Glycine Antagonists. Synthesis, Structure, and Biological Effects of Some Bicyclic 5-Isoxazolol Zwitterions

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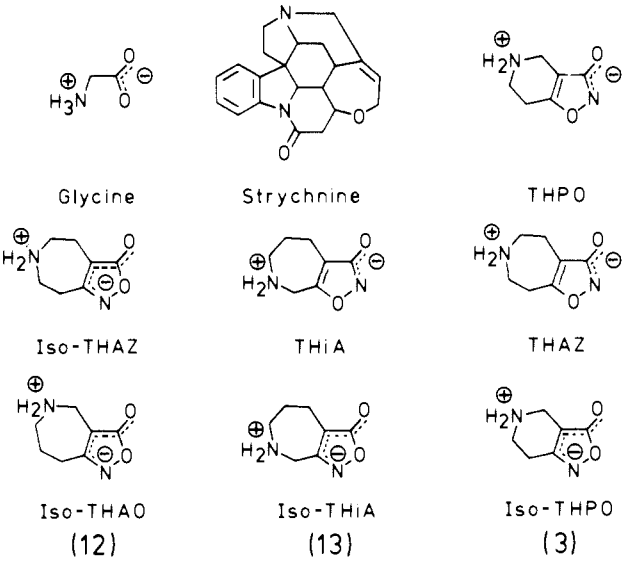
The bicyclic 5-isoxazolol zwitterions 4,5,6,7-tetrahydroisoxazolo[4,3-*c*]pyridin-3-ol (**3**, iso-THPO), 5,6,7,8-tetrahydro-4*H*-isoxazolo[4,3-*c*]azepin-3-ol (**12**, iso-THAO), and 5,6,7,8-tetrahydro-4*H*-isoxazolo[3,4-*c*]azepin-3-ol (**13**, iso-THIA), which are structurally related to the glycine antagonist 5,6,7,8-tetrahydro-4*H*-isoxazolo[3,4-*d*]azepin-3-ol (iso-THAZ), have been synthesized and tested biologically. All of these compounds were glycine antagonists approximately equipotent with iso-THAZ during microelectrophoretic ejection near cat spinal neurons. In contrast to iso-THAZ, which also interacts with 4-aminobutyric acid (GABA) receptors in rat brains, neither **12** nor **13** show any significant affinities for GABA binding or uptake mechanisms in vitro. The glycine antagonist **3** was, however, shown also to be a moderately potent inhibitor of GABA uptake. The structure of **12** was established by an X-ray analysis. The bond lengths of the 5-isoxazolol anionic moiety of **12** are in agreement with a pronounced delocalization of the negative charge of this compound.

A number of amino acids play a major role in the function of the mammalian central nervous system (CNS).[1] While 4-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the brain, glycine is the major inhibitory neurotransmitter in the spinal cord.[1-3]

Compared with GABA,[4-6] the development of the pharmacology of the glycine neurotransmitter system is at a very early stage. The alkaloid strychnine (Chart I), which is a selective and very potent antagonist at glycine receptors,[3,7] appears to interact directly or allosterically with glycine receptors.[8,9] There is a striking lack of similarity between the structures of glycine and strychnine (Chart I), and, accordingly, electrophysiological and binding studies using strychnine as a pharmacological tool and radioactive ligand, respectively, have not shed much light on the molecular pharmacology of the glycine receptors.

We recently discovered that certain heterocyclic zwitterionic compounds, including 5,6,7,8-tetrahydro-4*H*-isoxazolo[3,4-*d*]azepin-3-ol (iso-THAZ),[10] 5,6,7,8-tetrahydro-4*H*-isoxazolo[4,5-*d*]azepin-3-ol (THAZ),[11] and 5,6,7,8-tetrahydro-4*H*-isoxazolo[5,4-*c*]azepin-3-ol (THIA)[12] (Chart I), antagonize the inhibitory effect of glycine, and, to some extent, that of GABA when administered microelectrophoretically near cat spinal neurons.[13,14] Within this new class of glycine antagonists iso-THAZ is the most selective compound. We now report the synthesis and biological testing of the iso-THAZ analogues 5,6,7,8-tetrahydro-4*H*-isoxazolo[4,3-*c*]azepin-3-ol (**12**, iso-THAO), 5,6,7,8-tetrahydro-4*H*-isoxazolo[3,4-*c*]azepin-3-ol (**13**, iso-THIA), and 4,5,6,7-tetrahydroisoxazolo[4,3-*c*]pyridin-3-ol (**3**, iso-THPO). Since iso-THAZ also binds weakly to GABA receptors in the brain in vitro,[13,15] apparently as an antagonist,[15] and since 4,5,6,7-tetrahydroisoxazolo[4,5-*c*]-pyridin-3-ol (THPO), an isomer of **3** (iso-THPO) (Chart I), is an inhibitor of GABA uptake,[14,16] the affinities of **3**, **12**, and **13** for GABA receptor and uptake sites in vitro were also tested.

Chart I. Schematic Drawings of the Structures of Glycine, the Glycine Receptor-Ionophore Antagonist Strychnine, the GABA Uptake Inhibitor THPO, and a Number of 3- and 5-Isoxazolol Zwitterionic Glycine Antagonists



Chemistry. A mixture of the cyclic β-oxo esters **6** and **7** (Chart II) was obtained from **5** by ring expansion with ethyl diazoacetate. Attempts to separate **6** and **7** on a preparative scale using chromatographic procedures or via selective copper(II) chelate formation of **6**, in analogy with a procedure described for the separation of the *N*-methoxycarbonyl analogues of **6** and **7**,[17] were unsuccessful. Treatment of a mixture of **6** and **7** with hydroxylamine under basic conditions gave as the major products **8** and

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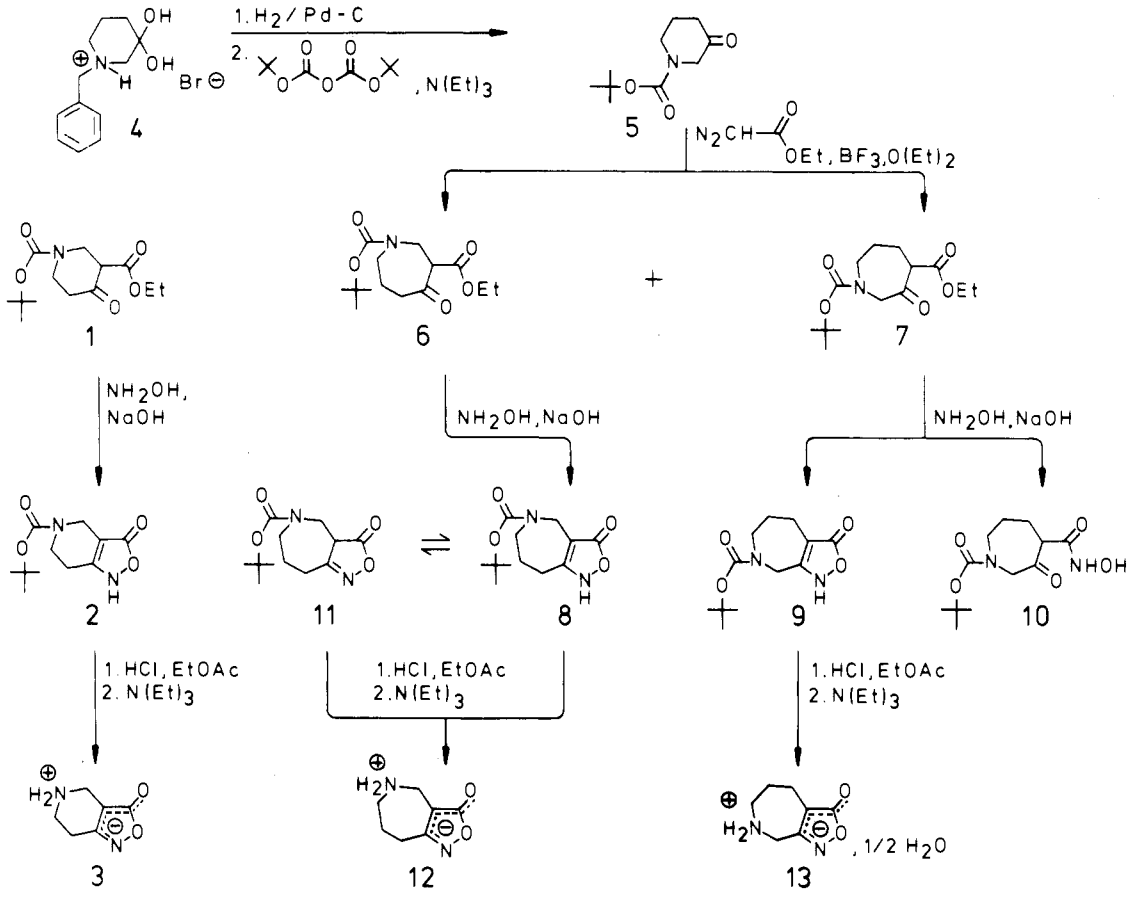
Chart II



9 and varying, but small amounts of the β-oxo hydroxamic acid **10**. During the column chromatographic (CC) separation procedures using silica gel a new compound was formed in varying quantities depending on the duration of the CC procedures. This latter compound, which could also be made by treatment of **8** with silica gel and the particular CC eluent, was shown to be the 2-isoxazolin-5-one tautomer (**11**) of **8**. This structure assignment is in agreement with the ^{1}H NMR and IR spectra of **8** and **11**. The IR spectra of **8** and **11** show carbonyl absorption bands at 1715 and 1785 cm^{-1}, respectively, for the 3-isoxazolin-5-one and 2-isoxazolin-5-one nuclei[18] of **8** and **11**, respectively. Deprotection of **8** as well as **11** gave **12** as the only product. Compound **9**, which is quite unstable, shows isoxazolin-5-one carbonyl absorption bands at 1700 and 1800 cm^{-1} and an increased number of signals in the ^{1}H NMR spectrum compared to the number of signals expected for **9**. Both observations suggest that **9** may exist as a tautomeric mixture of 2- and 3-isoxazolin-5-ones. Deprotection of **9** under conditions similar to those used for the conversion of **8** and **11** into **12** gave **13** as the only product. Attempts to crystallize **9** resulted in the formation of a new crystalline product ($C_{12}H_{18}N_2O_5$) of unknown structure. The structure of this compound is at present under investigation using X-ray diffraction methods.

Table I. pK_a Values and in Vitro Effects of Some Isoxazole Amino Acids

compd	pK_a values	inhibition of GABA binding:[a] IC_{50}, μM	inhibition of GABA uptake:[a] IC_{50}, μM
GABA	4.0, 10.7[b]	0.033[b]	2[b]
THPO	4.3, 9.1[b]	72[b]	160[b]
THAZ	4.8, 9.2[b]	15[c]	>300[d]
iso-THAZ	3.3, 9.6[e]	15[c]	>300[d]
3	3.0, 9.3	100	130
12	2.6, 10.4	>100	>300
13	2.5, 8.9	>100	>300

[a] See Experimental Section. [b] See ref 14. [c] See ref 15. [d] See ref 22. [e] See ref 10.

The β-oxo ester **1** was converted into the 5-isoxazolol zwitterion **3** under conditions similar to those used for the preparation of **12** and **13**. The IR spectral data of the intermediate **2** are in agreement with **2** existing predominantly in the 3-isoxazolin-5-one tautomeric form in the crystalline state ($\nu_{C=O}$ = 1725 cm^{-1}). The UV spectra of **2**, **8**, **9**, as well as **11** show strong absorption bands in the range of 257–261 nm (log ε 3.7–3.9), indicating that in methanol solution all of these compounds exist, to different extents, in the 3-isoxazolin-5-one tautomeric forms.[18]

The IR and UV spectroscopic data for **3**, **12**, and **13** are very similar and in agreement with the spectral data reported for other 5-isoxazolol zwitterions.[10,19,20] Spectro-

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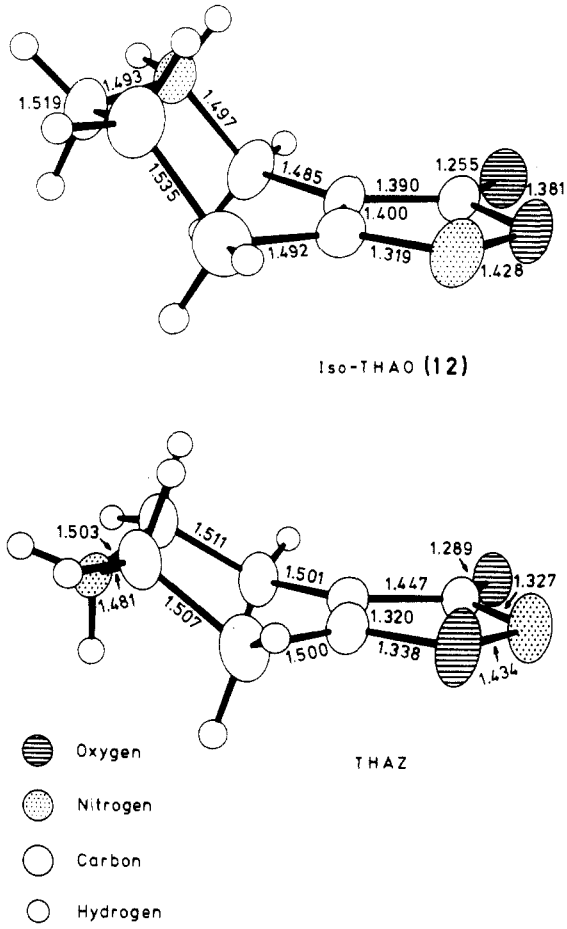

Figure 1. Perspective drawings[21] of the molecules of THAZ and 12 as determined by X-ray analyses. Bond lengths (angstroms) esd's 0.004–0.007 Å (THAZ)[23] and 0.002 Å (12). Thermal ellipsoids for non-hydrogen atoms are scaled to 50% probability; H atoms are represented as spheres of arbitrary radius. The drawing of THAZ is the mirror image of that shown in ref 23.

scopic data for 8–11 and the final products 3, 12, and 13 are given in the Experimental Section. pK_a values for 3, 12, and 13 are given in Table I. The low pK_a I values for iso-THAZ, 3, 12, and 13 as compared with those of the 3-isoxazolols THPO and THAZ are consistent with the high degree of delocalization of the negative charges of the former group of compounds.

In order to establish the molecular structure of 12, an X-ray analysis was carried out. A drawing of the molecule is shown in Figure 1 in which some distances are also indicated. The molecule has the expected zwitterionic structure. The bond lengths of the 5-isoxazolol anionic moiety are in agreement with a pronounced delocalization of the negative charge. The five-membered ring is planar within the limits of experimental error. The exocyclic oxygen atom O3 and the carbon atoms C8 and C4 are at distances ±0.03, ±0.04, and ±0.05 Å, respectively, from the least-squares plane through the five-membered ring. The seven-membered ring adopts a chair conformation. The packing of the molecules in the crystals is stabilized by hydrogen bonds.

Discussion and Results

Glycine is the major inhibitory neurotransmitter in the spinal cord,[1] and, hence, agents active at glycine receptors are of both pharmacological and therapeutic interest.

We have recently shown that THIA, THAZ, and iso-THAZ (Chart I) are capable of antagonizing the depressant effect of glycine on spinal neurons.[13,14] Within this new class of zwitterionic glycine antagonists iso-THAZ is the most potent compound. Iso-THAZ does, however, also appear to be a weak antagonist for GABA receptors in rat brains.[15] Because of this apparent lack of specificity of iso-THAZ as a glycine antagonist, its mechanism of interaction with the glycine receptors has not been studied in further detail.

The iso-THAZ analogues 12 and 13 were shown to be equipotent with or, perhaps, slightly less active than iso-THAZ as glycine antagonists (Figure 2). However, these compounds do not affect GABA receptor binding or uptake in vitro (Table I). In contrast, 3, which is the 5-isoxazolol isomer of the GABA uptake inhibitor THPO[14,16] (Chart I), is a moderately potent inhibitor of GABA uptake, and it marginally affects GABA binding in vitro (Table I) in

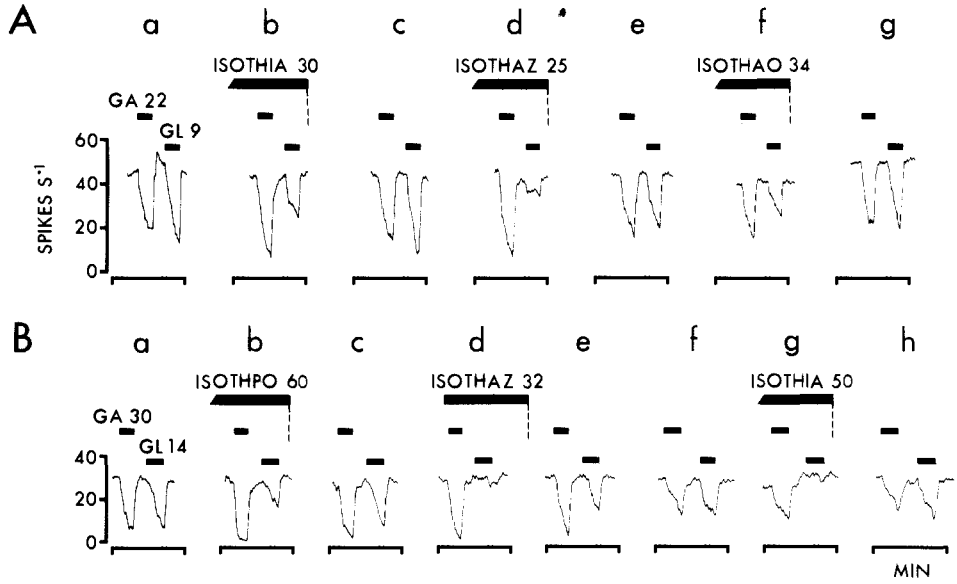

Figure 2. Effects of iso-THAZ, 3 (iso-THPO), 12 (iso-THAO), and 13 (iso-THIA) on the inhibition of firing of two cat spinal interneurons (A, a–g and B, a–h) by GABA (GA) and glycine (GL). A: a, before, b, during 13; c, 1 min after b; d, during iso-THAZ; e, 1 min after d; f, during 12; g, 1 min after f. B: a, before; b, during 3; c, 1 min after b; d, during iso-THAZ; e, 3 min after d; f, before; g, during 13; h, 2 min after g. Periods of microelectrophoretic ejection are indicated by horizontal bars and vertical lines. Currents are expressed in nanoamperes. Ordinates: firing rate, spikes per second. Abscissae: time, minutes.

addition to its glycine antaognist effect (Figure 2). These findings make **12** and **13** potentially useful tools for studies of glycine receptor mechanisms.

The bond lengths of the zwitterions THAZ[23] and **12** (Figure 1), as determined by X-ray analyses, are in agreement with the negative charge of the 3-isoxazolol moiety being much less delocalized, compared to the 5-isoxazolol moiety. In both crystal structures the exocyclic oxygen and isoxazole ring nitrogen atoms are acceptors for intermolecular hydrogen bonds. The hydrogen-bond systems of the respective crystal structures are, however, different as a result of the interchange of the oxygen and nitrogen atoms in the 3- compared to the 5-isoxazolol moiety. The differences in the degree of delocalization of the negative charges and the hydrogen-bonding characteristics of 3- and 5-isoxazolol moieties may to some extent explain the differences in potencies between the two classes of compounds.[13]

The position of the nitrogen atom of the six- or seven-membered ring in relation to the isoxazolol ring (charge separation) may contribute to the differences in potencies and/or selectivities between the compounds with respect to the glycine and GABA receptors. On the basis of X-ray structures of THAZ and **12** (Figure 1) or, in the case of iso-THAZ, **13** and **3**, measurements on Dreiding stereomodels, all in chair conformations, the approximate distances between the positively charged nitrogen atom and the exocyclic oxygen atom or the nitrogen atom of the isoxazole ring are 5.4 and 5.2 Å (THAZ), 5.4 and 4.5 Å (iso-THAZ), 5.5 and 3.7 Å (**13**), 4.3 and 4.3 Å (**12**), and 4.7 and 4.3 Å (**3**). Alteration of the chair to boat conformation causes only changes in the mentioned distances of THAZ (4.6 and 4.2 Å) and iso-THAZ (4.6 and 3.7 Å).

Further structural, neurochemical, and pharmacological studies on **12** and **13** may shed some light on the mechanism of action of these glycine antagonists. Hopefully, continued structure–activity studies in this field may eventually lead to the design and development of glycine agonists of therapeutic interest.

Experimental Section

Chemistry. General. Melting points are corrected and were determined in capillary tubes. Elemental analyses were performed by G. Cornali, Microanalytical Laboratory, Leo Pharmaceutical Products, Copenhagen, and by P. Hansen, Chemical Laboratory II, University of Copenhagen. IR spectra were recorded on a Perkin-Elmer grating infrared spectrophotometer (Model 247).

UV spectra were recorded in methanol on a Perkin-Elmer 402 spectrophotometer. The 60-MHz ^{1}H NMR spectra were recorded on a Varian 360L spectrometer. The 270-MHz ^{1}H NMR spectra were recorded on a Bruker HX 270 S instrument. A Fourier transform method was used to obtain the spectra with spectral widths of 3000 or 5000 Hz with digital resolution of 0.3 Hz. Me_4Si was used as an internal standard except for the compounds dissolved in D_2O, where, unless otherwise stated, 3-(trimethylsilyl)propanesulfonate was used. Thin-layer chromatography (TLC) and gravity column chromatography (CC) were performed with silica gel F_{254} plates (Merck) and silica gel (Woelm, 0.063–0.200 mm), respectively. The pK_a (H_2O, 25 °C) values were determined as described previously,[24,25] except that after adding 0.1 N HCl to pH ca. 2, the titration was carried out with 0.1 N NaOH to pH ca. 10. The ionic strength was kept constant with use of 0.15 M KCl.

***tert*-Butyl 1,3,4,5,6,7-Hexahydro-3-oxoisoxazolo[4,3-*c*]pyridine-5-carboxylate (2).** To a stirred solution of hydroxyammonium chloride (0.59 g, 8.5 mmol) in water (2 mL) was added sodium hydroxide (7 mL, 2 N) and the mixture was cooled to 0 °C. At this temperature a solution of **1**[26] (2.0 g, 7.4 mmol) in ethanol (5 mL) was added dropwise. The solution was left at 5 °C for 24 h and was then acidified (0 °C) with hydrochloric acid (4 N) to pH ca. 2.5. The precipitate was collected, washed with cold water, and dried to give **2** (0.69 g, 39%). An analytical sample was recrystallized (aqueous 2-propanol) to give pure **2**: mp 140–142 °C dec; IR (KBr) $\nu_{C=O}$ 1725 (s) cm^{-1}; UV λ_{max} 261 nm (log ϵ 3.91). Anal. ($C_{11}H_{16}N_2O_4$) C, H, N.

4,5,6,7-Tetrahydroisoxazolo[4,3-*c*]pyridin-3-ol Zwitterion (3). To a stirred solution of hydrogen chloride in ethyl acetate (6 mL, 3.9 M) was added at 25 °C a solution of **2** (0.50 g, 2.1 mmol) in ethyl acetate (2 mL). Stirring was continued for 4 h. The solution was evaporated in vacuo, and after drying the TLC-pure residue was dissolved in water (0.5 mL) and a solution of triethylamine (0.24 g, 2.4 mmol) in ethanol (8 mL) was added to pH ca. 7. The precipitate was collected, dried, and recrystallized (aqueous ethanol) to give **3** (0.22 g, 76%): mp 195 °C dec; IR (KBr) ν 3430 (br m), 3100–2200 (several bands), 1660 (s), 1620 (s), 1520 (s), 1495 (s), 1480 (s), 1460 (m-s), 1420 (m-s) cm^{-1}; UV λ_{max} 257 nm (log ϵ 3.94); ^{1}H NMR (60 MHz, D_2O, internal standard CH_3CN, δ 2.00) δ 3.85 (2 H, s, H_4), 3.41 (2 H, t, J = 6 Hz, H_6), 2.79 (2 H, t, J = 6 Hz, H_7); pK_a values 3.01 and 9.34. Anal. ($C_6H_8N_2O_2$) C, H, N.

1-(*tert*-Butyloxycarbonyl)-3-piperidone (5). A solution of **4**[17] (23.4 g, 81.2 mmol) in aqueous ethanol (200 mL, 50%) was hydrogenated (294 kPa) in a Parr hydrogenation apparatus using Pd–C (10%) as a catalyst (1.25 g). The reaction mixture was filtered and evaporated to dryness in vacuo. To an ice-cooled solution of the residue in water (40 mL) were added triethylamine (24.6 g, 244 mmol) and a solution of di-*tert*-butyl dicarbonate (21.3 g, 97.4 mmol) in tetrahydrofuran (40 mL), and the mixture was stirred at 0 °C for 2 h. After evaporation of the tetrahydrofuran under reduced pressure, chloroform (100 mL) was added. The mixture was cooled in an ice bath and acidified with acetic acid. The phases were separated, and the aqueous phase was extracted with chloroform (2 × 100 mL). The combined chloroform phases were washed with a saturated aqueous solution of sodium hydrogen carbonate (50 mL), dried, and evaporated in vacuo. The residue was distilled (104–105 °C, 60 Pa) to give **5** (13.1 g, 81%). Anal. ($C_{10}H_{17}NO_3$) C, H, N.

Ethyl (±)-1-(*tert*-Butyloxycarbonyl)-4-oxoperhydroazepine-3-carboxylate (6) and Ethyl (±)-1-(*tert*-Butyloxycarbonyl)-3-oxoperhydroazepine-4-carboxylate (7). To a stirred solution of **5** (13.1 g, 65.9 mmol) in dry ether (150 mL) maintained at −70 to −60 °C were added simultaneously and drop by drop freshly distilled boron trifluoride etherate (10.3 g, 72.5 mmol) and ethyl diazoacetate (9.8 g, 85.7 mmol), both of which were dissolved in dry ether (25 mL). The addition of the reagents took 10–15 min, respectively. Stirring was continued at the same temperature for an additional 30 min and subsequently until the temperature of the reaction mixture reached 20 °C. The reaction mixture was stirred with an aqueous solution of potassium carbonate (50 mL, 30%) and the ether phase was separated. The aqueous phase was extracted with ether (2 × 75 mL). The combined ether phases were dried and evaporated in vacuo. The residue was distilled (147–148 °C, 60 Pa) to give an oil, which was shown to consist of a mixture of **6** and **7** (12.9 g, 69%). TLC: R_f 0.47 and 0.50 [eluent, toluene–ethyl acetate (4:1)]. Anal. ($C_{14}H_{23}NO_5$) C, H, N.

***tert*-Butyl 1,3,5,6,7,8-Hexahydro-3-oxo-4*H*-isoxazolo[4,3-*c*]azepine-5-carboxylate (8), *tert*-Butyl 1,3,5,6,7,8-Hexahydro-3-oxo-4*H*-isoxazolo[3,4-*c*]azepine-7-carboxylate (9), and 1-(*tert*-Butyloxycarbonyl)-3-oxoperhydroazepine-4-hydroxamic Acid (10).** To a stirred solution of hydroxyammonium chloride (1.6 g, 23.0 mmol) and sodium hydroxide (1.7 g, 43 mmol) in water (14 mL) was added dropwise at 0 °C a solution of the mixture of **6** and **7** (5.71 g, 20.0 mmol) in ethanol

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(14 mL). Stirring was continued at 0 °C for 90 min. After evaporation of the ethanol under reduced pressure, chloroform (40 mL) was added. The mixture was cooled in an ice bath and acidified with acetic acid (ca. 5 mL). The phases were separated, and the aqueous phase was extracted with chloroform (2 × 40 mL). The combined and dried chloroform phases were evaporated under reduced pressure, and the residue was subjected to CC [silica gel, 250 g; eluents, toluene containing ethyl acetate (33–50%) and acetic acid (2%)] to give crude products 8 (2.70 g, 53%) and 9 (1.93 g, 38%). Compound 10 was isolated in small and varying amounts in different experiments.

Data for Compound 8. An analytical sample was recrystallized (ethyl acetate–light petroleum): mp 94–97 °C dec; TLC R_f 0.32 [eluent, ethyl acetate–toluene (2:1) and 2% acetic acid]; IR (KBr) $\nu_{C=O}$ 1795 (w), 1715 (s) cm^{-1}; UV λ_{max} 257 nm (log ϵ 3.86); ^{1}H NMR (270 MHz, $CDCl_3$) δ 5.05 (1 H, s, NH), 4.35 (1 H, d, $J_{H_{4a}H_{4e}} = -15$ Hz, H_{4e}), 4.17 (1 H, dd, $J_{H_{6a}H_{6e}} = -14$ Hz, $J_{H_{6e}H_7} = 5$ Hz, H_{6e}), 2.45–3.60 (4 H, m, H_{4a},H_{6a}, H_{8a},H_{8e}), 1.8 (2 H, m, H_{7a},H_{7e}), 1.41 (s) and 1.43 (s) (a total of 9 H, *t*-Bu). Anal. ($C_{12}H_{18}N_2O_4$) C, H, N.

Data for the 2- and 3-Isoxazolin-5-one Tautomers of 9. TLC-pure oil R_f 0.50 [eluent, ethyl acetate–toluene (2:1) and 2% acetic acid]; IR (film) $\nu_{C=O}$ 1800 (w), 1700 (s) cm^{-1}; UV λ_{max} 260 nm (log ϵ 3.76): ^{1}H NMR (270 MHz, $CDCl_3$): 2-isoxazolin-5-one, δ 5.1 (1 H, d, $J_{H_{8a}H_{8e}} = -17$ Hz, H_{8e}), 4.0 (1 H, d, $J_{H_{8a}H_{8e}} = -17$ Hz, H_{8a}), 4.1 (1 H, d, $J_{H_{6a}H_{6e}} = -14$ Hz, H_{6e}), 3.59 (1 H, b, H_{4e}), 3.33 (1 H, dd, $J_{H_{4a}C_{3a}H} = 10$ Hz, $J_{H_{4e}C_{3a}H} = 3$ Hz, C_{3a}H), 2.79 (1 H, dd, $J_{H_{6a}H_{6e}} = -14$ Hz, $J_{H_{6a}H_{5a}} = 10$ Hz, H_{6a}), 2.35–2.45 (1 H, m, H_{4a}), 1.60–1.85 (2 H, m, H_{5a},H_{5e}), 1.43 and 1.45 (a total of 9 H, *t*-Bu); 3-isoxazolin-5-one, δ 6.44 (1 H, s, NH), 4.98 (1 H, d, $J_{H_{8a}H_{8e}} = -17$ Hz, H_{8e}), 4.28 (1 H, m, H_{6e}), 4.05 (1 H, d, $J_{H_{8a}H_{8e}} = -17$ Hz, H_{8a}), 3.97 (tentative assignment) (1 H, m, H_{4e}), 2.44 (1 H, m, H_{6a}), 2.40 (tentative assignment) (1 H, m, H_{4a}), 1.60–1.85 (2 H, m, H_{5a},H_{5e}), 1.45 and 1.43 (a total of 9 H, *t*-Bu).

Data for Compound 10. Recrystallization (ethanol) mp 145 °C dec; R_f 0.33 [eluent, ethyl acetate–toluene (2:1) and 2% acetic acid]; IR (KBr) $\nu_{C=O}$ 1685 (s), 1670 (s) cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 6.0 (1 H, b, NH), 4.07 (1 H, d, $J_{H_{2a}H_{2e}} = -15$ Hz, H_{2e}), 3.95 (1 H, m, $J_{H_{7a}H_{7e}} = -14$ Hz, H_{7e}), 3.27 (1 H, d, $J_{H_{2a}H_{2e}} = -15$ Hz, H_{2a}), 2.96 (1 H, m, $J_{H_{7a}H_{7e}} = -14$ Hz, H_{7a}), 2.79 (1 H, m, H_4), 2.0–2.3 (4 H, m, H_5,H_6) 1.44 (9 H, s, *t*-Bu). Anal. ($C_{12}H_{20}N_2O_5$) C, H, N.

***tert*-Butyl 3,3a,4,6,7,8-Hexahydro-3-oxo-4*H*-isoxazolo[4,3-*c*]azepine-5-carboxylate (11).** To a solution of 8 (0.59 g, 2.3 mmol) in toluene (5 mL), ethyl acetate (5 mL), and acetic acid (2%) was added silica gel (Woelm, 0.063–2.00 mm, 2 g). The reaction mixture was heated (70 °C) for 4.5 h. The reaction mixture was filtered and evaporated in vacuo. The residue was subjected to CC [silica gel, 40 g; eluent, toluene–ethyl acetate–acetic acid (25:25:1)] and recrystallized (ethyl acetate–light petroleum) to give 11 (0.33 g, 56%): mp 150 °C dec; R_f 0.23 [eluent, ethyl acetate–toluene (2:1) and 2% acetic acid]; IR (KBr) $\nu_{C=O}$ 1785 (s), 1690 (br s); UV λ_{max} 261 nm (log ϵ 3.73); ^{1}H NMR (270 MHz, $CDCl_3$) δ 4.86 (1 H, t, $J_{H_4C_{3a}H} = 7$ Hz, C_{3a}H), 4.35 (1 H, d, $J_{H_{4a}H_{4e}} = -15$ Hz, H_{4e}), 4.17 (1 H, dd, $J_{H_{6a}H_{6e}} = -15$ Hz, $J_{H_7H_{6e}} = 5$ Hz, H_{6e}), 2.49–3.17 (4 H, m, H_{4a},H_{6a},H_{8a},H_{8e}), 1.8 (2 H, m, H_7), 1.42 (s) and 1.45 (s) (a total of 9 H, *t*-Bu). Anal. ($C_{12}H_{18}N_2O_4$) C, H, N.

5,6,7,8-Tetrahydro-4*H*-isoxazolo[4,3-*c*]azepin-3-ol Zwitterion (12). Compound 12 was synthesized as described above for 3 with use of 8 (0.76 g, 3.0 mmol) in ethyl acetate (2 mL) and hydrogen chloride in ethyl acetate (5 mL, 2.5 M). To a solution of the residue in water (3.5 mL) was added ethanol (1 mL). A solution of triethylamine (0.30 g, 3.0 mmol) in ethanol (2 mL) was added to pH ca. 7. The precipitate was collected, washed with aqueous ethanol (90%), and dried to give 12 (0.37 g, 80%): recrystallized (aqueous ethanol) mp 202 °C dec; IR (KBr) ν 3430 (br m), 3100–2000 (several bands, m-s), 1650 (s), 1605 (w), 1515 (s), 1490 (m), 1475 (m), 1460 (m-s), 1435 (m), 1425 (m); UV λ_{max} 248 nm (log ϵ 3.90); ^{1}H NMR (270 MHz, D_2O) δ 4.86 (2 H, s, H_4), 4.39 (2 H, m, H_6), 3.67 (2 H, m, H_8), 2.96 (2 H, m, H_7); pK_a values 2.64 and 10.38. Anal. ($C_7H_{10}N_2O_2$) C, H, N.

From 11. Compound 12 was synthesized from 11 (0.10 g, 0.4 mmol) as described above. Recrystallization (aqueous ethanol) gave 12 (34 mg, 33%). The IR spectrum was identical with that of 12 synthesized from 8.

5,6,7,8-Tetrahydro-4*H*-isoxazolo[3,4-*c*]azepin-3-ol Zwitterion Hemihydrate (13). Compound 13 was synthesized as described above for 3 with use of 9 (0.69 g, 2.7 mmol), ethyl acetate (2 mL), and hydrogen chloride in ethyl acetate (4 mL, 2.5 M). To a solution of the residue in water (3 mL) was added ethanol (1 mL). A solution of triethylamine (0.27 g, 2.7 mmol) in ethanol (2 mL) was added to pH ca. 7. The precipitate was collected, washed with aqueous ethanol (90%), and dried to give 13 (170 mg, 41%). An analytical sample was recrystallized (aqueous ethanol): mp 176 °C dec; IR (KBr) ν 3425 (br m), 3100–2300 (several bands, m-s), 1630 (s) 1620 (s), 1610 (s), 1520 (s), 1470 (m), 1440 (m), 1430 (m), 1410 (m) cm^{-1}; UV λ_{max} 262 nm (log ϵ 3.87); ^{1}H NMR (270 MHz, D_2O) δ 5.11 (2 H, s, H_8), 4.45 (2 H, m, H_6), 3.36 (2 H, m, H_4), 2.87 (2 H, m, H_5); pK_a values 2.51 and 8.87. Anal. ($C_7H_{10}N_2O_2 \cdot {}^1/_2H_2O$) C, H, N.

X-ray Crystallographic Analysis of 5,6,7,8-Tetrahydro-4*H*-isoxazolo[4,3-*c*]azepin-3-ol Zwitterion (12, iso-THAO). Crystal data are as follows: $C_7H_{10}N_2O_2$, M = 154.17; monoclinic, space group $P2_1/n$, a = 7.022 (4) Å, b = 14.594 (7) Å, c = 7.567 (4) Å, β = 109.07 (4)°, V = 733(1) Å^3, D_{calcd} = 1.397 (2) Mg m^{-3}, Z = 4, μ(Mo Kα) = 0.97 cm^{-1}, F(000) = 328.

Colorless prismatic needle shaped crystals were grown at 5 °C from an aqueous ethanol (50%) solution of the compound.

A single crystal of the size 0.30 × 0.35 × 0.50 mm was used for the determination of the unit cell parameters and for the collection of intensity data on a Picker FACS-1 diffractometer using graphite monochromated Mo Kα radiation (λ = 0.710 69 Å). The general techniques employed are as previously described.[27] Intensities were measured for 4066 reflections, h, k, $\pm l$ with $5.6 < 2\theta < 63.0°$ and $-h,k,\pm l$ with $5.6 < 2\theta < 45.0°$. The equivalent reflections were averaged with a merging R = 0.019. Of the 2445 unique reflections so obtained, 1793 had net intensities greater than 3.0 $\sigma(I)$, where $\sigma(I)$ is the estimated standard deviation of an intensity as calculated from counting statistics. These were regarded as observed reflections and used in the refinement procedure. No absorption corrections were made.

The structure was solved by direct methods and refined by full-matrix least-squares methods. The quantity minimized was $\Sigma w(|F_o| - k|F_c|)^2$, where the weights were initially taken as unity. The position of the hydrogen atoms were obtained from a difference map.

In subsequent full-matrix least-squares calculations an overall scale factor, atomic coordinates for all atoms, and anisotropic thermal parameters for the non-hydrogen atoms were refined. The thermal parameters for the hydrogen atoms were fixed at isotropic values. The weights used in the final cycles of refinement were given by $w = x \cdot y$, $x = 1$ for $\sin\theta \geq 0.25$, else $x = \sin\theta/0.25$, $y = 1$ for $F_o \leq 6.0$, else $y = 6.0/F_o$. The final R and R_w values for the observed reflections are 0.041 and 0.051, respectively. The extreme fluctuations of the electron density on the final difference Fourier map were in the range −0.23 to 0.25 e Å^{-3}.

Table II lists the final positional and equivalent isotropic thermal parameters. Lists of structure factors and anisotropic thermal parameters of the non-hydrogen atoms are available as supplementary material.

Calculations were carried out with use of the MULTAN program[28] and the X-RAY 76 program system.[29]

Microelectrophoretic Studies. Experiments were performed on lumbar dorsal horn interneurons and Renshaw cells of cats anesthetized with pentobarbitone sodium (35 mg/kg intraperitoneally initially, supplemented intravenously when required). Extracellular action potentials were recorded by means of the center barrel of seven-barrel micropipets, which contained 3.6 M NaCl. The compounds were administered electrophoretically from the outer barrels of the micropipets,[30] which contained aqueous

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Table II. Atomic Positions and Equivalent Isotropic/Isotropic Temperature Factors ($\times 10^2$ Å^2) for 12[a]

atom	x	y	z	U_{eq}/U_{iso}[b]
N1	−0.3009 (2)	0.6152 (1)	0.6922 (2)	4.10
O2	−0.3509 (1)	0.59245 (7)	0.4988 (2)	3.98
C3	−0.1805 (2)	0.60182 (9)	0.4472 (2)	3.01
C3a	−0.0260 (2)	0.63206 (8)	0.6036 (2)	2.73
C4	0.1855 (2)	0.6483 (1)	0.6119 (2)	3.69
N5	0.3329 (1)	0.59571 (9)	0.7668 (2)	3.16
C6	0.3628 (2)	0.6320 (1)	0.9584 (2)	3.83
C7	0.1924 (2)	0.6108 (1)	1.0344 (2)	4.20
C8	−0.0032 (2)	0.6649 (1)	0.9466 (2)	3.88
C8a	−0.1090 (2)	0.6386 (8)	0.7481 (2)	2.94
O3	−0.1938 (2)	0.5825 (8)	0.2821 (1)	4.21
H41	0.228 (2)	0.713 (1)	0.631 (2)	3.9
H42	0.200 (3)	0.627 (1)	0.494 (3)	3.9
H51	0.292 (2)	0.538 (1)	0.759 (2)	3.3
H52	0.452 (3)	0.597 (1)	0.745 (2)	3.3
H61	0.381 (2)	0.700 (1)	0.950 (2)	3.7
H62	0.487 (3)	0.604 (1)	1.039 (2)	3.7
H71	0.162 (3)	0.544 (1)	1.025 (2)	4.4
H72	0.246 (3)	0.627 (1)	1.163 (3)	4.4
H81	0.030 (3)	0.730 (1)	0.950 (2)	4.2
H82	−0.095 (3)	0.655 (1)	1.015 (2)	4.2

[a] Estimated standard deviations are given in parentheses. [b] For the non-hydrogen atoms, $U_{eq} = {}^1/_3(U_{11} + U_{22} + U_{33} + 2U_{13} \cos \beta)$.

solutions of the following compounds: GABA (GA, 0.2 M, pH 3), glycine (GL, 0.5 M, pH 3), iso-THAZ (0.1 M, pH 3), **3** (iso-THPO, 0.1 M, pH 3), **12** (iso-THAO, 0.1 M, pH 3.5), and **13** (iso-THIA, 0.1 M, pH 3.5). Cell firing rates were maintained by the continuous microelectrophoretic ejection of (*RS*)-homocysteate (0.2 M, pH 7.5, -12 nA). GABA and glycine were administered for times and with currents sufficient to obtain approximate equal and submaximal inhibition of firing. Antagonism by the other compounds was apparent from a slower onset and reduced degree of inhibition.

GABA Receptor Binding. [^{3}H]GABA binding was studied with use of rat brain synaptic membranes prepared as previously described.[31] Aliquots of synaptic membranes (0.8–1.2 mg of protein) were incubated in triplicate in 2 mL of 50 mM Tris-citrate (pH 7.1) containing 5 nM [^{3}H]GABA. Different concentrations of compounds were added. The samples were incubated for 15 min at 2 °C followed by centrifugation at 48000*g* for 10 min. The pellets were rinsed twice with 5-mL portions of cold water and suspended in water (0.5 mL). The radioactivity was measured by conventional scintillation counting methods. Nonspecific binding in the presence of 1 mM GABA was always subtracted. The IC_{50} values for the compounds tested were estimated by measuring the inhibition of at least four different concentrations of the respective compounds and performing log probit analyses of the results.

Inhibition of Neuronal (Synaptosomal) GABA Uptake. The crude synaptosomal preparation was prepared from rat brains as previously described.[32] The whole brains were homogenized in 10 volumes of ice-cold 0.32 M sucrose and the homogenate centrifuged at 600*g* at 4 °C for 10 min. The pellet was discarded and the supernatant 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. Five hundred microliters of the synaptosome suspension was preincubated for 10 min at 25 °C with 1.9 mL of phosphate medium containing the inhibitor. Then [^{3}H]GABA was added to give a final GABA concentration of 50 nM, and the incubation was continued for a further 10 min. The synaptosomes were isolated by rapid filtration through Whatman GF/C glass fiber filters, and the filters were washed with ice-cold phosphate medium (10 mL). The filters were transferred to scintillation vials, and the radioactivity was measured by conventional scintillation counting methods.

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Registry No. 1, 98977-34-5; **2**, 98977-35-6; **3**, 72551-83-8; **4**, 61995-16-2; **5**, 98977-36-7; **6**, 98977-37-8; **7**, 98977-38-9; **8**, 98977-39-0; **9**, 98977-40-3; **9** (3-isoxazolin-5-one tautomer), 98977-45-8; **10**, 98977-41-4; **11**, 98977-42-5; **12**, 98977-43-6; **13**, 98977-44-7.

Supplementary Material Available: Lists of structure factors and anisotropic thermal parameters of the non-hydrogen atoms (10 pages). Ordering information is given on any current masthead page.

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