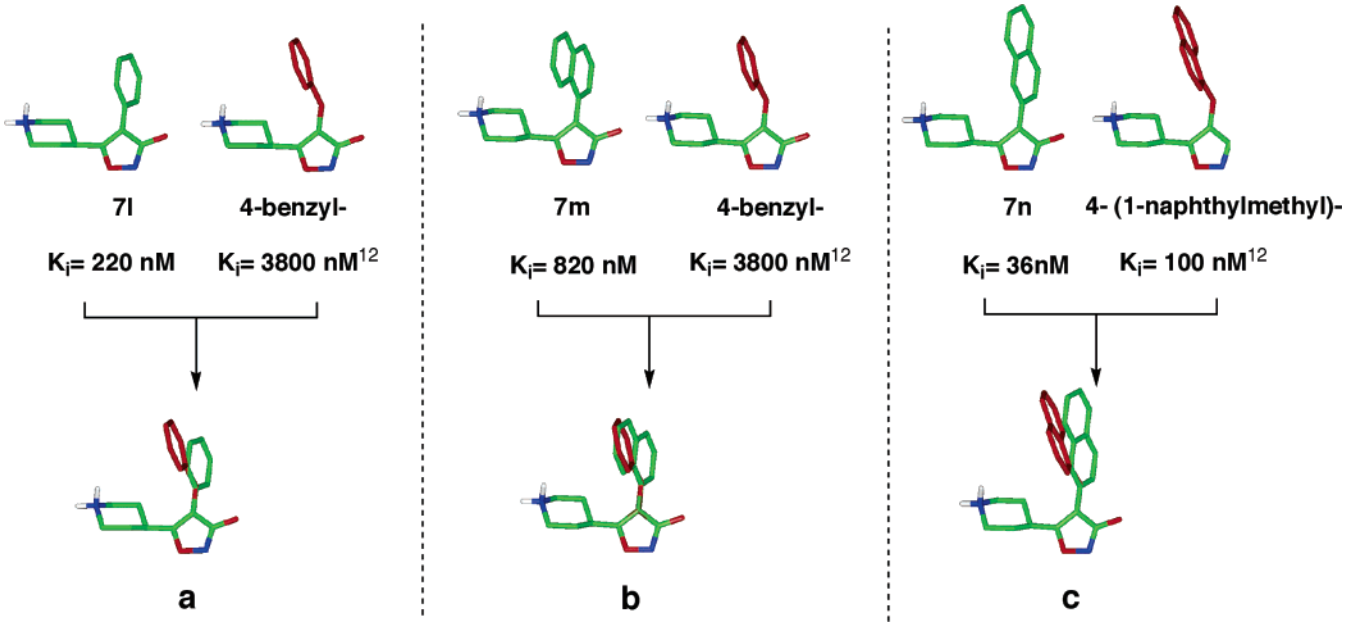


Figure 6. Superposition of the deduced bioactive conformations of (a) compound **7l** and the 4-benzyl-substituted 4-PIOL compound (red benzyl group), (b) **7m** and the 4-benzyl-substituted 4-PIOL compound (red benzyl group), and (c) **7n** and the 4-(1-naphthylmethyl)-substituted 4-PIOL compound (red naphthylmethyl group).

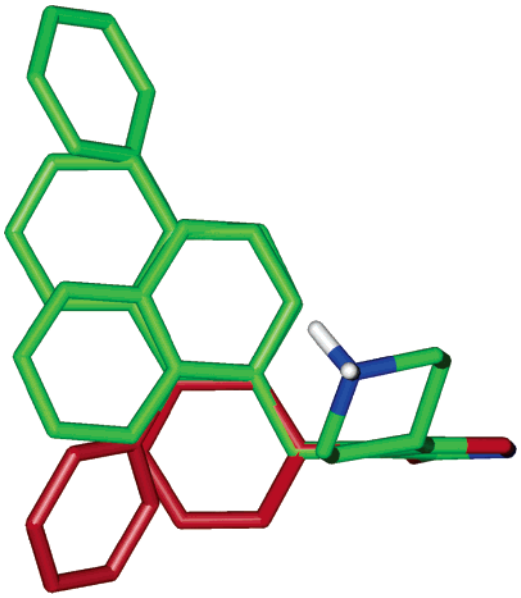


Figure 7. Regions in the vicinity of the 4-position of compound **4** in the direction of the C4−H bond of the 3-isoxazolol ring previously explored by aromatic substituents[12] (green) and the new regions explored by compounds **6k** and **7l** (red).

been introduced in different positions in the 2-naphthyl ring system, and aryl substituents have been coupled directly to the 4-position of the 3-isoxazolol ring of **4**.

By introduction of a bromo substituent in different positions in the 2-naphthyl ring system of **5**, the 1-position was shown to be the most favorable position in terms of affinity to the $GABA_A$ receptor. Introduction of a chloro, fluoro, cyano, methylthio, or even as large as a phenyl substituent in the 1-position of the 2-naphthylmethyl ring system resulted in slightly higher to markedly higher affinity compared to that of **5**. These substituents seem to occupy a previously unexplored receptor region in the pharmacophore model (Figure 7). In this series, the 1-bromo analogue showed the highest affinity of the series, markedly higher than that of **5**. Introduction of a phenyl group directly into the 4-position of the 3-isoxazolol ring in 4-PIOL gave rise to compound **7l** with an unexpectedly high affinity as compared to that of the parent compound **4**. On the basis of molecular modeling studies, this phenyl group represents an additional region, which has not been studied previously (Figure 7). All of the compounds tested were shown to be selective and potent $GABA_A$ antagonists.

On the basis of the structure−activity relationships of the compounds described in this study, additional information concerning the structure and properties of the ligand-binding site in the $GABA_A$ receptor has been obtained.

Experimental Section

Chemistry. General Procedures. All reactions involving air-sensitive reagents were performed under N_2 using syringe−septum cap techniques. Column chromatography (CC) was performed on Merck silica gel 60 (0.06−0.200 mm). Flash column chromatography (FC) was performed using Merck silica gel 60 (0.040−0.063 mm). Dry column vacuum chromatography[16] was performed using Merck silica gel 60 (0.015−0.040 mm). Analytical thin-layer chromatography (TLC) was carried out using Merck silica gel 60 F_{254} plates. All compounds were detected as single spots on TLC plates and visualized using UV light and $KMnO_4$ spraying reagent. Compounds containing amino groups were also visualized using a ninhydrin spray reagent. Compounds containing the 3-isoxazolol unit were visualized using an $FeCl_3$ spraying reagent. Melting points were determined in capillary tubes and are uncorrected. NMR spectra were recorded on a 300-MHz Varian spectrometer in $CDCl_3$ solutions using TMS as an internal standard or in D_2O solutions using 1,4-dioxane as an internal standard. 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.

3-Benzyloxy-5-(1-methoxycarbonyl-4-piperidyl)isoxazol (9). A mixture of 5-(1-methoxycarbonyl-4-piperidyl)-3-isoxazolol **(8)**[23] (1.4 g, 6.2 mmol) and potassium carbonate (1.71 g, 9.29 mmol) in acetone (18 mL) was stirred at 70 °C for 1 h.

Benzyl bromide (1.59 g, 9.29 mmol) was added dropwise to the reaction, and stirring was continued for 2 h at 70 °C followed by 20 h at room temperature. The reaction mixture was filtered through a sintered glass, and the filter was washed with acetone (3 × 15 mL). The combined organic phases were evaporated. Dry CC (toluene/EtOAc (3:1)) of the crude product gave the product as colorless crystals (0.95 g, 50%): mp 51−53 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.34−7.44 (5H, m), 5.64 (1H, s), 5.24 (2H, s), 4.18 (2H, broad s), 3.71 (3H, s), 2.82−2.97 (3H, m), 1.99−2.04 (2H, m), 1.56−1.69 (2H, m). ^{13}C NMR ($CDCl_3$) δ: 175.9, 171.1, 155.2, 135.3, 135.2, 128.5, 128.0, 127.9, 127.7, 124.7, 90.8, 70.9, 52.1, 42.8, 34.4, 29.1, 21.0. Anal. ($C_{17}H_{20}N_2O_4$) C,H,N.

3-Benzyloxy-4-iodo-5-(1-methoxycarbonyl-4-piperidyl)-isoxazol (11). To a solution of **9** (3.38 g, 10.7 mmol) in AcOH (40 mL), a solution of iodomonochloride (2.78 g, 17.1 mmol) in AcOH (15 mL) was added followed by water (75 mL). The reaction mixture was stirred, and the temperature was raised to 80 °C over 1 h and stirred for 21 h. The reaction was cooled, and sodium thiosulfate was added until the iodine color dissappeared followed by water (150 mL), and the mixture was extracted with Et_2O (5 × 100 mL). The combined extracts were washed with a 2% sodium thiosulfate solution (2 × 50 mL), dried, and evaporated. Dry CC (toluene/EtOAc (3:1)) of the crude product gave the product as colorless crystals (3.55 g, 75%): mp 99−101 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.28−7.39 (5H, m), 5.22 (2H, s), 4.16 (2H, broad s), 3.65 (3H, s), 2.84−2.91 (3H, m), 1.72−1.80 (4H, m). ^{13}C NMR ($CDCl_3$) δ: 175.3, 171.1, 156.2, 135.8, 128.9 (2C), 128.9, 128.4 (2C), 72.3, 53.1, 48.4, 43.9 (2C), 36.0, 29.1 (2C). Anal. ($C_{17}H_{19}IN_2O_4$) C,H,N.

3-Benzyloxy-4-[(1-bromo-2-naphthyl)hydroxymethyl]-5-(1-methoxycarbonyl-4-piperidyl)isoxazol (13a). A solution of **11** (0.80 g, 1.81 mmol) in dry THF (7 mL) was added dropwise to a solution of ^{i}PrMgCl (1.48 M in hexane, 1.22 mL, 1.81 mmol) at −30 °C under N_2 atmosphere. The reaction mixture was stirred and allowed to warm to 0 °C over 1 h. A solution of **17a**[22] (0.47 g, 1.99 mmol) in dry THF (3 mL) was added at 0 °C, and stirring was continued for 25 h at room temperature. The reaction mixture was quenched with saturated aqueous ammonium chloride (15 mL) and extracted with Et_2O (3 × 25 mL). The combined extracts were dried and evaporated. Dry CC (toluene/EtOAc (3:1)) of the crude product gave the product as colorless crystals (0.73 g, 72%): mp 151−152 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.27 (1H, d, J = 8.3 Hz), 7.77−7.84 (3H, m), 7.51−7.63 (2H, m), 7.26−7.29 (5H, m), 6.31 (1H, s), 5.24 (2H, s), 4.17 (2H, broad s), 3.65 (3H, s), 2.57−2.63 (2H, m), 2.33−2.41 (1H, m), 1.58−1.80 (4H, m). ^{13}C NMR ($CDCl_3$) δ: 172.7, 169.9, 156.1, 138.7, 136.0, 134.5, 132.4, 128.8(2C), 128.7, 128.5, 128.3(2C), 128.2, 128.1, 127.6, 127.3, 125.4, 122.5, 105.9, 72.0, 67.1, 53.0, 44.1, 43.9, 35.1, 29.8, 29.6. Anal. ($C_{28}H_{27}BrN_2O_5$) C,H,N.

3-Benzyloxy-4-[(1-bromo-2-naphthyl)methyl]-5-(1-methoxycarbonyl-4-piperidyl)isoxazol (14a) To a solution of **13a** (0.45 g, 0.82 mmol) and triethylsilane (0.21 mL, 1.3 mmol) in dry CH_2Cl_2 (6 mL) was added dropwise TFA (1.78 mL) at 0 °C, the reaction was then stirred at 0 °C for 2.5 h, and the stirring was continued at room temperature for 19 h. Water (10 mL) was added to the mixture, and the organic phase was extracted with Et_2O (3 × 25 mL). The combined extracts were dried and evaporated. Dry CC (toluene/EtOAc (4:1)) of the crude product gave the product as colorless oil (0.35 g, 80%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.31 (1H, d, J = 8.8 Hz), 7.80 (1H, d, J = 7.9 Hz), 7.68 (1H, d, J = 8.5 Hz), 7.56−7.64 (1H, m), 7.47−7.54(1H, m), 7.30−7.35 (4H, m), 7.19−7.27 (2H,m), 5.27 (2H, s), 4.00−4.19 (4H, m), 3.67 (3H, s), 2.64−2.84 (3H, m), 1.62−1.81 (4H, m). ^{13}C NMR ($CDCl_3$) δ: 172.1, 171.0, 156.2, 136.5, 136.2, 133.7, 129.4, 128.8, 128.7, 128.6, 128.4, 128.3, 128.1, 128.0, 127.7, 126.7, 102.6, 71.7, 53.0, 44.1, 35.00, 29.6, 28.8.

4-[(1-Bromo-2-naphthyl)methyl]-5-(4-piperidyl)-3-isoxazolol Hydrobromide (6a). Compound **14a** (0.1 g, 0.19 mmol) was dissolved in a solution of HBr in AcOH (33%, 3 mL), and the mixture was stirred at room temperature for 18 h. After evaporation, the residue was recrystallized (MeOH/Et_2O) to give **6a** (60 mg, 70%) as colorless crystals: mp > 210 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.28 (1H, d, J = 8.3 Hz), 7.84 (1H, d, J = 8.5 Hz), 7.81 (1H, d, J = 8.5 Hz), 7.47−7.64 (2H, m), 7.36 (1H, d, J = 8.5 Hz), 4.06 (2H, s), 3.31−3.39 (2H, m), 3.03−3.13 (1H, m), 2.90 (2H, dt, J = 12.6 and 4.1 Hz), 1.79−2.01 (4H, m). Anal. ($C_{19}H_{19}BrN_2O_2\cdot HBr\cdot 0.5H_2O$) C,H,-Br,N.

4-[(8-Bromo-2-naphthyl)hydroxymethyl]-3-isopropoxy-5-(1-methoxycarbonyl-4-piperidyl)isoxazol (13b). From the same procedure as described for **13a**, the title compound was prepared using **12**[12] (0.75 g, 1.9 mmol) in dry THF (3 mL), EtMgBr (0.94 M in THF, 2.04 mL, 1.9 mmol), and **17b** (0.45 g, 1.9 mmol) in dry THF (3 mL). FC (toluene/EtOAc (1:1)) of the residue gave the product as viscous oil (0.38 g, 40%). ^{1}H NMR ($CDCl_3$) δ: 8.28 (1H, s), 7.77−7.81 (3H, m), 7.52 (1H, d, J = 8.4 Hz), 7.31 (1H, dd, J = 7.6 and 7.6 Hz), 5.93 (1H, s), 4.84−4.96 (1H, m), 4.07 (2H, br. s), 3.66 (3H, s), 2.88−2.99 (1H, m), 2.57−2.78 (2H, m), 1.58−1.77 (3H, m), 1.45−1.49 (1H, m), 1.36 (3H, d, J = 6.0 Hz), 1.34 (3H, d, J = 6.0 Hz). ^{13}C NMR ($CDCl_3$) δ: 171.2, 168.5, 155.5, 141.4, 133.7, 131.4, 130.1, 128.5, 127.5, 126.2, 124.8, 123.2, 122.6, 107.0, 73.6, 66.3, 52.6, 43.6, 43.5, 34.6, 29.5, 29.0, 22.0, 21.9.

4-[(8-Bromo-2-naphthyl)methyl]-3-isopropoxy-5-(1-methoxycarbonyl-4-piperidyl)isoxazol (14b). Compound **14b** was prepared as described for **14a** using **13b** (0.55 g, 1.1 mmol) and triethylsilane (280 μL, 1.76 mmol) in dry CH_2Cl_2 (4 mL) and TFA (2.26 mL). FC (toluene/EtOAc (4:1)) afforded the title compound (0.43 g, 81%) as a viscous oil, which crystallized to a colorless solid within several days: mp 95−100 °C. ^{1}H NMR ($CDCl_3$) δ: 8.00 (1H, s), 7.75−7.79 (3H, m), 7.37 (1H, dd, J = 8.1 and 1.8 Hz), 7.29 (1H, dd, J = 7.3 and 7.3 Hz), 4.92 (1H, hep, J = 5.7 Hz), 4.17 (2H, m), 3.82 (2H, s), 3.69 (3H, s), 2.74−2.89 (3H, m), 1.56−1.85 (4H, m), 1.37 (6H, d, J = 5.7 Hz). ^{13}C NMR ($CDCl_3$) δ: 170.4, 169.6, 155.4, 138.4, 133.0, 131.6, 129.9, 128.4, 127.4, 125.6, 125.3, 122.0, 102.1, 73.0, 52.4, 43.5, 29.2, 27.1, 21.9.

4-[(8-Bromo-2-naphthyl)methyl]-5-(4-piperidyl)-3-isoxazolol Hydrobromide (6b). Compound **14b** (100 mg, 0.2 mmol) was dissolved in a solution of HBr in water (48%, 2 mL), and the mixture was heated at 100 °C for 1 h. After evaporation, the residue was recrystallized (MeOH/Et_2O) to give **6b** (80 mg, 83%) as light brown crystals: mp > 210 °C. ^{1}H NMR (CD_3OD) δ: 8.02 (1H, s), 7.84−7.87 (2H, m), 7.79 (1H, dd, J = 7.8 and 1.8 Hz), 7.50 (1H, dd, J = 8.5 and 1.8 Hz), 7.33 (1H, dd, J = 7.8 and 7.8 Hz), 3.95 (2H, s), 3.39−3.45 (2H, m), 3.21−3.32 (1H, m), 3.01−3.11 (2H, m), 1.94−2.10 (4H, m). Anal. ($C_{19}H_{19}BrN_2O_2\cdot HBr$) C,H,N.

4-[(7-Bromo-2-naphthyl)hydroxymethyl]-3-isopropoxy-5-(1-methoxycarbonyl-4-piperidyl)isoxazol (13c). From the same procedure as described for **13b**, the title compound was prepared using **12**[12] (190 mg, 0.81 mmol) in THF (1.5 mL), EtMgBr (0.94 M in THF, 0.86 mL, 8.1 mmol), and **17c** (0.32 g, 0.81 mmol) in THF (1.5 mL). FC (toluene/EtOAc (1:1)) gave **13c** (0.24 g, 59%) as viscous oil. ^{1}H NMR ($CDCl_3$) δ: 7.96 (1H, J = 1.9 Hz), 7.75−7.78 (2H, m), 7.69 (1H, d, J = 8.8 Hz), 7.54 (1H, dd, J = 8.8 and 1.9 Hz), 7.44 (1H, 8.7 and 1.6 Hz), 4.83−4.95 (1H, m), 4.09 (2H, br s), 3.65 (3H, s), 3.18 (1H, br s), 2.54−2.82 (3H, m), 1.45−1.81 (4H, m), 1.34 (3H, d, J = 6.0 Hz), 1.29 (3H, d, J = 6.0 Hz). ^{13}C NMR ($CDCl_3$) δ: 171.5, 168.7, 155.5, 140.6, 134.0, 131.0, 129.7, 129.3, 129.2, 128.0, 124.5, 123.1, 120.2, 106.9, 73.7, 66.1, 52.6, 43.6, 43.5, 34.6, 29.6, 29.0, 22.0.

4-[(7-Bromo-2-naphthyl)methyl]-3-isopropoxy-5-(1-methoxycarbonyl-4-piperidyl)isoxazol (14c). Compound **14c** was prepared as described for **14a** using **13c** (0.13 g, 0.26 mmol) in dry CH_2Cl_2 (1 mL), triethylsilane (66 μL, 0.41 mmol) and TFA (0.54 mL). The product was a viscous oil that crystallized to a colorless solid within several days: mp 112−117 °C. ^{1}H NMR ($CDCl_3$) δ: 7.91 (1H, d, J = 2.1 Hz), 7.73 (1H, d, J = 8.1 Hz), 7.67 (1H, d, J = 8.7 Hz), 7.51 (1H, dd, J = 8.7 Hz), 7.46 (1H, s), 7.32 (1H, dd, J = 8.1 and 1,5 Hz), 4.92 (1H, hep, J = 5.7 Hz), 4.15 (2H, br s), 3.78 (2H, s), 3.68 (3H, s), 2.72−2.81 (3H, m), 1.54−1.84 (4H, m), 1.34 (6H, d, J = 5.7 Hz). ^{13}C NMR ($CDCl_3$) δ: 170.7, 169.9, 155.6, 137.9, 130.4,

129.3, 129.2, 128.8, 128.0, 127.1, 125.1, 120.1, 103.0, 73.2, 52.7, 43.7, 34.6, 29.4, 27.0, 22.1.

4-[(7-Bromo-2-naphthyl)methyl]-5-(4-piperidyl)-3-isoxazolol Hydrobromide (6c). Compound **6c** was prepared as described for **6b** using **14c** (100 mg, 0.2 mmol) in a solution of HBr in water (48%, 2 mL). Recrystalllization ($MeOH/Et_2O$) gave **6c** (70 mg, 73%) as pale brown crystals: mp > 210 °C. ^1H NMR (CD_3OD) δ: 8.00 (1H, s), 7.81 (1H, d, J = 8.4 Hz), 7.74 (1H, J = 8.8 Hz), 7.64 (1H, s), 7.52 (1H, d, J = 8.8 Hz), 7.44 (1H, d, J = 8.4 Hz), 3.89 (2H, s), 3.38−3.42 (2H, m), 3.17−3.27 (1H, m), 3.01−3.10 (2H, m), 1.91−2.07 (4H, m). ^{13}C NMR (CD_3OD) δ: 171.1, 170.8, 139.7, 136.0, 131.9, 130.5, 130.4, 129.7, 129.2, 128.4, 126.2, 120.9, 105.3, 44.6, 33.0, 27.5. Anal. ($C_{19}H_{19}BrN_2O_2 \cdot HBr$) C,H,N.

4-[(5-Bromo-2-naphthyl)hydroxymethyl]-3-isopropoxy-5-(1-methoxycarbonyl-4-piperidyl)isoxazol (13d). From the same procedure as described for **13b**, the title compound was prepared using **12**[12] (2.5 g, 6.34 mmol) in THF (9 mL), EtMgBr (0.94 M in THF, 6.75 mL, 6.43 mmol), and **17d** (1.49 g, 6.34 mmol) in THF (6.8 mL). FC (toluene/EtOAc (1:1)) gave **13d** (0.95 g, 30%) as viscous oil. ^1H NMR ($CDCl_3$) δ: 8.21 (1H, d, J = 8.8 Hz), 7.85 (1H, s), 7.93 (2H, d, J = 7.8 Hz), 7.55 (1H, dd, J = 8.8 and 1.6 Hz), 7.34 (1H, dd, J = 7.8 and 7.8 Hz), 5.90 (1H, s), 4.86−4.98 (1H, m), 4.09 (2H, br s), 3.66 (3H, s), 2.56−2.79 (4H, m), 1.50−1.83 (4H, m), 1.36 (3H, d, J = 6.2 Hz), 1.31 (3H, d, J = 6.2 Hz). ^{13}C NMR ($CDCl_3$) δ: 171.7, 168.9, 155.7, 140.5, 134.3, 131.4, 130.1, 127.9, 127.5, 126.8, 125.7, 124.7, 122.7, 107.0, 74.0, 66.2, 52.8, 43.8, 43.7, 34.8, 29.8, 29.2, 22.2, 22.1.

4-[(5-Bromo-2-naphthyl)methyl]-3-isopropoxy-5-(1-methoxycarbonyl-4-piperidyl)isoxazol (14d). Compound **14d** was prepared as described for **14b** using **13d** (0.88 g, 1.75 mmol) in in dry CH_2Cl_2 (7 mL), triethylsilane (450 μL, 2.8 mmol), and TFA (3.65 mL). Dry CC (toluene/EtOAc (4:1)) gave the product as a viscous oil (0.81 g, 95%). ^1H NMR ($CDCl_3$) δ: 8.15 (1H, d, J = 8.7 Hz), 7.69−7.74 (2H, m), 7.54 (1H, s), 7.41 (1H, dd, J = 8.7 and 1.8 Hz), 7.30 (1H, dd, J = 7.8 and 7.8 Hz), 4.92 (1H, hep, J = 6.2 Hz), 4.14 (2H, br s), 3.80 (2H, s), 3.68 (3H, s), 2.73−2.83 (3H, m), 1.67−1.87 (4H, m), 1.34 (6H, d, J = 6.2 Hz). ^{13}C NMR ($CDCl_3$) δ: 170.9, 170.1, 155.8, 137.8, 134.7, 130.8, 129.6, 128.2, 127.5, 126.6, 126.5, 122.7, 103.1, 73.4, 52.8, 43.8, 34.8, 29.5, 26.8, 22.2.

4-[(5-Bromo-2-naphthyl)methyl]-5-(4-piperidyl)-3-isoxazolol Hydrobromide (6d). Compound **6d** was prepared as described for **6b** using **13d** (140 mg, 0.29 mmol) in a solution of HBr in water (48%, 3 mL). Recrystalllization ($MeOH/Et_2O$) gave **6d** (100 mg, 74%) as colorless crystals: mp > 210 °C. ^1H NMR (CD_3OD) δ: 8.14 (1H, d, J = 8.7 Hz), 7.82 (1H, d, J = 7.9 Hz), 7.75 (1H, d, J = 7.9 Hz), 7.72 (1H, s), 7.53 (1H, d, J = 8.7 Hz), 7.35 (1H, dd, 7.9 and 7.9 Hz), 3.92 (2H, s), 3.18−3.43 (3H, m), 3.01−3.10 (2H, m), 1.95−2.07 (4H, m), Anal. ($C_{19}H_{19}BrN_2O_2 \cdot HBr \cdot 0.5\ H_2O$) C,H,N.

3-Benzyloxy-4-[(1-fluoro-2-naphthyl)hydroxymethyl]-5-(1-methoxycarbonyl-4-piperidyl)isoxazol (13e). Compound **13e** was prepared as described for **13a** using **11** (0.40 g, 0.9 mmol) in dry THF (3 mL), ^{i}PrMgCl (1.9 M in hexane, 0.47 mL, 0.9 mmol), and **17e** (0.17 g, 1.0 mmol) in dry THF (2 mL). Dry CC (toluene/EtOAc (3:1)) of the crude product gave the product as a colorless oil (0.15 g, 35%). ^1H NMR (300 MHz, $CDCl_3$) δ: 8.03−8.07 (1H, m), 7.84−7.87 (1H, m), 7.60−7.70 (2H, m), 7.52−7.56 (2H, m), 7.23−7.32 (4H, m), 7.18 (1H, d, J = 8.5 Hz), 6.28 (1H, s), 5.23 (2H, s), 4.12−4.21 (2H, m), 3.69 (3H, s), 2.60−2.97 (3H, m), 1.58−1.83 (4H, s). ^{13}C NMR ($CDCl_3$) δ: 172.1, 169.5, 156.0, 135.8, 134.5, 129.3, 128.6, 128.5, 128.1, 127.7, 127.2, 126.8, 125.5, 124.3, 123.7, 120.8, 120.7, 106.4, 71.9, 61.1, 53.0, 44.1, 44.0, 35.0, 29.8.

3-Benzyloxy-4-[(1-fluoro-2-naphthyl)methyl]-5-(1-methoxycarbonyl-4-piperidyl)isoxazol (14e). Compound **14e** was prepared as described for **14a** using **13e** (0.13 g, 0.3 mmol), triethylsilane (0.07 mL, 0.43 mmol) in dry CH_2Cl_2 (2 mL), and TFA (0.65 mL, 8.4 mmol). Dry CC (toluene/EtOAc (4:1)) of the crude product gave **14e** as a colorless oil (80 mg, 58%). ^1H NMR (300 MHz, $CDCl_3$) δ: 8.04 (1H, d, J = 8.7 Hz), 7.79−7.83 (1H, m), 7.46−7.56 (3H, m), 7.19−7.31 (6H, m), 5.25 (2H, s), 4.14−4.20 (2H, m), 3.83 (2H, s), 3.67 (3H, s), 2.75−2.94 (3H, m), 1.69−1.78 (4H, m). ^{13}C NMR ($CDCl_3$) δ: 171.6, 170.8, 156.0, 154.3, 136.2, 129.3, 128.7(2C), 128.5, 128.1, 127.7, 126.7, 123.7, 120.6, 120.5, 102.8, 71.7, 53.1, 44.1, 34.9, 29.7, 20.4.

4-[(1-Fluoro-2-naphthyl)methyl]-5-(4-piperidyl)-3-isoxazolol Hydrobromide (6e). Compound **6e** was prepared as described for **6a** using **14e** (80 mg, 0.17 mmol) in a solution of HBr in AcOH (33%, 6 mL). Recrystalllization ($MeOH/Et_2O$) gave **6e** (30 mg, 44%) as colorless crystals: mp > 210 °C. ^1H NMR (300 MHz, $CDCl_3$) δ: 8.03 (1H, d, J = 8.5 Hz), 7.83−7.86 (1H, m), 7.61 (1H, d, J = 8.5), 7.46−7.56 (2H, m), 7.31−7.36 (1H, m), 3.88 (2H, s), 3.36−3.43 (1H, m), 3.06−3.21 (2H, m), 2.97−3,07(2H, m), 1.88−2.00 (4H, m). Anal. ($C_{19}H_{19}FN_2O_2 \cdot HBr$) C,H,N.

4-[(1-Chloro-2-naphthyl)hydroxymethyl]-3-isopropoxy-5-(1-methoxycarbonyl-4-piperidyl)isoxazol (13f). Compound **13f** was prepared as described for **13a** using **12**[12] (0.27 g, 0.69 mmol), in dry THF (5 mL), ^{i}PrMgCl (2.0 M in hexane, 0.37 mL, 0.74 mmol), and **17f** (0.14 g, 0.74 mmol) in dry THF (2 mL). Dry CC (toluene/EtOAc (3:1)) of the crude product gave **13f** as a colorless oil (0.17 g, 54%). ^1H NMR (300 MHz, $CDCl_3$) δ: 8.26 (1H, d, J = 9.2 Hz), 7.79−7.87 (2H, m), 7.49−7.62 (2H, m), 7.11−7.17 (1H, m), 6.28 (1H, d, J = 1.3 Hz), 4.89 (1H, hep, J = 6.1 Hz), 4.01−4.17 (2H, m), 3.65 (3H, s), 2.31−2.62 (3H, m), 1.51−1.80 (4H, s), 1.37 (3H, d, J = 6.1 Hz), 1.31 (3H, d, J = 6.1 Hz). ^{13}C NMR ($CDCl_3$) δ: 171.8, 169.2, 156.0, 134.2, 131.0, 129.3, 128.5, 128.4, 127.8, 127.2, 127.1, 124.8, 124.7, 106.1, 74.3, 64.7, 53.1, 44.2, 44.0, 35.1, 29.9, 29.8, 22.5.

4-[(1-Chloro-2-naphthyl)methyl]-3-isopropoxy-5-(1-methoxycarbonyl-4-piperidyl)isoxazol (14f). Compound **14f** was prepared as described for **14a** using **13f** (0.16 g, 0.35 mmol), triethylsilane (0.09 mL, 0.56 mmol) in dry CH_2Cl_2 (5 mL), and TFA (1.22 mL). Dry CC (toluene/EtOAc (4:1)) of the crude product gave **14f** as a yellow oil (0.13 g, 87%). ^1H NMR (300 MHz, $CDCl_3$) δ: 8.28 (1H, d, J = 8.5 Hz), 7.79 (1H, d, J = 8.0 Hz), 7.66 (1H, d, J = 8.5 Hz), 7.45−7.60 (2H, m), 7.28 (1H, d, J = 8.5 Hz), 4.29 (1H, hep, J = 6.1), 4.03−4.17 (2H, m), 3.94 (2H, s), 3.66 (3H, s), 2.66−2.85 (3H, m), 1.56−1.82 (4H, m), 1.34 (6H, d, J = 6.1 Hz). ^{13}C NMR ($CDCl_3$) δ: 171.4, 170.3, 156.0, 134.4, 133.6, 131.3, 130.5, 128.3, 127.6, 127.1, 126.6, 124.7, 102.7, 73.6, 53.1, 44.1, 35.0, 29.7, 25.7, 22.5.

4-[(1-Chloro-2-naphthyl)methyl]-5-(4-piperidyl)-3-isoxazolol Hydrobromide (6f) Compound **14f** (0.12 g, 0.26 mmol) was dissolved in a solution of HBr in water (48%, 4 mL), and the mixture was refluxed for 1 h. The mixture was cooled and evaporated, and the residue was evaporated twice from toluene (5 mL) and recrystallized ($MeOH/Et_2O$) to give **6f** (72 mg, 66%) as colorless crystals: mp > 210 °C. ^1H NMR (300 MHz, $CDCl_3$) δ: 8.26 (1H, d, J = 8.3 Hz), 7.84 (1H, d, J = 8.0 Hz), 7.77 (1H, d, J = 8.5 Hz), 7.49−7.59 (2H, m), 7.38 (1H, d, J = 8.5 Hz), 4.03 (2H, s), 3.33−3.39 (2H, m), 3.08−3.17 (1H, m), 2.94 (2H, dt, J = 12.4 and 3.6 Hz), 1.84−2.03 (4H, m). Anal. ($C_{19}H_{19}ClN_2O_2 \cdot HBr$) C,H,N.

1-Bromo-7-bromomethylnaphthalene (16b).[17] A solution of **15b**[17] (0.2 g, 0.9 mmol) in CCl_4 (5 mL) was treated under reflux with *N*-bromosuccinimide (NBS) (a total of 170 mg, 0.95 mmol) and benzoyl peroxide (a total of 3 mg, 0.012 mmol) over a period of 3 h. Each of the reagents was added in three equal portions every 1 h. After being stirred for 40 h at 75 °C, the reaction mixture was filtered while warm and concentrated to give the crude product. FC (petroleum ether (80−100 °C)) and recrystalllization (petroleum ether (40−65 °C)) gave the product as colorless crystals (170 mg; 62%): mp 112−116 °C. ^1H NMR ($CDCl_3$) δ: 8.22 (1H, s), 7.78−7.84 (3H, m), 7.56 (1H, dd, J = 8.5 and 1.8 Hz), 7.33 (1H, dd, J = 8.5 and 7.4 Hz), 4.70 (2H, s). ^{13}C NMR ($CDCl_3$) δ: 136.8, 134.3, 131.8, 130.6, 129.4, 127.8, 127.1, 127.0, 122.9, 33.6. Anal. ($C_{11}H_8Br_2$) C,H,N.

8-Bromonaphthalene-2-carbaldehyde (17b). 2-Nitropropane (350 μL, 3.9 mmol) was added to a solution of NaOEt (3.8 mmol) in EtOH (8 mL). A colorless precipitate was formed immediately. Compound **16b** (1.13 g, 3.8 mmol) was added to the mixture, and stirring was continued for 65 h at room temperature. The reaction mixture was filtered and concentrated, and to the residue were added Et_2O (100 mL) and water

(15 mL). The organic phase was washed with aqueous sodium hydroxide (10%, 2 × 10 mL) and water (10 mL), dried, and concentrated. FC (toluene) gave **17b** (640 mg, 72%) as colorless crystals: mp 83−84°. ^{1}H NMR ($CDCl_3$) δ: 10.21 (1H, s), 8.69 (1H, s), 7.84−8.00 (4H, m), 7.46 (1H, dd, J = 7.5 and 7.5 Hz). ^{13}C NMR δ: 192.2, 137.7, 135.2, 134.0, 131.6, 131.2, 129.7, 129.4, 128.0, 124.4, 123.4.

2-Bromo-7-methylnaphthalene (15c) and 1-Bromo-6-methylnaphthalene (15d).[18] To a suspension of (1,1-diethoxy-3-butyl)triphenylphosphonium bromide[19] (2.9 g, 6.0 mmol) in dry THF (20 mL), *n*-BuLi (1.6 M in hexane, 3.88 mL, 6.2 mmol) was added slowly at −70 °C. The mixture was warmed to 0 °C for 30 min causing the formation of a deep red colored solution, which was cooled to −75 °C. A solution of 3-bromobenzaldehyde (0.8 mL, 6.8 mmol) in dry THF (2 mL) was added dropwise, and the reaction mixture was stirred at room temperature for 23 h. Triethylamine (97 μL) was added, and the mixture was evaporated on silica gel (20 mL), put on the top of a pad of silica gel (25 mL), and eluted with toluene (5 × 50 mL). The solvent was evaporated, and AcOH (2 × 10 mL) was added and evaporated again. The residue was dissolved in AcOH (10 mL) and treated with HBr in water (48%, 6 mL) for 2 h at 95 °C. The mixture was poured on ice (20 mL) and extracted with CH_2Cl_2 (3 × 20 mL). The organic phases were dried and evaporated. FC (petroleum ether (80−100 °C)) of the crude product gave a mixture of **15c** and **15d** (0.69 g, 46%, 1:1). Fractional crystallization (EtOH) gave **15c** (310 mg, 21%) as colorless crystals. ^{1}H and ^{13}C NMR spectra were identical with those earlier described[18] for **15c**. The residue containing a mixture of **15c** and **15d** (380 mg, 25%, 1:10) was used in the synthesis of **16d** without further purification.

2-Bromo-7-bromomethylnaphthalene (16c).[18] Compound **16c** was prepared as described above for **16b** by using **15c**[18] (2.0 g, 9.0 mmol) in CCl_4 (50 mL), NBS (1.7 g, 9.5 mmol), and benzoyl peroxide (30 mg, 0.1 mmol). FC (petroleum ether (80−100 °C)) and recrystalllization (heptane) gave the product as colorless crystals (1.89 g, 70%): mp 150−151 °C. ^{1}H NMR ($CDCl_3$) δ: 7.97−7.98 (1H, m), 7.80 (1H, d, J = 8.5 Hz), 7.73 (1H, m), 7.69 (1H, J = 8.8 Hz), 7.56 (1H, dd, J = 8.8 and 1.9 Hz), 7.52 (1H, dd, J = 8.5 and 1.8 Hz), 4.64 (2H, s). ^{13}C NMR ($CDCl_3$) δ: 136.4, 134.3, 131.5, 130.0, 129.4, 128.8, 127.3, 126.9, 120.6, 33.4. Anal. ($C_{11}H_8Br_2$) C,H,N.

7-Bromonaphthalene-2-carbaldehyde (17c). Compound **17c** was prepared as described above for **17b** by using **16c** (1.72 g, 5.7 mmol), NaOEt (5.7 mmol), and 2-nitropropane (537 μL). FC (toluene) gave **17c** (670 mg, 50%) as colorless crystals: mp 93−94 °C. ^{1}H NMR ($CDCl_3$) δ: 10.13 (1H, s), 8.19 (1H, s), 8.11 (1H, m), 7.94 (1H, dd, J = 8.5 and 1.5 Hz), 7.87 (1H, d, J = 8.5 Hz), 7.74 (1H, d, J = 8.6 Hz), 7.67 (1H, dd, J = 8.6 and 1.9 Hz). ^{13}C NMR ($CDCl_3$) δ: 192.0, 134.8, 133.7, 133.2, 132.4, 131.4, 129.7, 129.1, 123.3, 121.1.

1-Bromo-6-bromomethylnaphthalene (16d). Compound **16d** was prepared according to the procedure described for **16b**, using a mixture of **15c** and **15d** in a ratio of 1:10 (3.15 g, 14.3 mmol) in CCl_4 (50 mL), NBS (2.7 g, 15 mmol), and benzoyl peroxide (45 mg, 0.2 mmol). Recrystalllization (petroleum ether (80−100 °C)) gave **16d** (2.36 g, 55%): mp 118−120 °C. ^{1}H NMR ($CDCl_3$) δ: 8.24 (1H, d, J = 8.8 Hz), 7.83−7.84 (1H, m), 7.79 (2H, d, J = 7.7. Hz), 7.62 (1H, dd, J = 8.8 and 1.8 Hz), 7.35 (1H, dd, J = 7.7 and 7.7 Hz), 4.68 (2H, s). ^{13}C NMR ($CDCl_3$) δ: 136.1, 134.4, 131.7, 130.6, 128.2, 128.1, 128.0, 126.9, 122.8, 33.4.

5-Bromonaphthalene-2-carbaldehyde (17d). Compound **17d** was prepared as described for **17b** using **16d** (2.36 g, 7.9 mmol), NaOEt (7.9 mmol) in EtOH (16 mL), and 2-nitropropane (737 μL, 8.2 mmol). FC (toluene) gave the product (1.49 g, 81%) as colorless crystals: mp 77−79 °C. ^{1}H NMR ($CDCl_3$) δ: 10.20 (1H, s), 8.35 (1H, d, J = 8.7 Hz), 8.33 (1H, s), 8.05 (1H, dd, J = 8.7 and 1.6 Hz), 7.98 (1H, d, J = 7.8 Hz), 7.94 (1H, dd, J = 7.8 and 1.0 Hz), 7.44 (1H, dd, J = 7.8 and 7.8 Hz). ^{13}C NMR ($CDCl_3$) δ: 191.6, 134.8, 134.5, 134.2, 133.8, 132.9, 129.4, 128.4, 127.4, 124.2, 122.9.

1-Fluoro-2-methylnaphthalene (15e).[20] A solution of **15a** (0.40 g, 1.8 mmol) in dry THF (4 mL) was stirred at −78 °C under N_2 atmosphere. *n*-BuLi (1.41 M in hexane, 1.29 mL, 1.8 mmol) was added dropwise, and the mixture was stirred at −78 °C for 15 min. This mixture was added to a solution of *N*-fluorobis(phenylsulfonyl)amine (0.57 g, 1.8 mmol) in dry THF (2 mL) at −78 °C. The mixture was stirred and allowed to warm from −78 °C to 0 °C over 2 h. Water (20 mL) was added, and the mixture was extracted with CH_2Cl_2 (3 × 25 mL). The combined extracts were dried and evaporated to give **15e** as brown oil (0.22 g, 76%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.10 (1H, d, J = 8.2 Hz), 7.78 (1H, d, J = 7.6 Hz), 7.41−7.53 (3H, m), 7.25 (1H, t, J = 7.3 Hz), 2.43 (3H, s). ^{13}C NMR ($CDCl_3$) δ: 129.3, 129.2, 127.7, 127.6, 126.4, 126.1, 123.3, 123.3, 120.4, 120.4, 14.9.

1-Fluoro-2-bromomethylnaphthalene (16e).[21] Compound **16e** was prepared according to the procedure described for **16b**, using **15e** (0.55 g, 3.4 mmol) in CCl_4 (10 mL), NBS (0.67 g, 3.74 mmol), and benzoyl peroxide (20 mg, 0.09 mmol). The mixture was cooled, water (15 mL) was added, and the mixture was extracted with CH_2Cl_2 (3 × 15 mL). The combined extracts were dried and evaporated. Dry CC (petroleum ether (80−100 °C)) of the crude product gave the product as colorless crystals (0.35 g, 44%): mp 85−88 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.10−8.14 (1H, m), 7.81−7.85 (1H, m), 7.63 (1H, d, J = 8.5 Hz), 7.53−7.57 (2H, m), 7.44 (1H, t, J = 8.5 Hz), 4.73 (2H, s). ^{13}C NMR ($CDCl_3$) δ: 127.8, 127.5, 127.0, 124.4, 121.2, 26.6.

1-Fluoronaphthalene-2-carbaldehyde (17e).[21] Compound **17e** was prepared as described for **17b** using **16e** (0.30 g, 1.26 mmol), NaOEt (1.26 mmol) in EtOH (4 mL), and 2-nitropropane (0.12 mL, 1.39 mmol), which gave **17e** as a yellow oil (0.19 g, 87%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 10.60 (1H, s), 8.25 (1H, d, J = 8.3 Hz), 7.83−7.91 (2H, m), 7.56−7.76 (3H, m). ^{13}C NMR ($CDCl_3$) δ: 187.4, 130.4, 128.2, 127.8, 127.6, 127.1, 122.4, 122.3, 122.2.

2-(1-Chloro-2-naphthyl)-1,3-dioxolane (19). Compound **18**[22] (0.56 g, 2.0 mmol) was stirred and cooled to −78 °C in THF (2 mL), *n*-BuLi (1.43 M in hexane, 1.46 mL, 2,1 mmol) was added, and the mixture was stirred at −78 °C for 1 h. A solution of hexachloroethane (0.62 g, 2.60 mmol) in THF (4 mL) was added dropwise at −78 °C, and the mixture was stirred and allowed to warm to room temperature over 3.5 h. Water (15 mL) was added, and the mixture was extracted with CH_2Cl_2 (2 × 20 mL), dried, and evaporated. Dry CC (petroleum ether (80−100 °C)/EtOAc (4:1)) of the crude product gave the product as a yellow oil (0.23 g, 50%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.33 (1H, d, J = 9.3 Hz), 7.77−7.84 (2H, m), 7.46−7.69 (3H, m), 6.42 (1H, s), 4.03−4.27 (4H, m). ^{13}C NMR ($CDCl_3$) δ: 135.0, 131.1, 128.7, 128.4, 128.3, 127.5, 127.5, 127.4, 125.0, 124.1, 101.5, 66.1.

1-Chloronaphthalene-2-carbaldehyde (17f). Toluene-*p*-sulfonic acid monohydrate (0.08 g, 0.4 mmol) was added to a solution of **19** (0.22 g, 0.94 mmol) in acetone (15 mL) and stirred at room temperature for 5 h. Water (10 mL) was added, and acetone was removed under reduced pressure. The aqueous residue was extracted with EtOAc (2 × 15 mL). The organic phase was washed with saturated aqueous sodium hydrogen carbonate (2 × 15 mL) and brine (15 mL), dried, and evaporated to give **17f** as yellow crystals (0.16 g, 90%): mp 91−94 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 10.74 (1H, s), 8.45−8.48 (1H, m), 7.93 (1H, d, J = 8.5 Hz), 7.80 (1H, d, J = 8.8 Hz), 7.66−7.70 (2H, m), 7.50−7.60 (1H, m). ^{13}C NMR ($CDCl_3$) δ: 190.7, 137.3, 130.0, 128.7, 128.3, 127.8, 127.4, 127.1, 125.7, 123.7. Anal. ($C_{11}H_7ClO$) C,H,N.

3-Benzyloxy-4-[(1-methylthio-2-naphthyl)methyl]-5-(1-methoxycarbonyl-4-piperidyl)isoxazol (20g). A solution of **14a** (0.4 g, 0.75 mmol) in dry THF (3 mL) was stirred at −78 °C under N_2 atmosphere. *n*-BuLi (1.52 M in hexane, 0.6 mL, 0.9 mmol) was added dropwise, and the mixture was stirred at −78 °C for 15 min. Dimethyl disulfide (0.35 g, 3.75 mmol) in dry THF (1 mL) was added dropwise, and the mixture was stirred at −78 °C for 4 h. The reaction mixture was quenched with water (5 mL) and extracted with EtOAc (3 × 15 mL). The combined extracts were dried and evaporated.

Dry CC (toluene/EtOAc (3:1)) of the crude product gave **20g** as a colorless oil (90 mg, 23%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.65 (1H, d, J = 8.4 Hz), 7.84 (1H, d, J = 8.3 Hz), 7.73 (1H, d, J = 8.5 Hz), 7.48−7.64 (2H, m), 7.19−7.33 (6H, m), 5.24 (2H, s), 4.12−4.26 (4H, m), 3.68 (3H, s), 2.71−2.90 (3H, m), 2,21 (3H, s), 1.59−1.74 (4H, m). ^{13}C NMR ($CDCl_3$) δ: 171.8, 171.1, 156.1, 141.9, 136.3, 133.5, 132.3, 129.6, 128.9, 128.8, 128.7, 128.6, 128.3, 127.7, 127.6, 126.9, 126.1, 103.9, 71.6, 53.0, 44.1, 35.0, 29.7, 26.9, 20.0.

4-[(1-Methylthio-2-naphthyl)methyl]-5-(4-piperidyl)-3-isoxazolol Hydrobromide (6g). Compound **6g** was prepared as described for **6a** using **20g** (70 mg, 0.14 mmol) in a solution of HBr in water (33%, 3 mL). Recrystalllization (MeOH/Et_2O) gave **6g** (50 mg, 83%) as colorless crystals: mp >210 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.64 (1H, d, J = 8.5 Hz), 7.80−7.85 (2H, m), 7.56−7.61 (1H, m), 7.41−7.51 (2H, m), 4.27 (2H, s), 3.32−3.41 (2H, m), 2.85−3.15 (3H, m), 3.00 (3H, s), 1.81−1.98 (4H, m). Anal. ($C_{20}H_{22}N_2O_2S$·HBr) C,H,N.

3-Benzyloxy-4-[(1-phenylthio-2-naphthyl)methyl]-5-(1-methoxycarbonyl-4-piperidyl)isoxazol (20h). From the same procedure as described for **20g**, the title compound is prepared using **14a** (0.3 g, 0.56 mmol) in dry THF (3 mL), n-BuLi (1.52 M in hexane, 0.45 mL, 0.67 mmol), and diphenyl disulfide (0.15 g, 0.67 mmol) in dry THF (1 mL). Dry CC (toluene/EtOAc (3:1)) of the crude product gave **20h** as a colorless oil (0.13 g, 36%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.48−8.51 (1H, m), 7.83−7.87 (2H, m), 7.43−7.52 (2H, m), 7.23−7.28 (4H, m), 7.16−7.19 (2H, m), 7.02−7.10 (3H, m), 6.85−6.88 (2H, m), 5.24 (2H, s), 4.06−4.17 (4H, m), 3.67 (3H, m), 2.62−2.84 (3H, m), 1.58−1.71 (4H, m). ^{13}C NMR ($CDCl_3$) δ: 172.0, 156.1, 143.6, 138.3, 138.2, 136.3, 135.8, 133.6, 130.8, 129.4, 128.8, 128.7, 128.6, 128.3, 128.0, 127.9, 127.0, 126.5, 126.1, 125.7, 125.4, 103.2, 71.7, 53.0, 44.0, 34.9, 29.6, 26.9.

4-[(1-Phenylthio-2-naphthyl)methyl]-5-(4-piperidyl)-3-isoxazolol Hydrobromide (6h). Compound **6h** was prepared as described for **6a** using **20h** (90 mg, 0.23 mmol) in a solution of HBr in AcOH (33%, 5 mL). Recrystalllization (MeOH/Et_2O) gave **6h** (55 mg, 48%) as colorless crystals: mp > 210 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.38−8.43 (1H, m), 7.95 (1H, d, J = 8.5 Hz), 7.87−7.90 (1H, m), 7.58 (1H, d, J = 8.5 Hz), 7.41−7.44 (2H, m), 7.11−7.16 (3H, m), 6.88 (2H, d, J = 7.6 Hz), 4.17 (2H, s), 3.28−3.33 (2H, m), 2.78−3.05 (3H, m), 1.74−1.93 (4H, m). Anal. ($C_{25}H_{24}N_2O_2S$·HBr·H_2O) C,H,N.

4-[(1-Formyl-2-naphthyl)methyl]-3-benzyloxy-5-(1-methoxycarbonyl-4-piperidyl)isoxazol (20i). From the same procedure as described for **20g**, the title compound was prepared using **14a** (0.15 g, 0.28 mmol) in dry THF (2 mL), n-BuLi (1.52 M in hexane, 0.19 mL, 0.28 mmol), and DMF (0.21 g, 2.8 mmol). Dry CC (toluene/EtOAc (3:1)) of the crude product gave **20i** as a colorless oil (0.08 g, 54%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 11.01 (1H, s), 8.71 (1H, d, J = 8.5 Hz), 7.92 (1H, d, J = 8.5 Hz), 7.86−7.89 (1H, m), 7.56−7.65 (2H, m), 7.16−7.34 (6H, m), 5.21 (2H, s), 4.11−4.20 (4H, m), 3.68 (3H, s), 2.64−2.83 (3H, m), 1.67−1.79 (4H,m). ^{13}C NMR ($CDCl_3$) δ: 193.7, 172.0, 170.8, 156.1, 141.6, 136.1, 134.4, 132.9, 129.4, 129.1, 129.0, 128.8, 128.7, 128.6, 128.4(2C), 128.3, 126.8, 124.0, 103.0, 71.8, 53.0, 44.0, 34.9, 29.7, 24.7.

3-Benzyloxy-4-[(1-cyano-2-naphthyl)methyl]-5-(1-methoxycarbonyl-4-piperidyl)isoxazol (20j). A solution of **20i** (0.14 g, 0.29 mmol), NH_3 (aq) (25%, 8 mL), and THF (2 mL) was stirred at room temperature. Iodine (0.08 g, 0.33 mmol) was added, and the mixture was stirred at room temperature for 18 h. Additional iodine (a total of 0.16 g, 0.67 mmol) and NH_3 (aq) (25%, a total of 8 mL) were added over a period of 30 h. Aqueous sodium thiosulfate solution (2%, 15 mL) was added, and the mixture was extracted with EtOAc (3 × 25 mL). The combined extracts were dried and evaporated. Dry CC (toluene/EtOAc (2:1)) of the crude product gave **20j** as a yellow viscous oil (0.09 g, 23%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.20 (1H, d, J = 8.8 Hz), 7.76−7.94 (2H, m), 7.67−7.75 (1H, m), 7.54−7.64 (1H, m), 7.39−7.48 (1H, m), 7.29−7.36 (5H, m), 5.26 (2H, s), 4.12−4.30 (2H, m), 4.08 (2H, s), 3.69 (3H, s), 2.80−3.13 (3H, m), 1.59−1.79 (4H, m).

4-[(1-Cyano-2-naphthyl)methyl]-5-(4-piperidyl)-3-isoxazolol Hydrobromide (6j). Compound **6j** was prepared as described for **6a** using **20j** (40 mg, 0.08 mmol) in a solution of HBr in AcOH (33%, 2 mL). Recrystalllization (MeOH/Et_2O) gave **6j** (20 mg, 33%) as colorless crystals: mp > 210 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.12 (1H, t, J = 8.6 Hz), 7.97 (1H, d, J = 8.3 Hz), 7.58−7.78 (3H, m), 7.33−7.43 (1H, m), 4.11 (2H, s), 3.32−3.47 (3H, m), 2.93−3.19 (2H, m), 1.97−2.10 (4H, m). Anal. ($C_{20}H_{19}N_3O_2$·HBr·H_2O) C,H,N.

3-Benzyloxy-4-[(1-phenyl-2-naphthyl)methyl]-5-(1-methoxycarbonyl-4-piperidyl)isoxazol (21). To a solution of **14a** (0.2 g, 0.37 mmol) in DMF (2 mL), phenylboronic acid (70 mg, 0.56 mmol), $Pd(PPh_3)_2Cl_2$ (30 mg, 0.037 mmol), $NaHCO_3$ (90 mg, 1.11 mmol), and water (2 mL) were added. The mixture was stirred at 50 °C under N_2 atmosphere for 18 h. EtOAc (15 mL) was added, and the organic phase was washed with water (10 mL), NaOH (2 M, 2 × 10 mL), and water (10 mL), dried, and evaporated. Dry CC (toluene/EtOAc (3:1)) of the crude product gave **21** as a yellow oil (0.15 g, 76%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.31 (1H, d, J = 8.5 Hz), 7.75−7.86 (1H, m), 7.67 (1H, d, J = 8.5), 7.57−7.62 (1H, m), 7.42−7.55 (2H, m), 7.11−7.35 (10H, m), 5.27 (2H, s), 4.03−4.21 (4H, m), 3.67 (3H, s), 2.64−2.86 (3H, m), 1.57−1.75 (4H, m). ^{13}C NMR ($CDCl_3$) δ: 172.1, 171.0, 156.1, 139.4, 138.2, 136.3, 130.7, 129.4, 128.9, 128.8, 128.7, 128.6, 128.5, 128.3, 128.2, 128.1, 128.0, 127.7, 126.7, 125.7, 102.6, 71.7, 53.0, 44.1, 35.0, 29.6, 28.8, 21.9.

4-[(1-Phenyl-2-naphthyl)methyl]-5-(4-piperidyl)-3-isoxazolol Hydrobromide (6k). Compound **6k** was prepared as described for **6a** using **21** (0.15 g, 0.28 mmol) in a solution of HBr in AcOH (33%, 4 mL). Recrystalllization (MeOH/Et_2O) gave **6k** (60 mg, 46%) as colorless crystals: mp 168−171 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.31 (1H, d, J = 8.5 Hz), 7.81−8.88 (3H, m), 7.51−7.68 (4H, m), 7.38 (1H, d, J = 8.5 Hz), 7.25−7.33 (2H, m), 4.07 (2H, s), 3.33−3.38 (2H, m), 2.99−3.17 (3H, m), 1.78−2.10 (4H, m). Anal. ($C_{25}H_{24}N_2O_2$·HBr·H_2O) C,H,N.

3-Benzyloxy-4-phenyl-5-(1-methoxycarbonyl-4-piperidyl)isoxazol (22l). To a solution of **11** (0.2 g, 0.45 mmol) in DMF (3 mL), phenylboronic acid (90 mg, 0.9 mmol), $Pd(PPh_3)_2Cl_2$ (32 mg, 0.045 mmol), and aqueous K_2CO_3 (3 M, 0.29 mL, 0.9 mmol) were added. The mixture was stirred at 70 °C under N_2 atmosphere for 18 h. Et_2O (10 mL) was added, and the organic phase was washed with water (10 mL), NaOH (2 M, 2 × 10 mL), and water (10 mL), dried, and evaporated. Dry CC (toluene/EtOAc (3:1)) of the crude product gave **22l** as pale brown crystals (0.14 g, 85%): mp 114−117 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.33−7.45 (10H, m), 5.34 (2H, s), 4.09−4.25 (2H, m), 3.71 (3H, s), 2.80−3.08 (3H, m), 1.80−1.93 (4H, m). ^{13}C NMR ($CDCl_3$) δ: 171.4, 169.6, 156.1, 136.3, 135.6, 129.2, 129.1, 128.8, 128.6, 128.3, 128.0, 106.9, 71.9, 53.1, 44.1, 30.1. Anal. ($C_{23}H_{24}N_2O_4$·H_2O) C,H,N.

4-Phenyl-5-(4-piperidyl)-3-isoxazolol Hydrobromide 7l. Compound **7l** was prepared as described for **6a** using **22l** (0.24 g, 0.61 mmol) in a solution of HBr in AcOH (33%, 10 mL). Recrystalllization (MeOH/Et_2O) gave **7l** (0.15 g, 76%) as colorless crystals: mp > 210 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.31−7.46 (5H, m), 3.33−3.47(3H, m), 3.05−3.16 (2H, m), 2.06−2.12 (4H, m). Anal. ($C_{14}H_{16}N_2O_2$·HBr·1.5 H_2O) C,H,N.

3-Benzyloxy-4-(1-naphthyl)-5-(1-methoxycarbonyl-4-piperidyl)isoxazol (22m). Compound **22m** was prepared as described above for **22l** using **11** (0.5 g, 1.13 mmol) in DMF (9 mL), 1-naphthylboronic acid (0.39 g, 2.26 mmol), $Pd(PPh_3)_2Cl_2$ (80 mg, 0.11 mmol), and aqueous K_2CO_3 (3 M, 0.75 mL, 2.26 mmol). Dry CC (toluene/EtOAc (4:1)) of the crude product gave **22m** as pale brown crystals (0.29 g, 58%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.95 (2H, d, J = 8.2 Hz), 7.71 (1H, d, J = 8.0 Hz), 7.48−7.60 (3H, m), 7.38−7.41 (1H, m), 7.28−7.33 (3H, m), 7.22 (2H, d, J = 8.2 Hz), 5.33 (2H, q, J = 11.9 Hz), 4.11−4.21 (2H, m), 3.71 (3H, s), 2.66−2.90 (3H, m), 1.73−1.90 (4H, m). ^{13}C NMR ($CDCl_3$) δ: 172.5, 170.3, 156.0, 138.1, 136.1, 132.7, 129.3, 129.0, 128.7, 128.6, 128.5, 128.4, 128.2, 126.7, 126.5, 125.7, 125.6, 105.5, 71.7, 53.0, 43.9, 35.1, 30.0, 29.7. Anal. ($C_{27}H_{26}N_2O_4$·0.25 H_2O) C,H,N.

4-(1-Naphthyl)-5-(4-piperidyl)-3-isoxazolol Hydrobromide (7m). Compound **7m** was prepared as described for **6a**

using **22m** (0.28 g, 0.63 mmol) in a solution of HBr in AcOH (33%, 10 mL). Recrystalllization (EtOH) gave **7m** (0.16 g, 68%) as pale brown crystals: mp > 210 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.90−7.96 (2H, m), 7.62−7.66 (1H, m), 7.48−7.56 (3H, m), 7.41 (1H, dd, J = 1.2 and 7.0 Hz), 3.30−3.37 (2H, m), 2.83−3.00 (3H, m), 1.91−2.04 (4H, m). Anal. ($C_{18}H_{17}N_2O_2$·HBr·0.75H_2O) C,H,N.

3-Benzyloxy-4-(2-naphthyl)- 5-(1-methoxycarbonyl-4-piperidyl)isoxazol (22n). Compound **22n** was prepared as described for **22l** using **11** (0.3 g, 0.68 mmol) in DMF (4 mL), 2-naphthylboronic acid (0.23 g, 1.36 mmol), $Pd(PPh_3)_2Cl_2$ (65 mg, 0.07 mmol), and aqueous K_2CO_3 (3 M, 0.45 mL, 1.36 mmol). Dry CC (toluene/EtOAc (4:1)) of the crude product gave **22n** as yellow crystals (0.20 g, 67%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.87 (1H, d, J = 8.8 Hz), 7.79−7.82 (3H, m), 7.46−7.52 (2H, m), 7.39−7.44 (2H, m), 7.29−7.37 (2H, m), 7.21−7.25 (1H, m), 7.15 (1H, d, J = 7.8 Hz), 5.35 (2H, s), 4.11−4.23 (2H, m), 3.70 (3H, s), 3.07−3.11 (1H, m), 2.78−2.87 (2H, m), 1.88−1.96 (4H, m). ^{13}C NMR ($CDCl_3$) δ: 171.5, 169.6, 156.0, 136.2, 133.5, 132.8, 128.7, 128.5, 128.2, 128.1, 128.0, 126.9, 126.7, 126.6, 126.2, 106.9, 71.8, 53.1, 44.0, 35.0, 30.0. Anal. ($C_{27}H_{26}N_2O_4$) C,H,N.

4-(2-Naphthyl)-5-(4-piperidyl)-3-isoxazolol Hydrobromide (7n). Compound **7n** was prepared as described for **6a** using **22n** (0.18 g, 0.41 mmol) in a solution of HBr in AcOH (33%, 4 mL). Recrystalllization (MeOH/Et_2O) gave **7n** (0.11 g, 72%) as colorless crystals: mp > 210 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.83−7.96 (4H, m), 7.46−7.52 (3H, m), 3.35−3.48 (3H, m), 3.04−3.14 (2H, m), 2.08−2.15 (4H, m). Anal. ($C_{18}H_{18}N_2O_2$·HBr·0.5 H_2O) C,H,N.

Receptor Binding Assays. Muscimol 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.[23] On the day of the assay, the membrane preparation was quickly thawed, suspended in 40 volumes of 50 mM Tris-HCl buffer (pH 7.4) using an Ultra-Turrax homogenizer, and centrifuged at 48 000*g* for 10 min at 4 °C. This step was repeated 4 times. The final pellet was resuspended in incubation buffer for the relevant binding assay.

Muscimol binding was carried out in triplicate and in total volumes of 250 mL by incubation of synaptic membranes (100 mg protein/aliquot) in 200 mL Tris-HCl buffer (50 mM, pH 7.4), 25 μL [^{3}H]muscimol (final concentration 5−6 nM), and 25 μL of the test substance in various concentrations. After incubation at 0 °C for 60 min, binding was determined by filtration through Whatman GF/B filters, using a 96-well Packard Filtermate cell harvester, and filters were washed with 3 × 250 mL of ice-cold buffer. Nonspecific binding was determined using 1.0 mM GABA.

$GABA_B$ binding was carried out in triplicate by incubation of membranes (200 mg protein/aliquot) in 0.75 mL Tris-HCl buffer (50 mM + 2.5 mM $CaCl_2$, pH 7.4), 0.1 mL isoguvacine (400 mM), 0.05 mL [^{3}H]GABA (final concentration 3−5 nM), and 0.1 mL of the test substance in various concentrations. After incubation at 25 °C for 45 min, binding was determined by filtration through Whatman GF/C filters, using a Brandell M-48R cell harvester, and filters were washed with 3 × 3 mL of ice-cold buffer. Nonspecific binding was determined using 0.1 mM baclofen.

IC_{50} values were estimated by measuring the inhibition of at least five different test concentrations, and were estimated from the function $B = B_{max} - (B_{max}[\text{inhibitor}]^n)/(IC_{50}{}^n + [\text{inhibitor}]^n)$ using the nonlinear curve fitting program GraphPad Prism v. 3.00 (GraphPad Software, San Diego, CA). K_i values were calculated from the Cheng-Prusoff equation: $K_i = IC_{50}/(1 + [\text{radioligand}]/K_D)$.

Electrophysiology in Vitro. Cerebral cortical neurons were cultured essentially as described by Herts et al.[24] from 15-day-old mouse embryos. Whole-cell patch-clamp recordings were made from cerebral cortical neurons cultured for 7−9 days. Glass cover slips with the neurons were placed on the stage of an Olympus BX50WI microscope (Olympus, Japan), where the individual neurons were viewed at ×400. The neurons were perfused with artificial balanced salt solution (ABSS) at a rate of 2 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 7.35 at 22 °C. Standard patch-clamp techniques[25] 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 PP-830 electrode puller (Narishige, Japan) 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 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.[26] 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 **6c**,**g**,**h**,**k** and **7m**, inhibition of isoguvacine-induced currents was quantified by measuring the maximum currents recorded during application of drugs. For **6c**,**g**,**h**,**k** and **7m**, the inhibition was quantified using the response magnitude after 5 s of application. The equation $I = I_0/(1 + \text{antilog}([B] - \log IC_{50})n_H)$ was fitted to the experimental concentration response data. I is the current, I_0 is the current induced by 20 mM isoguvacine 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.

Molecular Modeling. All of the calculations were performed as previously described[12] using the MM3* force field as implemented in MacroModel v. 8.[27] Conformational searching was performed by using the Monte Carlo multiple minimum method (MCMM)[28] as implemented in MacroModel v. 8.

Conformational energy penalties for adopting the bioactive conformation were calculated by subtracting the internal energy of the global energy minimum conformation in water (GB/SA solvation model)[29] from the internal energy of the bioactive conformation identified by using the pharmacophore model, as previously described in detail.[11,12]

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Supporting Information Available: Elemental analyses. This information is available free of charge via the Internet at http://pubs.acs.org.

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