3-Hydroxyisoxazole Bioisosteres of GABA. Synthesis of a Series of 4-Substituted Muscimol Analogues and Identification of a Bicyclic 2-Isoxazoline Rearrangement Product

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3-Hydroxy-4-(2-hydroxyethyl)-5-methylisoxazole (**1**) was used as the starting material for the syntheses of the muscimol (5-aminomethyl-3-hydroxyisoxazole) analogues **9**, **10** and **14**, containing 2-acetoxyethyl, 2-hydroxyethyl, and 2-chloroethyl substituents, respectively, in the 4-position of the ring. These analogues were synthesized via bromination of the 5-methyl groups of the di-*O*-protected derivative of **1** (**5**) followed by a Gabriel phthalimide reaction. *N*-Deprotection of 4-(2-chloroethyl)-3-methoxy-5-phthalimidomethylisoxazole (**12**), an intermediate for the preparation of **14**, followed by cyclization and *O*-deprotection form the last steps of a new synthesis of the clinically active bicyclic muscimol analogue, (4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol) (THIP) (**16**). Whereas treatment of 4-(2-hydroxyethyl)-3-methoxy-5-methylisoxazole (**3a**) with bromine or with NBS gave the bicyclic 2-isoxazoline **17a**. A similar treatment of the 4-(3-hydroxypropyl) homologue (**3b**) of **3a** did not provide the 6-membered ring analogue of **17a** (**17b**). Whilst muscimol and THIP are very potent agonists at $GABA_A$ receptors, none of the muscimol analogues **9**, **10** or **14** showed significant affinity for $GABA_A$ receptor sites *in vitro*.

Reduced activity of the central 4-aminobutanoic acid (GABA) neurotransmitter system, probably reflecting degeneration of GABA neurones, is associated with certain neurological disorders such as Huntington's chorea,[1] epilepsy,[2,3] and tardive dyskinesia.[4,5] Although the specific $GABA_A$ agonists muscimol[6] and 4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol (THIP)[7] (Fig. 1) show limited clinical effects in these diseases,[8–10] $GABA_A$-receptor-stimulating drugs may have clinical interest. The lack of marked clinical effects of muscimol or THIP may reflect rapid desensitization of the target $GABA_A$ receptors[11] as a result of prolonged activation by these $GABA_A$ agonists showing full or almost full, respectively, $GABA_A$ agonist efficacy.[12–15] If this is the case, low-efficacy $GABA_A$ agonists

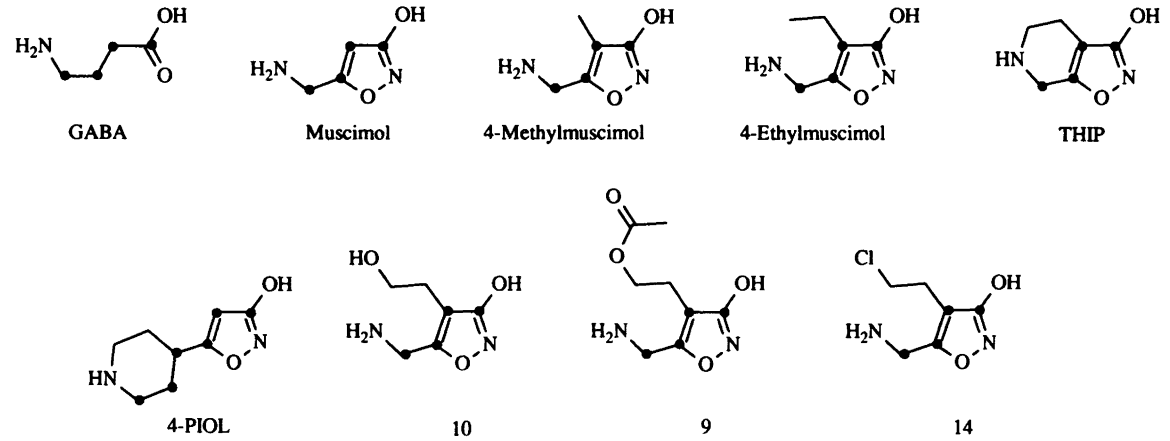

Fig. 1.

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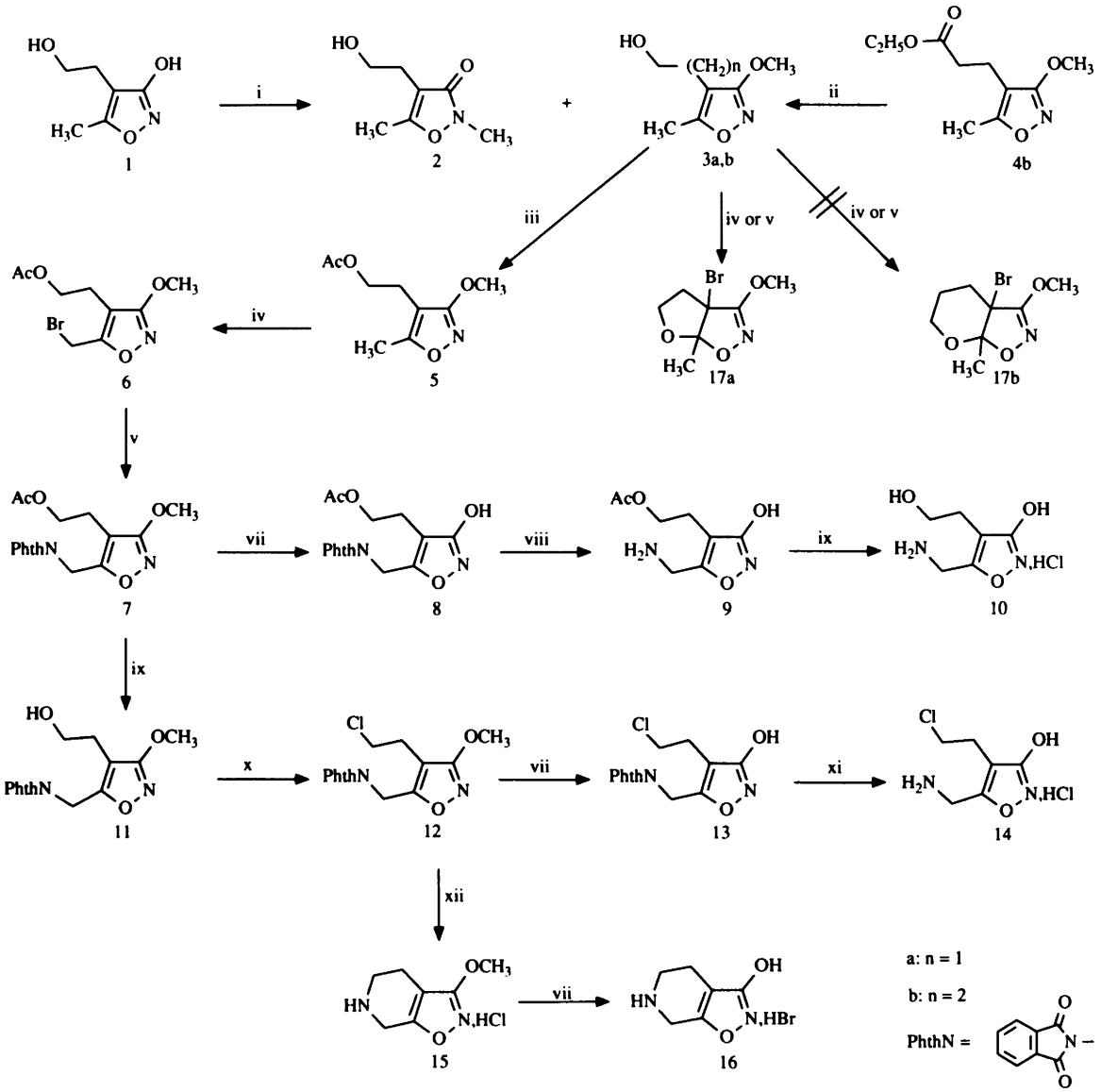
such as the non-fused THIP analogue 3-hydroxy-5-(4-piperidyl)isoxazole (4-PIOL)[16–19] (Fig. 1) may have therapeutic interest.

Unfortunately, 4-PIOL, which does not cause significant receptor desensitization,[19] is unable to cross the blood–brain barrier (BBB) making behavioural animal studies on this novel $GABA_A$ agonist very difficult.[18,20] THIP and muscimol, on the other hand, are capable of penetrating the BBB,[21,22] and since THIP shows reduced $GABA_A$ agonist efficacy as compared with muscimol in *in vitro* test systems,[14,15] we decided to synthesize the muscimol analogues **9**, **10** and **14** containing alkyl groups in the 4-position of the ring, equivalent to the position of the ethylene ring residue of THIP. Although 4-methylmuscimol is only weak and 4-ethylmuscimol virtually inactive as $GABA_A$ agonists,[23,24] we wished to investigate the influence of substituents of different size and polarity in the 4-alkyl groups (Fig. 1) on the $GABA_A$ receptor affinity of the compounds.

Results and discussion

3-Hydroxy-4-(2-hydroxyethyl)-5-methylisoxazole (**1**)[25] was methylated using diazomethane, and the *O*-methylated product **3a**, separated from the isomeric *N*-methylated compound **2**, was acetylated to give **5** (Scheme 1). Regioselective bromination of **5** and treatment of the bromomethyl product **6** with potassium phthalimide gave **7**, which was deprotected stepwise to give the desired muscimol analogues **9** and **10**.

Compound **12**, which was synthesized in two steps from **7**, was converted into the desired compound **14** in two steps using acid deprotection reagents. Cleavage of the phthal-



Scheme 1. i, CH_2N_2; ii, $NaBH_4$; iii, $(CH_3CO)_2O$–H_3PO_4; iv, Br_2; v, NBS; vi, potassium phthalimide; vii, HBr–CH_3COOH; viii, N_2H_4; ix, CH_3OH–HCl; x, $SOCl_2$; xi, 6 M HCl; xii, N_2H_4. HCl.

imide group of **12** by treatment with hydrazine resulted in spontaneous cyclization to 3-methoxy-4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridine (*O*-methyl-THIP, **15**). *O*-Demethylation of **15** gave THIP hydrobromide (**16**). These reactions represent an alternative synthesis of the $GABA_A$ receptor agonist, THIP.[26–28] The prospects of the new synthesis in terms of large scale preparation of THIP are under investigation.

In order to synthesize the key intermediate **7** in better yields, attempts to convert **3a** into the corresponding 5-bromomethyl analogue by treatment with *N*-bromosuccinimide (NBS) or with bromine unexpectedly resulted in cyclization of **3a** into **17a**. The mechanism(s) underlying this cyclization reaction are unknown. In order to elucidate the scope of this cyclization reaction, the homologue **3b** of **3a** was treated with NBS or with bromine under similar conditions. These reactions did, however, lead to very complex reaction mixtures, in/from which **17b** could not be detected/isolated.

All new compounds gave correct analyses and consistent IR and ^1H NMR spectra. The structure assigned to **17a** is supported by its IR spectrum in which OH stretching vibrations as well as the absorption bands at 1660 and 1530 cm^{-1}, characteristic of 3-oxygenated 4,5-disubstituted isoxazoles,[29] are absent. In the mass spectrum, peaks at *m/z* 235 and 237 are consistent with the formula $C_7H_{10}BrNO_3$ for **17a**. The pattern of resonance signals in the 250 MHz ^1H NMR spectrum of **17a** indicated that the ethylene chain is incorporated into a ring structure. The two quartets at δ 23 (C-6a–CH_3) and at δ 58 (O–CH_3) in the 62.9 MHz ^{13}C NMR spectrum support the proposed structure of **17a** (Fig. 2).

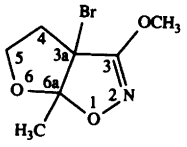


Fig. 2.

The new muscimol analogues **9**, **10** and **14** were tested *in vitro* as potential inhibitors of ^3H-GABA binding. Whereas muscimol (IC_{50} = 0.006 μM) and THIP (IC_{50} = 0.1 μM) are potent inhibitors[30] in this assay for $GABA_A$ receptor affinity, none of the compounds **9**, **10** and **14** showed significant inhibitory effect when tested at concentrations of 100 μM. Steric effects of the C-4 substituents may impede binding of these muscimol analogues to $GABA_A$ receptor sites. Alternatively, or in addition, these substituents may prevent the aminomethyl groups of these compounds from adopting receptor-active conformations similar to that reflected by the conformationally restricted muscimol analogue THIP (Fig. 1).

Experimental

Routine analytical procedures have been described in a previous paper.[31] The 250 MHz ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker AM-250 spectrometer. GC–MS analyses were carried out on a Finnigan 9611 gas chromatograph using a fused silica J&W, DB-5 column (30×0.25 mm, film 0.25 μm) coupled to a Finnigan MAT 4515B mass spectrometer in the EI mode. Thin layer and column chromatography (CC) were accomplished using silica gel GF_{254} plates (Merck) and silica gel, 0.05–0.20 mm (Merck), respectively. Flash chromatography was performed on silica gel 60 H (Merck). HPLC analyses were carried out on a Shimadzu LC-6A Liquid Chromatograph system [Column: 15.0 cm×4.6 mm ID Supelcosil LC-18-DB (5 μ); mobile phase: methanol–water (50 : 50); flow rate: 1.0 ml min^{-1}; room temperature; pressure: 1150 psi; detection: UV (254 nm)] combined with a C-R6A Chromatopac data processor. All evaporations were performed at ca. 15 mmHg using a rotary evaporator.

4-(2-Hydroxyethyl)-2,5-dimethylisoxazol-3(2H)-one (**2**) *and 4-(2-hydroxyethyl)-3-methoxy-5-methylisoxazole* (**3a**). Diazomethane (ca. 2.4 g, ca. 58 mmol), prepared from *N*-methyl-*N*-nitroso-4-toluenesulfonamide (15.5 g; 67.5 mmol), was added with stirring at room temperature to a mixture of **1**[25] (7.93 g, 55 mmol) and ether (150 ml). After an additional 2 h of stirring, the excess of diazomethane was destroyed by addition of a small excess of acetic acid. The reaction mixture was evaporated to dryness and the residue flash-chromatographed [eluents: ethyl acetate containing methanol (5–25 %)]. Yields: **2**, 4.01 g (46 %) and **3a**, 3.63 g (42 %) as colourless oils.

An analytical sample of crude **2** was distilled in a Kugelrohr apparatus at 190 °C/2 mmHg to give analytically pure **2**. Anal. $C_7H_{11}NO_3$: C, H, N. IR (film): 3600–3100 (several bands, s), 1640 (s), 1425 (m) cm^{-1}. ^1H NMR (60 MHz, $CDCl_3$): δ 3.9 (3 H, s), 3.7 (2 H, t, *J* 6 Hz), 2.7 (1 H, s), 2.5 (2 H, t, *J* 6 Hz), 2.3 (3 H, s).

A sample of crude **3a** was distilled in a Kugelrohr apparatus at 150 °C/1 mmHg to give analytically pure **3a**. Anal. $C_7H_{11}NO_3$: H, N. Found, C 52.29. Calc., C 53.49. IR (film): 3600–3100 (several bands, s), 3000–2750 (several bands, m), 1650 (m), 1520 (s), 1470 (s), 1410 (s) cm^{-1}. ^1H NMR (60 MHz, DMSO-d_6): δ 3.84 (1 H, s), 3.53 (2 H, t, *J* 7 Hz), 3.40 (3 H, s), 2.35 (2 H, t, *J* 7 Hz), 2.23 (3 H, s).

4-(3-Hydroxypropyl)-3-methoxy-5-methylisoxazole (**3b**). $NaBH_4$ (624 mg, 16.5 mmol) was added to a solution of **4**[31] (1.07 g, 5 mmol) in THF (15 ml), and the mixture was heated to 50 °C. Over a period of 30 min, MeOH (3.5 ml) was added with stirring, and heating and stirring were continued for a further 2 h. $NaBH_4$ (150 mg, 4 mmol) was added, and heating and stirring were continued for 1 h. Water (25 ml) was added to the cooled (room temperature) mixture which was then concentrated to ca. 15 ml. The mixture was extracted with ethyl acetate (3×30 ml). The

pooled extracts were dried ($MgSO_4$), filtered and evaporated to dryness to give pure **3b**. Yield 790 mg (92 %) as a colourless oil. Anal. $C_8H_{13}NO_3$: C, H, N. IR (film): 3700–3100 (s), 3000–2800 (several bands, s), 1650 (s), 1525 (s), 1470 (s), 1410 (s) cm^{-1}. ^1H NMR (60 MHz, CCl_4): δ 3.92 (3 H, s), 3.50 (2 H, t, *J* 7 Hz), 2.56 (1 H, s), 2.30 (2 H, t, *J* 7 Hz), 2.23 (3 H, s), 1.9–1.3 (2 H, m).

4-(2-Acetoxyethyl)-3-methoxy-5-methylisoxazole (**5**). Phosphoric acid (85 %, 5 drops) was added to a mixture of **3a** (3.14 g, 20 mmol) in acetic anhydride (6.0 ml). The solution was kept at 80 °C for 15 min then cooled to room temperature. Water (1 ml) was added carefully with stirring to avoid a vigorous reaction. More water (10 ml) was added with stirring, and the ice-cooled solution was extracted with dichloromethane (3×10 ml). The pooled extracts were dried ($MgSO_4$), filtered and evaporated to dryness. The crude product was distilled in a Kugelrohr apparatus at 140 °C/10^{-5} mmHg to give 3.20 g (80 %) of **5** as a colourless oil. Anal. $C_9H_{13}NO_4$: C, H, N. IR (film): 3100–2800 (several bands, m), 1740 (s), 1655 (m), 1520 (s), 1470 (s), 1410 (s), 1380 (w), 1360 (m) cm^{-1}. ^1H NMR (60 MHz, $CDCl_3$): δ 4.06 (2 H, t, *J* 7 Hz), 3.92 (3 H, s), 2.51 (3 H, t, *J* 10.0 Hz), 2.26 (3 H, s), 1.96 (3 H, s).

4-(2-Acetoxyethyl)-5-bromomethyl-3-methoxyisoxazole (**6**). A mixture of **5** (1.20 g, 6 mmol) and bromine (2.0 ml, 36 mmol) was stirred at room temperature for 8 days. The bromine was evaporated off and the residue was dissolved in dichloromethane (10 ml). After addition of water (10 ml) and $Na_2S_2O_5$ to decolourize the mixture, the organic layer was isolated, dried (K_2CO_3) and filtered. After evaporation to dryness the residue was submitted to CC [silica gel 50 g; eluent: toluene–ethyl acetate (9 : 1)]. The fractions containing pure **6** were pooled and evaporated to give 0.85 g (51 %) of **6** as a colourless oil. Anal. $C_9H_{12}BrNO_4$: C, H, Br, N. IR (film): 3100–2800 (several bands, m), 1740 (s), 1650 (m), 1520 (s), 1460 (m), 1410 (s), 1380 (w), 1360 (m) cm^{-1}. ^1H NMR (60 MHz, $CDCl_3$): δ 4.33 (2 H, s), 4.20 (2 H, t, *J* 7 Hz), 4.00 (3 H, s), 2.66 (2 H, t, *J* 7 Hz), 2.05 (3 H, s).

4-(2-Acetoxyethyl)-3-methoxy-5-phthalimidomethylisoxazole (**7**). A mixture of **6** (278 mg, 1 mmol), potassium phthalimide (204 mg, 1.1 mmol) and *N,N*-dimethylacetamide (10 ml) was kept at 120 °C for 90 min. The mixture was cooled to room temperature, water (20 ml) was added and the mixture was stirred for 15 min. The crystals were filtered off, washed with water and dried to give 220 mg (64 %) of **7** as colourless crystals. M.p. 99–100 °C. Anal. $C_{17}H_{16}N_2O_6$: C, H, N. IR (KBr): 3650–3200 (w), 3100–2800 (several bands, w), 1760 (m), 1740 (s), 1720 (s), 1660 (w), 1610 (w), 1525 (s), 1460 (m), 1410 (s), 1390 (s), 1350 (m) cm^{-1}. ^1H NMR (60 MHz, $CDCl_3$): δ 8.0–7.6 (4 H, m), 4.86 (2 H, s), 4.22 (2 H, t, *J* 7 Hz), 3.96 (3 H, s), 2.80 (2 H, t, *J* 7 Hz), 2.03 (3 H, s).

4-(2-Acetoxyethyl)-3-hydroxy-5-phthalimidomethylisoxazole (**8**). A mixture of **7** (172 mg, 0.5 mmol) and AcOH containing 33 % HBr (5 ml) was kept at 50 °C with stirring for 90 min. The mixture was cooled to room temperature and evaporated to dryness at 50 °C. The crude product was crystallized from toluene–ethyl acetate to give 90 mg (55 %) of **8** as colourless crystals. M.p. 149–150 °C. Anal. $C_{16}H_{14}N_2O_6$: C, H, N. IR (KBr): 3700–3300 (several bands, w), 3300–2300 (several bands, w), 1770 (w), 1710 (s), 1660 (w), 1525 (m), 1460 (w), 1410 (m), 1390 (s), 1340 (m) cm^{-1}. ^1H NMR (60 MHz, $CDCl_3$): δ 9.50 (1 H, s), 8.0–7.6 (4 H, m), 4.83 (2 H, s), 4.20 (2 H, t, *J* 7 Hz), 2.76 (2 H, t, *J* 7 Hz), 1.96 (3 H, s).

4-(2-Acetoxyethyl)-5-aminomethyl-3-hydroxyisoxazole hemihydrate (**9**) *and 5-aminomethyl-4-(2-hydroxyethyl)-3-hydroxyisoxazole hydrochloride* (**10**). Hydrazine monohydrate (250 µl, 5.0 mmol) was added to a solution of **8** (840 mg, 2.5 mmol) in MeOH (25 ml), and the mixture was refluxed for 1 h. The mixture was cooled to room temperature and the filtered solution was evaporated to ca. 10 ml and stored overnight at 5 °C. The crystals were filtered off and dissolved in water (3 ml) and after filtration the solution was evaporated to dryness. The residue was recrystallized from MeOH–water to give colourless crystals of **9** as a hemihydrate. Yield: 119 mg (23 %). M.p. 146–147 °C. Anal. $C_8H_{12}N_2O_4 \cdot \frac{1}{2}H_2O$: C, H, N. IR (KBr): 3700–3300 (several bands, m), 3200–2000 (several bands, m), 1730 (s), 1720 (s), 1670 (w), 1620 (w), 1480 (s), 1420 (m), 1370 (m) cm^{-1}. ^1H NMR (60 MHz, DMSO-d_6): δ 4.67 (ca. 6 H, s), 4.08 (2 H, t, *J* 7 Hz), 3.69 (2 H, s), 2.60 (2 H, t, *J* 7 Hz), 2.00 (2 H, s).

The pooled mother liquors were evaporated to dryness and after addition of MeOH containing 10 % HCl (5 ml), the solution was refluxed for 4 h. After being cooled to room temperature and filtered, the solution was evaporated to dryness. The residue was crystallized from MeOH–ether to give **10** as colourless crystals. Yield: 80 mg (17 %). M.p. 174–176 °C (decomp.). Anal. $C_6H_{11}ClN_2O_3$: C, H, Cl, N. IR (KBr): 3700–3250 (s), 3250–2300 (several bands, s), 2000 (w), 1660 (m), 1610 (w), 1545 (s), 1480 (m), 1360 (m) cm^{-1}. ^1H NMR (60 MHz, DMSO-d_6): δ 8.70 (3 H, s), 5.15 (2 H, s), 4.08 (2 H, q, *J* 7 Hz), 3.52 (2 H, t, *J* 7 Hz), 2.51 (2 H, t, *J* 7 Hz).

4-(2-Hydroxyethyl)-3-methoxy-5-phthalimidomethylisoxazole (**11**). A solution of **7** (2.0 g, 5.8 mmol) in MeOH containing 5 % HCl (100 ml) was refluxed for 2 h. After evaporation to dryness the residue was crystallized from toluene–light petroleum to give **11** as colourless crystals. Yield: 1.21 g (40 %). M.p. 95.5–96.5 °C. Anal. $C_{15}H_{14}N_2O_5$: C, H, N. IR (KBr): 3480 (s), 3000–2800 (several bands, w), 1770 (s), 1720 (s), 1710 (s), 1660 (m), 1610 (w), 1525 (s), 1410 (s), 1390 (s) cm^{-1}. ^1H NMR (60 MHz, $CDCl_3$): δ 8.0–7.7 (4 H, m), 4.86 (2 H, s), 3.93 (2 H, s), 3.76 (2 H, t, *J* 7 Hz), 2.67 (2 H, t, *J* 7 Hz).

4-(2-Chloroethyl)-3-methoxy-5-phthalimidomethylisoxazole (**12**). A mixture of **11** (1.0 g, 3.32 mmol), pyridine (265 mg, 3.32 mmol) and $SOCl_2$ (592 mg, 5.0 mmol) in toluene (20 ml) was heated with stirring at 60 °C for 10 h. After filtration and evaporation to dryness the residue was crystallized from toluene–light petroleum to give **12** as colourless crystals. Yield: 770 mg (72 %). M.p. 108–109 °C. Anal. $C_{15}H_{13}ClN_2O_4$: C, H, Cl, N. IR (KBr): 3700–3200 (several bands, m), 3100–2800 (several bands, w), 1770 (m), 1720 (s), 1710 (s), 1650 (m), 1525 (s), 1460 (s), 1450 (w), 1415 (s), 1385 (s) cm^{-1}. ^1H NMR (60 MHz, $CDCl_3$): δ 8.0–7.7 (4 H, m), 4.86 (2 H, s), 3.93 (3 H, s), 3.68 (2 H, t, *J* 7 Hz), 2.94 (2 H, t, *J* Hz).

4-(2-Chloroethyl)-3-methoxy-5-phthalimidomethylisoxazole (**13**). A mixture of **12** (750 mg, 2.34 mmol) and AcOH containing 33 % HBr (6 ml) was heated with stirring at 50 °C for 2 h. After evaporation to dryness another portion of AcOH containing 33 % HBr (6 ml) was added and heating and stirring were repeated at 50 °C for 2 h. The last procedure was repeated a third time. The residue after final evaporation, was crystallized from ethyl acetate to give **13**. Yield: 468 mg (65 %). M.p. 189–190 °C. Anal. $C_{14}H_{11}ClN_2O_4$: C, H, Cl, N. IR (KBr): 3700–3300 (m), 3200–2500 (several bands, m), 1770 (m), 1720 (s), 1710 (s), 1660 (m), 1540 (s), 1520 (m), 1470 (w), 1420 (m), 1400 (s), 1350 (m) cm^{-1}. ^1H NMR (60 MHz, DMSO-d_6): δ 11.60 (1 H, s), 7.89 (4 H, m), 4.81 (2 H, s), 3.73 (2 H, t, *J* 7 Hz), 3.32 (2 H, s), 2.84 (2 H, t, *J* 7 Hz).

5-Aminomethyl-4-(2-chloroethyl)-3-hydroxyisoxazole hydrochloride (**14**). A mixture of **13** (300 mg, 0.98 mmol) and 6 M HCl (12 ml) was refluxed for 3 h. After evaporation to dryness the residue was dissolved in water (10 ml) and extracted continuously for 2 h using ether–dichloromethane. The aqueous phase was evaporated to dryness and the residue was crystallized from MeOH–ether to give **14** as colourless crystals. Yield: 25 mg (11 %). M.p. 153–155 °C (decomp.). Anal. $C_6H_{10}Cl_2N_2O_2$: C, H, Cl, N. IR (KBr): 3700–3300 (m), 3300–2500 (several bands, s), 1670 (m), 1595 (m), 1570 (w), 1520 (s), 1480 (s), 1430 (m), 1375 (w) cm^{-1}. ^1H NMR (60 MHz, DMSO-d_6): δ 11.71 (1 H, s), 8.61 (3 H, s), 4.13 (2 H, s), 3.73 (2 H, t, *J* 7 Hz), 2.82 (2 H, t, *J* 7 Hz).

3-Methoxy-4,5,6,7-tetrahydroisoxazolo[5,4-c]-pyridinium chloride (**15**). A solution of **12** (200 mg, 0.62 mmol), MeOH (6 ml) and hydrazine hydrate (36 mg, 0.68 mmol) was refluxed with stirring for 12 h. The mixture was evaporated and the residue was acidified to pH ca. 2 with 4 M HCl. After extraction with four 10-ml portions of dichloromethane, to the aqueous phase was added 10 % aqueous $NaHCO_3$ to pH ca. 11. Continuous extraction of the alkaline solution with ether–dichloromethane (5 : 1) for 2 h gave a crude extract which was dried ($MgSO_4$), filtered and evaporated to dryness. The crude product was dissolved in 4 M HCl (1 ml) and evaporated to dryness. Crystallization from MeOH–ether gave **15** as colourless crystals. Yield: 70 mg (59 %). M.p., IR and ^1H NMR spectra were identical with those of an authentic sample.[32]

3-Hydroxy-4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridinium bromide (THIP hydrobromide) (**16**). A mixture of **15** (70 mg; 0.37 mmol) and AcOH containing 33 % HBr (3 ml) was kept at 50 °C for 90 min. The solution was dissolved in water (5 ml) and charcoal (200 mg) was added. The mixture was boiled for 2 min, filtered and evaporated to dryness. The residue was crystallized from ethanol–ether to give **16** as colourless crystals. Yield: 27 mg (33 %). M.p., IR and ^1H NMR spectra were identical with those of an authentic sample.[26]

*(3a*RS,*6a*RS)*-3a-Bromo-3-methoxy-6a-methyl-3a,4,5,6a-tetrahydrofuro[3,2-d]isoxazole* (**17a**). A solution of compound **3a** (1.6 g, 10 mmol) in tetrachloromethane (10 ml) was treated under reflux with NBS (a total of 1.8 g, 10 mmol) and benzoyl peroxide (50 mg) over a period of 2 h. Each of the reagents was added in four equal portions every 30 min, and 30 min after the addition of the last portion of the reagents the reaction mixture was filtered and evaporated to give crude **17a**. CC [eluent: toluene–ethyl acetate (3 : 1)] gave **17a** as an oil. Yield: 1.7 g (70 %). Almost the same yield was obtained by treatment of compound **3a** in tetrachloromethane with excess of bromine for 6 days at room temperature. Anal. $C_7H_{10}BrNO_3$: C, H, N. Found: Br 35.37; calc. Br 33.83. IR (film) 2990–2880 (several bands, w), 1620 (s), 1450 (s), 1380 (s), 1365 (s) cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): δ 4.0 (1 H, t d, *J* 0.8 Hz, *J* 7.7 Hz, *J* −9.1 Hz), 3.9 (3 H, s), 3.7 (t d, *J* 4.7 Hz, *J* 12.1 Hz, *J* −9.1 Hz), 2.8 (1 H, t d, *J* 0.8 Hz, *J* 4.7 Hz, *J* −12.8 Hz), 2.5 (1 H, t d, *J* 7.7 Hz, *J* 12.1 Hz, *J* −12.8 Hz), 1.7 (3 H, s). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 165 (C-3, s), 118 (C-6a, s), 66 (C-5, t), 63 (C-3a, s), 58 (O–CH_3, q), 41 (C-4, t), 23 (C–CH_3, q); *J* (C-5, H-5) 150 Hz, (O–CH_3, H-8) 148 Hz, (C-4, H-4), 138 Hz (C–CH_3, H-7) 129 Hz.

Inhibition of $GABA_A$ receptor binding. The ^3H-GABA binding assay was performed with rat brain synaptic membranes as described previously in detail.[28] Aliquots of synaptic membranes (0.8–1.2 mg of protein) were incubated in triplicate at 2 °C in 2 ml of Tris–citrate buffer (pH 7.1) containing 5 nM ^3H-GABA. Test substances were added in various concentrations. The samples were incubated for 15 min at 2 °C followed by centrifugation. The pellets were rinsed twice with 5 ml portions of cold water and suspended in water (0.4 ml). IC_{50} values were estimated by measuring the inhibition with at least four different concentrations. Non-specific binding in the presence of 1 mM GABA was subtracted.[28]

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