Synthesis and Binding Properties to GABA Receptors of 3-Hydroxypyridinyl- and 3-Hydroxypiperidinyl-Analogues of Baclofen

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The synthesis of 3-(3-hydroxy-2-pyridinyl)propanoic acid, 3-(3-hydroxy-2-pyridinyl)-4-aminobutanoic acid, their corresponding piperidine compounds, and of some cyclized derivatives is described.

In *in vitro* assays none of the new compounds shows any noteworthy affinity for $GABA_A$ or $GABA_B$ receptors; only (*R,S*)-3-(3-hydroxy-2-pyridinyl)-4-aminobutanoic acid and its lactam inhibited in some degree [^{3}H]GABA binding, at 10^{-4} M concentration, with low specificity as regards the two receptors.

Synthese und GABA-Rezeptoren-Affinität von 3-Hydroxypyridinyl- und 3-Hydroxypiperidinyl-Analogen des Baclofen

Die Synthesen von 3-(3-Hydroxy-2-pyridinyl)propansäure, 3-(3-Hydroxy-2-pyridinyl)-4-aminobutansäure, deren Piperidinyl-Analogen und einiger Cyclisierungsderivaten werden beschrieben.

In *in vitro* Bindungs-Studien zeigen die neuen Substanzen keine signifikante Affinität zu $GABA_A$- und $GABA_B$-Rezeptoren; nur (*R,S*)-3-(3-Hydroxy-2-pyridinyl)-4-aminobutansäure und ihr Lactam hemmten die [^{3}H]GABA-Bindung in 10^{-4} M Konzentration schwach. Die Spezifität für die beiden Rezeptoren ist gering.

A large variety of GABA analogues have been investigated in the last decade and many of them, showing a partial constrained structure, are selective agonists for the $GABA_A$ receptor[1]. On the contrary much less information is available on the structure-activity relationship for $GABA_B$ receptor agonists, where only (*R*)-β-*p*-chlorophenyl-GABA (Baclofen)[2,3] is a selective agonist, which has entered in clinical use as antispastic agent[4,5].

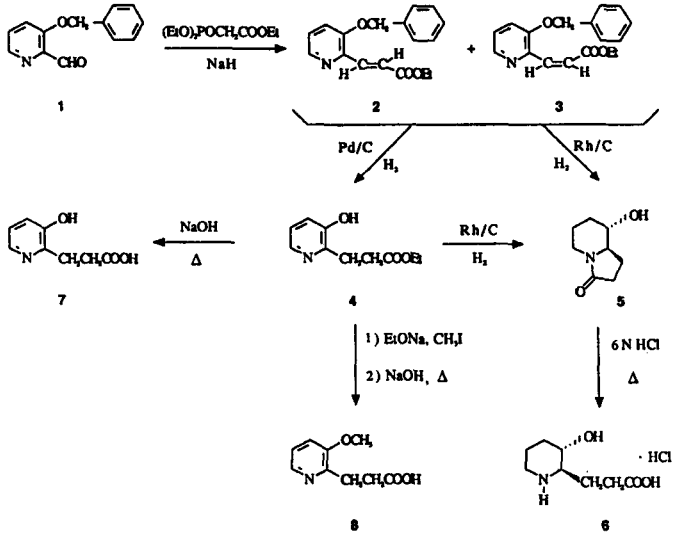
Small alterations in the Baclofen molecule result in inactivity of the compounds[6,7]: for instance replacing the chlorine atom in the phenyl ring with an hydroxy as well as different alkoxy groups gave inactive products[8].

Less than 1% of an oral dose of Baclofen crosses the blood-brain barrier into the CNS[9]; the hydroxy group would have been a useful function for introducing a lipophilic chain into the molecule of the gabergic agent. We considered it worthwhile to synthesize suitable derivatives of hydroxypyridine and hydroxypiperidine, with the basic nitrogen included in the heterocyclic ring in the γ position in respect to the carboxylic group (**5**, **6**, **7**, and **8**), or with an additional γ-amino group (**13**, **15**, **16**, **17**, **18**, and **19**). In the event of a positive result in the binding assay, we had in mind to prepare other alkylated and new acylated pro-drugs.

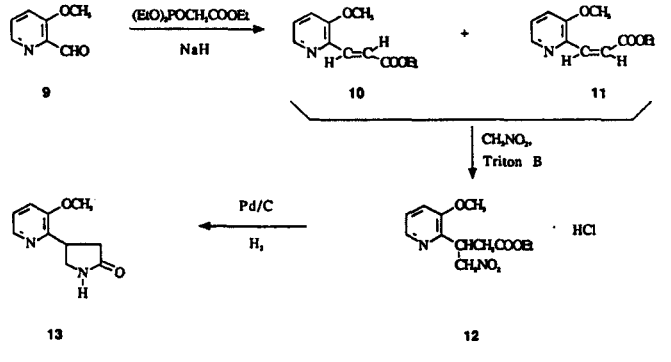
Chemistry

Starting material for all new compounds was 3-benzyloxy- or 3-methoxy-2-picolinaldehyde (**1** and **9**). The first one was recently described[10], **9** was prepared, by the same method, from 3-methoxy-2-hydroxymethylpyridine[11]; we obtained a solid, while it was reported as an oil[11,12].

A *Wadsworth-Emmons* reaction on the aldehydes **1** or **9** afforded a mixture of *E* and *Z* forms of the corresponding α,β-unsaturated esters **2**, **3**, and **10**, **11** with prevalence of the *E* forms (Schemes 1 and 2). It was possible to separate the isomers by column chromatography, but it was not necessary for the following reactions.



Scheme 1



Scheme 2

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The hydrogenation of the mixture of esters **2** and **3**, using Pd/C, afforded **4**. If Rh/C was used and the reaction was carried out in glacial acetic acid at 60 psi and 60°C for several h, the pyridine ring was also hydrogenated; subsequent intramolecular cyclization led to the indolizinone **5**. **5** was also prepared from **4** by catalytic hydrogenation under the above reported conditions.

Stereochemical assignments for **5** may be made on the basis of IR- and NMR-data. According to the *Bohlmann* correlation[13], a quinolizidine system shows one or more strong bands in the 2700-2800 cm^{-1} region, when two or more hydrogens on C-atoms adjacent to the N are *trans* diaxial to the unshared N-electron pair. This correlation is also applicable for stereochemical assignments of the ring fusion of the indolizidine system[14].

The absence of the *Bohlmann* bands in the IR-spectra of the lactam **5** let to hypothesize *cis* ring fusion. Analysis of NMR data provides a confirmation of this hypothesis and allows to establish the configuration of the C-atoms 8 and 8a. The equatorial position of H-8 follows without ambiguity from the width of the multiplet at half heigth (W/2 = 7 Hz); the vicinal coupling constants of H-8a ($J_{8aeq,8eq}$ = 2.0 Hz, $J_{8aeq,1'}$ = 8.2 Hz, $J_{8aeq,1''}$ = 5.0 Hz) suggest that this proton is in equatorial position on the piperidine ring.

The pyrrolidinonic ring was opened by acid hydrolysis and 3-(3-hydroxy-2-piperidinyl)propanoic acid (**6**) was isolated as its hydrochloride. The corresponding pyridinyl acid **7**, prepared by us from the ethyl ester **4**, was previously obtained by ring cleavage of 2-furylcarboxyethylketone with ammonia[15]. The 3-methoxy analogue **8** was also obtained from **4** by etherification and hydrolysis, without purification of the intermediate ester.

In order to join an aminomethyl group to the side chain, nitromethane was added to the mixture of esters **2** and **3** or **10** and **11** in presence of Triton B, and the intermediates **14** or **12** were hydrogenated under different conditions to achieve selective reductions (Schemes 2 and 3).

When the pyridine ring was not reduced, the lactams **13**, **15**, and **16** were obtained. Hydrolysis of **16** in alkaline condition, followed by purification on Dowex resin, gave the pyridinyl-GABA analogue **17**, as a zwitterion.

Treatment with Dowex resin was necessary to have the piperidinyl-GABA analogue **19** from the crude oil obtained by hydrogenation of **14** over Rh/C, or from the lactone **18**. This compound was obtained by heating the same oil in 6 N HCl.

Lactone **18** shows three chiral carbon atoms. Key assignments involve the H-4a and H-8a signals, which appear as a multiplet and a doublet with W/2 of 5.7 and 6.0 Hz, respectively. Absence of large coupling constants allows to exclude *trans* ring fusion which would involve a *trans* diaxial interaction between the two protons, with consequent large coupling constant. The positions, axial of H-4a and equatorial of H-8a, in the piperidine ring follow from the same observations.

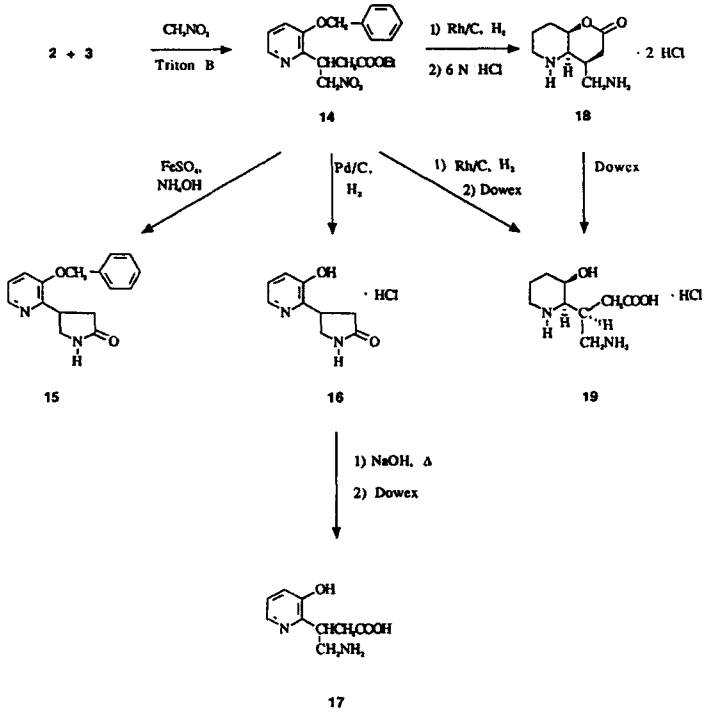
To establish the configuration of C-4, the signals of H-3 must be observed. They appear as two double doublet at δ 2.68 and 2.59, and collapse to two doublets by spin decoupling following the irradiation of the H-4 signal at δ 2.35. The 11.0 Hz coupling constant between H-4 and H-3a points to a *trans* diaxial relationship; the coupling constant between H-4 and H-3e (6.0 Hz) is congruous with this assignment.

The same configuration was assigned to the aminoacid **19** obtained from the lactone by hydrolysis.

Biological Results and Discussion

All the compounds were tested for their ability to displace [^{3}H]GABA from rat brain membranes in the absence or in the presence of isoguvacine ($GABA_A$ or $GABA_B$ sites, respectively).

The results were reported as percent of binding inhibition of [^{3}H] GABA to the $GABA_A$ and $GABA_B$ receptor sites, and were compared with those of GABA and (-)- or (+)-Baclofen (Table 1).



Scheme 3

Table 1: Binding inhibition of [^{3}H]GABA to $GABA_A$ and $GABA_B$ receptors

Compound	Concentration (M)	Binding inhibition (%)[a] $GABA_A$	$GABA_B$
GABA	10^{-7}	60	–
GABA	10^{-6}	95	97
(-)Baclofen	10^{-7}	–	48
(-)Baclofen	10^{-6}	–	87
(+)Baclofen	10^{-6}	–	0
4	10^{-4}	26	22
5	10^{-4}	7	6
6	10^{-4}	11	13
7	10^{-4}	14	5
8	10^{-4}	9	21
13	10^{-4}	0	5
15	10^{-4}	10	11
16	10^{-4}	18	61
17	10^{-4}	40	23
18	10^{-4}	10	7
19	10^{-4}	8	10

[a] Results were means of two experiments done in triplicate

The new GABA analogues were not active below 10^{-4} M; in any case at this concentration some activity, with low selectivity in relation to the two receptors, is shown by **16** and **17**. These compounds are the racemic 3-(3-hydroxy-2-pyridinyl)-4-aminobutanoic acid and its lactam.

Berthelot et al.[8], in a study on some Baclofen analogues, established that the coplanarity of the aromatic ring with the C-3 atom of the GABA chain was necessary for $GABA_B$ receptor affinity. Although in the pyridine derivatives **13**, **15**, **16**, and **17** such condition is respected, only **16** and **17** show a certain activity.

The nature of the ring, the position, and the kind of its substituent therefore appear to be determinant. Anyhow, these results do not encourage further research on other pyridinyl and piperidinyl derivatives.

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Experimental Part

Chemistry

Melting points: Büchi SMP-20 apparatus (uncorrected).- IR: Perkin-Elmer 297.- NMR: Varian EM-390 (90 MHz) and/or Varian XL-300 (300 MHz), TMS as internal standard.- Elemental Analyses: Laboratorio di Microanalisi, Dipartimento di Scienze farmaceutiche, Univ. di Padova.- Column chromatography: silica gel RS, 0.05-0.20 mm (Carlo Erba).- 3-Benzyloxy-2-picolinaldehyde (**1**) was prepared as reported[10]. 3-Methoxy-2-hydroxymethylpyridine, described by French[11], was synthesized by us from 2-hydroxymethyl-3-pyridinol according to *Nedenskov* for 3-pyridinol ethers[16].- Temp. in °C.

3-Methoxy-2-picolinaldehyde (**9**)

To a solution of 3-methoxy-2-hydroxymethylpyridine (0.1 moles) in 1,2-dimethoxyethane (150 ml) heated at 100°C, MnO_2 (0.9 moles), previously activated at 120°C for 2 h, was added under stirring over a period of 1 h; heating was continued for another h. After cooling, the suspension was filtered and the filtrate evaporated under reduced pressure. The aldehyde was purified by column chromatography (cc) eluting with AcOEt. Yield 78%. Mp. 64-68° from ethyl ether (oil in ref. 11,12).

Wadsworth-Emmons reaction on compounds **1** *and* **9**

A suspension of NaH (0.01 moles) in dry ethyl ether (10 ml) was added to a solution of triethyl phosphonoacetate (0.01 moles) in dry ethyl ether (20 ml) and the mixture was stirred 1 h at room temp.. 3-Benzyloxy-2-picolinaldehyde (**1**)[10] or 3-methoxy-2-picolinaldehyde (**9**) (0.01 moles) in dry ethyl ether (45 ml) was then added dropwise at room temp.. The mixture was stirred overnight, washed with water, dried (Na_2SO_4) and evaporated under reduced pressure. The obtained oil was chromatographed on silica gel eluting with ethyl ether/petroleum ether (3/1) to separate the Z- from the *E*-form.

(E)-Ethyl 3-(3-benzyloxy-2-pyridinyl)propenoate (**2**)

Yield 80%. Mp. 59-60° from ethyl ether/petroleum ether.- IR (KBr): 1690 (C=O) cm^{-1}.- ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 8.25 (m, 1H, H-6), 8.20 (d, 1H, PyCH=, J = 16 Hz), 7.45 (m, 5H, Ph), 7.25 (m, 2H, H-4, H-5), 7.10 (d, 1H, =CHCOO, J = 16 Hz), 5.15 (s, 2H, OCH_2), 4.25 (q, 2H, $COOCH_2$), 1.30 (t, 3H, CH_3).- $C_{17}H_{17}NO_3$ (283.3) Calcd. C 72.1 H 6.05 N 4.9 Found C 72.1 H 5.86 N 5.2.

(Z)-Ethyl 3-(3-benzyloxy-2-pyridinyl)propenoate (**3**)

Yield 10%. Mp. 27-28° from ethyl ether/petroleum ether.- IR (KBr): 1720 (C=O) cm^{-1}.- ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 8.10 (dd, 1H, H-6, $J_{5,6}$ = 4 Hz, $J_{4,6}$ = 2 Hz), 7.35 (m, 5H, Ph), 7.10 (m, 2H, H-4, H-5), 7.05 (d, 1H, PyCH=, J = 12 Hz), 6.00 (d, 1H, =CHCOO, J = 12 Hz), 5.00 (s, 2H, OCH_2), 4.05 (q, 2H, $COOCH_2$), 1.15 (t, 3H, CH_3).- $C_{17}H_{17}NO_3$ (283.3) Calcd. C 72.1 H 6.05 N 4.9 Found C 72.0 H 6.15 N 5.1.

(E)-Ethyl 3-(3-methoxy-2-pyridinyl)propenoate (**10**)

Yield 38%. Mp. 63-66° from ethyl ether/petroleum ether.- IR (KBr): 1690 (C=O) cm^{-1}.- ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 8.25 (m, 1H, H-6), 8.10 (d, 1H, PyCH=, J = 16 Hz), 7.20 (m, 2H, H-4, H-5), 7.05 (d, 1H, =CHCOO, J = 16 Hz), 4.30 (q, 2H, $COOCH_2$), 3.85 (s, 3H, OCH_3), 1.30 (t, 3H, CH_3).- $C_{11}H_{13}NO_3$ (207.2) Calcd. C 63.7 H 6.32 N 6.8 Found C 63.4 H 6.30 N 6.9.

(Z)-Ethyl 3-(3-methoxy-2-pyridinyl)propenoate (**11**)

Yield 5%.- IR (film): 1710 (C=O) cm^{-1}.- ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 8.20 (m, 1H, H-6), 7.20 (m, 2H, H-4, H-5), 7.10 (d, 1H, PyCH=, J = 12 Hz), 6.15 (d, 1H, =CHCOO, J = 12 Hz), 4.20 (q, 2H, $COOCH_2$), 3.80 (s, 3H, OCH_3), 1.15 (t, 3H, CH_3).- $C_{11}H_{13}NO_3$ (207.2) Calcd. C 63.7 H 6.32 N 6.8 Found C 63.5 H 6.48 N 6.7.

Ethyl 3-(3-hydroxy-2-pyridinyl)propanoate (**4**)

A solution of a mixture of *E* and *Z* esters **2** and **3** (0.01 moles) in ethanol (200 ml) was hydrogenated for 6 h at 45 psi over 5% Pd/C (500 mg). After filtration, the solvent was evaporated *i. vac.* and the residue crystallized from ethyl acetate. Yield 95%. Mp. 102-104°.- IR (KBr): 2700-2300 (OH); 1730 (C=O) cm^{-1}.- ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 9.4-9.1 (bs, 1H, OH), 8.15 (m, 1H, H-6), 7.3-7.0 (m, 2H, H-4, H-5), 4.15 (q, 2H, $COOCH_2$), 3.15 (t, 2H, $PyCH_2$), 2.85 (t, 2H, CH_2COO), 1.20 (t, 3H, CH_3).- $C_{10}H_{13}NO_3$ (195.2) Calcd. C 61.5 H 6.71 N 7.2 Found C 61.9 H 6.90 N 7.0.

3-(3-Hydroxy-2-pyridinyl)propanoic acid (**7**)

A solution of ester **4** (0.01 moles) in 2N NaOH (100 ml) was refluxed for 4 h. After cooling, pH was adjusted to ≈ 6 with 2N HCl and the mixture extracted continuously for 4 d with ethyl ether. The org. solution was dried (Na_2SO_4) and evaporated *i. vac.* to give a solid. Yield 58%. Mp. 189-191° from ethanol, as reported[15].

3-(3-Methoxy-2-pyridinyl)propanoic acid (**8**)

4 (0.01 moles) was added to a solution of Na° (0.01 moles) in absol. ethanol (7 ml). After 1 h at room temp. under stirring, DMSO (11 ml) was added and the ethanol was completely removed under reduced pressure. CH_3I (0.012 moles) was added and the suspension was stirred overnight at room temp.. After dilution with water, the mixture was extracted with chloroform, the org. layer was dried (Na_2SO_4) and evaporated *i. vac.* to give an oil. 2N NaOH (40 ml) was added and the suspension was refluxed for 4 h. After cooling, the mixture was extracted with ethyl acetate, the aqueous solution was brought to pH 6 with 2N HCl and extracted continuously for 4 days with ethyl acetate. The org. solution was dried (Na_2SO_4) and evaporated *i. vac.* to give **8**. Yield 13%. Mp. 97-103° from ethyl acetate.- IR (KBr): 2700-2300 (OH); 1710 (C=O) cm^{-1}.- ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 10.7-10.4 (bs, 1H, COOH), 8.15 (m, 1H, H-6), 7.30 (m, 2H, H-4, H-5), 3.85 (s, 3H, OCH_3), 3.20 (dd, 2H, $PyCH_2$ J_1 = 6 Hz, J_2 = 4 Hz), 2.80 (dd, 2H, CH_2COO, J_1 = 6 Hz, J_2 = 4 Hz).- $C_9H_{11}NO_3$ (181.2) Calcd. C 59.7 H 6.12 N 7.7 Found C 60.0 H 6.30 N 7.5.

(8SR,8aRS)-Hexahydro-8-hydroxy-3(2H)-indolizinone (**5**)

A solution of ester **4** or a mixture of *E* and *Z* esters **2** and **3** (0.01 moles) in glacial acetic acid (125 ml) was hydrogenated for 24 h at 60 psi and 60° over 850 mg of Rh/C. After cooling, the catalyst was filtered off and the solvent evaporated *i. vac.*. The obtained oil was chromatographed on silica gel/ethyl acetate. Yield 66% from **4** and 42% from the mixture of esters. Mp. 93-96° from ethyl acetate.- IR (KBr): 3270 (OH); 1660 (C=O) cm^{-1}.- ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) = 4.09 (dd, 1H, H-5eq, J_{gem} = 13.3 Hz, $J_{5eq,6ax}$ = 5 Hz), 3.82 (m, 1H, H-8eq, W/2 = 7 Hz, $J_{8aeq,8eq}$ = 2 Hz), 3.54 (m, 1H, H-8aeq, $J_{8aeq,1'}$ = 8.2 Hz, $J_{8aeq,1''}$ = 5 Hz, $J_{8aeq,8eq}$ = 2 Hz), 3.2-2.9 (bs, 1H, OH), 2.66 (td, 1H, H-5ax, J_{gem} = 13.3 Hz, $J_{5ax,6ax}$ = 13.3 Hz, $J_{5ax,6eq}$ = 3.6 Hz), 2.5-2.2 (m, 2H, H-2), 2.2-1.9 (m, 3H, H-1, H-7eq), 1.82 (m, 1H, H-6ax), 1.63 (m, 1H, H-7ax), 1.47 (m, 1H, H-6eq).- $C_8H_{13}NO_2$ (155.2) Calcd. C 61.9 H 8.44 N 9.0 Found C 61.6 H 8.55 N 9.2.

(2RS,3SR)-3-(3-Hydroxy-2-piperidinyl)propanoic acid hydrochloride (**6**)

A solution of the indolizinone **5** (0.01 moles) in 6N HCl (60 ml) was refluxed for 24 h. After cooling, the solvent was evaporated *i. vac.* and the residue crystallized from water/acetone. Yield 67%. Mp. 174-175°.- IR (KBr): 3420 (OH alcohol); 3300-2300 (NH_2^+, OH); 1720 (C=O) cm^{-1}.- ^{1}H-NMR (90 MHz, DMSO d_6): δ (ppm) = 10.3-9.7 (bs, 1H, COOH), 9.7-8.3 (bs, 1H, NH), 6.1-5.0 (bs, 1H, OH), 4.10 (s, 1H, H-3), 3.6-2.8 (m, 3H, H-2, 2 H-6), 2.50 (m, 2H, CH_2COO), 2.3-1.4 (m, 6H, 2 H-4, 2 H-5, CH_2).- $C_8H_{16}NO_3Cl$ (209.7) Calcd. C 45.8 H 7.69 N 6.7 Cl 16.9 Found C 46.2 H 8.03 N 6.7 Cl 16.6.

Reaction with nitromethane

Triton B (5 ml of a 35% solution in methanol) and nitromethane (0.35 moles) were added to a solution of the α,β-unsaturated esters **2** and **3** or **10** and **11** (0.01 moles). The mixture was heated at 80° under stirring for 18 h. After cooling, water was added and the mixture was extracted with ethyl ether. The org. layer was dried (Na_2SO_4) and evaporated. The oil containing **12** or **14** was chromatographed on silica gel/ethyl ether/petroleum ether (3/1) or ethyl ether, respectively.

(RS)-Ethyl3-(3-methoxy-2-pyridinyl)-4-nitrobutanoate (**12**)

Yield 78%. IR (film): 1720 (C=O); 1545, 1370 (NO_2) cm^{-1}.- ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 8.00 (m, 1H, H-6), 7.10 (m, 2H, H-4, H-5), 5.1-4.3 (m, 3H, $CHCH_2NO_2$), 3.95 (q, 2H, $COOCH_2$), 3.70 (s, 3H, OCH_3), 2.80 (dd, 1H, CHCOO, J_{gem} = 16 Hz, J = 6 Hz), 2.50 (dd, 1H, CHCOO, J_{gem} = 16 Hz, J = 7 Hz), 1.10 (t, 3H, CH_3).- The hydrochloride crystallized from ethanol/ethyl acetate. Mp. 146-147° dec.- IR (KBr): 2800-1900 (NH^+); 1715 (C=O); 1540, 1370 (NO_2) cm^{-1}.- $C_{12}H_{17}N_2O_5Cl$ (304.7) Calcd. C 47.3 H 5.62 N 9.2 Cl 11.6 Found C 47.4 H 5.33 N 9.0 Cl 11.7.

(RS)-Ethyl3-(3-benzyloxy-2-pyridinyl)-4-nitrobutanoate (**14**)

Yield 85%. Mp. 46-47° from ethyl ether/petroleum ether.- IR (KBr): 1730 (C=O); 1545, 1380 (NO_2) cm^{-1}.- ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 8.15 (m, 1H, H-6), 7.5-7.2 (m, 5H, Ph), 7.2-7.0 (m, 2H, H-4, H-5), 5.10 (s, 2H, OCH_2), 5.0-4.5 (m, 3H, $CHCH_2NO_2$), 4.05 (q, 2H, $COOCH_2$), 2.95 (dd, 1H, CHCOO, J_{gem} = 16 Hz, J = 6 Hz), 2.65 (dd, 1H, CHCOO, J_{gem} = 16 Hz, H = 7 Hz), 1.10 (t, 3H, CH_3).- $C_{18}H_{20}N_2O_5$ (344.4) Calcd. C 62.8 H 5.85 N 8.1 Found C 62.6 H 5.87 N 8.3.

(RS)-4-(3-Benzyloxy-2-pyridinyl)pyrrolidin-2-one (**15**)

A hot solution of **14** (0.01 moles) in ethanol (100 ml) was added to a suspension of $FeSO_4 \cdot 7H_2O$ (0.1 moles) in water (100 ml) and conc. ammonia solution (6 ml) under stirring. The mixture was heated at 100° for 1 h under stirring; during this period conc. ammonia solution (35 ml) was added dropwise. After cooling, the solid was filtered off and washed with ethanol. The filtrate was concentrated *i. vac.* and extracted with ethyl acetate. The org. layer was dried (Na_2SO_4) and evaporated *i. vac.*. The residue was chromatographed on silica gel/ethyl acetate. Yield 32%. Mp. 126-129° from ethyl acetate.- IR (KBr): 3190, 3070 (NH); 1690 (C=O) cm^{-1}.- ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 8.20 (dd, 1H, H-6, $J_{5,6}$ = 4 Hz, $J_{4,6}$ = 2 Hz), 7.30 (m, 5H, Ph), 7.15 (m, 2H, H-4, H-5), 6.9-6.6 (bs, 1H, NH), 5.05 (s, 2H, OCH_2), 4.5-3.9 (m, 1H, CH), 3.70 (d, 2H, CH_2N, J = 7 Hz), 2.70 (dd, 1H, CHCO, J_{gem} = 16 Hz, J = 9 Hz), 2.55 (dd, 1H, CHCO, J_{gem} = 16 Hz, J = 9 Hz).- $C_{16}H_{16}N_2O_2$ (268.3) Calcd. C 71.6 H 6.01 N 10.4 Found C 71.3 H 6.05 N 10.5.

(RS)-4-(3-Hydroxy-2-pyridinyl)pyrrolidin-2-one hydrochloride (**16**)

A solution of compound **14** (1.5 mmoles) in ethanol (50 ml) and conc. HCl (0.05 ml) was hydrogenated for 7 h at 60 psi over 5% Pd/C (100 mg). After filtration, the solvent was evaporated *i. vac.* and the residue crystallized from ethanol/ethyl ether. Yield 30%. Mp. 216-220° dec.- IR (KBr): 3200-1900 (OH, NH, NH^+); 1670 (C=O) cm^{-1}.- ^{1}H-NMR (90 MHz, DMSO d_6): δ (ppm) = 10.1-9.6 (bs, 2H, OH, NH), 8.10 (dd, 1H, H-6, $J_{5,6}$ = 4 Hz, $J_{4,6}$ = 2 Hz), 7.20 (m, 2H, H-4, H-5), 4.0-3.5 (m, 3H, $CHCH_2N$), 2.75-2.45 (m, 2H, CH_2CO).- $C_9H_{11}N_2O_2Cl$ (214.6) Calcd. C 50.4 H 5.16 N 13.1 Cl 16.5 Found C 50.6 H 5.30 N 13.4 Cl 16.2.

(RS)-3-(3-Hydroxy-2-pyridinyl)-4-aminobutanoic acid (**17**)

A solution of pyrrolidinone **16** (1 mmole) in ethanol (5 ml) and 10 N NaOH (1 ml) was refluxed for 1 h. After cooling, the pH was adjusted to ≈ 6 with acetic acid. Ethanol was evaporated *i. vac.* and the residue was chromatographed on Dowex 50Wx4 (H^+ form, 20-50 mesh, Fluka) eluting with N-NH_4OH. The residue from eluate was washed with ethanol; the compound crystallized from water/acetone as zwitterion. Yield 50%. Mp. 188-190°.- IR (KBr): 3260 (OH pyridinol); 3100-2700, 2700-2300, 2300-2000 (NH_3^+); 1620 (C=O) cm^{-1}.- ^{1}H-NMR (90 MHz, DMSO d_6): δ (ppm) = 8.05 (dd, 1H, H-6, $J_{3,6}$ = 4 Hz, $J_{4,6}$ = 2 Hz), 7.20 (m, 2H, H-4, H-5), 7.1-5.2 (bs, 4H, OH, NH_3^+), 4.2-3.4 (m, 3H, $CHCH_2N$), 2.8-2.4 (m, 2H, CH_2CO).- $C_9H_{12}N_2O_3$ (196.2) Calcd. C 55.1 H 6.17 N 14.3 Found C 55.2 H 6.32 N 14.3.

(RS)-4-(3-Methoxy-2-pyridinyl)pyrrolidin-2-one (**13**)

The hydrochloride **12** (3 mmoles) was suspended in a saturated solution of $NaHCO_3$. The free base was extracted with ethyl acetate, the org. layer was dried (Na_2SO_4) and evaporated *i. vac.*. The obtained oil was solubilized in ethanol (200 ml) and hydrogenated for 2 h at 45 psi over 5% Pd/C (200 mg). The catalyst was then filtered off and the solvent evaporated *i. vac.*. The

residue was purified on silica gel eluting at first with ethyl acetate, then with ethanol. Yield 43%. Mp. 127-129° from ethyl ether.- IR (KBr): 3200-3020 (NH); 1680 (C=O) cm^{-1}.- ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 8.20 (dd, 1H, H-6, $J_{5,6}$ = 4 Hz, $J_{4,6}$ = 2 Hz), 7.4-7.2 (bs, 1H, NH), 7.15 (m, 2H, H-4, H-5), 4.5-3.9 (m, 1H, CH), 3.80 (s, 3H, OCH_3), 3.70 (dd, 2H, CH_2N, J_{gem} = 8 Hz, J = 3 Hz), 2.90 (dd, 1H, CHCO, J_{gem} = 17 Hz, J = 8 Hz), 2.55 (dd, 1H, CHCO, J_{gem} = 17 Hz, J = 9 Hz).- $C_{10}H_{12}N_2O_2$ (192.2) Calcd. C 62.5 H 6.29 N 14.6 Found C 62.3 H 6.30 N 14.9.

(4SR,4aRS,8aRS)-Octahydro-4-aminomethyl-2H-pyrano[3,2-b]pyridin-2-one dihydrochloride (**18**)

A solution of ester **14** (5 mmoles) in glacial acetic acid (200 ml) was hydrogenated for 24 h at 60 psi and 60° over 1 g of Rh/C. After cooling, the catalyst was filtered off and the solvent evaporated *i. vac.*. The residue was dissolved in 6N HCl (50 ml) and refluxed for 24 h. After cooling and concentrating *i. vac.*, acetone was added, the precipitate being filtered and crystallized from water/acetone. Yield 12%. Mp. 225-235°.- IR (KBr): 3100-2300, 2020-1950 (NH_2^+, NH_3^+); 1720 (C=O) cm^{-1}.- ^{1}H-NMR (300 MHz, DMSO d_6): δ (ppm) = 9.0-8.2 (bs, 5H, NH, NH_2, 2 HCl), 4.79 (d, 1H, H-8aeq, $J_{8aeq,8ax}$ = 1.2 Hz, W/2 = 6 Hz), 3.75 (m, 1H, H-4aax, W/2 = 5.7 Hz), 3.33 (dd, 1H, 1 CH_2N, J_{gem} = 12.7 Hz, $J_{CHN,4}$ = 6.3 Hz), 3.24 (d, 1H, H-6eq, J_{gem} = 12.6 Hz), 3.02 (dd, 1H, 1 CH_2N, J_{gem} = 12.7 Hz, $J_{CHN,4}$ = 7.5 Hz), 2.91 (m, 1H, H-6ax), 2.68 (dd, 1H, H-3ax, J_{gem} = 15.6 Hz, $J_{3ax,4}$ = 11.7 Hz), 2.59 (dd, 1H, H-3eq, J_{gem} = 15.6 Hz, $J_{3eq,4}$ = 6.3 Hz), 2.4-2.3 (m, 1H, H-4) 2.1-1.6 (m, 4H, H-8, H-7).- $C_9H_{18}N_2O_2Cl_2$ (257.2) Calcd. C 42.0 H 7.05 N 10.9 Cl 27.6 Found C 42.1 H 7.35 N 10.7 Cl 27.4.

(2'RS,3'RS,3SR)-3-(3'-Hydroxy-2'-piperidinyl)-4-aminobutanoic acid hydrochloride (**19**)

18 or the crude oil obtained by hydrogenation of **14** over Rh/C was chromatographed on Dowex 50Wx4 (H^+ form, 20-50 mesh, Fluka) eluting with N-NH_4OH. The eluate was evaporated *i. vac.*, the residue was taken up with ethanol and an ether solution of HCl was added. The white solid was filtered and recrystallized from ethanol/ethyl ether. Yield 41% from **18** and 20% from **14**. Mp. 190-195°.- IR (KBr): 3500-3200 (OH alcohol, NH_2); 2900-2500 (OH, NH_2^+); 1690 (C=O) cm^{-1}.- ^{1}H-NMR (300 MHz, CD_3OD): δ (ppm) = 4.01 (m, 1H, H-3eq, W/2 = 8 Hz), 3.55 (dd, 1H, 1 CH_2N, J_{gem} = 9.6 Hz, J = 8.4 Hz), 3.4-3.2 (m, 3H, 1 CH_2N, H-2ax, H-6eq), 3.1-2.8 (m, 2H, H-6ax, CH), 2.45 (dd, 1H, 1 CH_2CO, J_{gem} = 16.8 Hz, J = 9 Hz), 2.34 (dd, 1H, 1 CH_2CO, J_{gem} = 16.8 Hz, J = 10.2 Hz), 2.2-1.8 (m, 2H, H-5), 1.8-1.6 (m, 2H, H-4).- $C_9H_{19}N_2O_3Cl$ (238.7) Calcd. C 45.3 H 8.02 N 11.7 Cl 14.9 Found C 45.6 H 8.20 N 12.0 Cl 15.0.

Pharmacology

$GABA_A$ and $GABA_B$ binding

The preparation of synaptosomal membranes from whole rat brain was carried out basically according to *Zukin*[17].

$GABA_A$ receptor binding was measured by incubating synaptic membranes (0.5 mg protein) for 15 min at 4° with appropriate concentrations of the test compounds in 0.05 M Tris-citrate buffer, pH 7.1, in the presence of 10 nM [^{3}H]GABA (Amersham, 47.5 Ci/mmol). Bound [^{3}H]GABA was separated from the medium by centrifugation at 48 000 g for 10 min. Non specific binding was determined by including 0.1 mM cold GABA in the incubation buffer.

In the $GABA_B$ binding assay the synaptic membranes (0.8 mg protein) were incubated for 15 min at room temp. in 0.05 M Tris-HCl buffer, pH 7.4, containing 2.5 mM $CaCl_2$, with the compounds under investigation in the presence of 40 µM isoguvacine (RBI) and 10 nM [^{3}H]GABA. The subsequent steps of the procedure[18] were the same as those for $GABA_A$ receptor binding. All compounds were tested at the maximal 10^{-4} M concentration.

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