Hydroxy- and Amino-Substituted Piperidinecarboxylic Acids as γ-Aminobutyric Acid Agonists and Uptake Inhibitors

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The syntheses of (3*RS*,4*RS*)-4-hydroxypiperidine-3-carboxylic acid (**4**), (3*RS*,5*SR*)-5-hydroxypiperidine-3-carboxylic acid (**20**), (3*RS*,4*SR*)-4-acetamidopiperidine-3-carboxylic acid (**10**), and (3*RS*,5*SR*)-5-acetamidopiperidine-3-carboxylic acid (**18**), related to the specific γ-aminobutyric acid (GABA) uptake inhibitors (*RS*)-piperidine-3-carboxylic acid (nipecotic acid) and (3*RS*,4*SR*)-4-hydroxypiperidine-3-carboxylic acid (**21**), are described. Furthermore, (3*RS*,4*SR*)-3-hydroxypiperidine-4-carboxylic acid (**14**), related to the specific GABA agonist piperidine-4-carboxylic acid (isonipecotic acid), has been synthesized. The structures of **4**, **10**, **14**, **18**, and **20** have been established by 270-MHz ^{1}H NMR spectroscopic analyses. The affinity of the compounds for the GABA receptors and for the neuronal (synaptosomal) GABA uptake system in vitro has been measured. Compound **14** interacts selectively with the GABA receptors but less effectively than isonipecotic acid and the cis-isomer **22**. Compounds **4**, **18**, and **20** are inhibitors of the GABA uptake system, although much weaker than nipecotic acid and (3*RS*,4*SR*)-4-hydroxypiperidine-3-carboxylic acid (**21**). Compound **10** is inactive in both test systems.

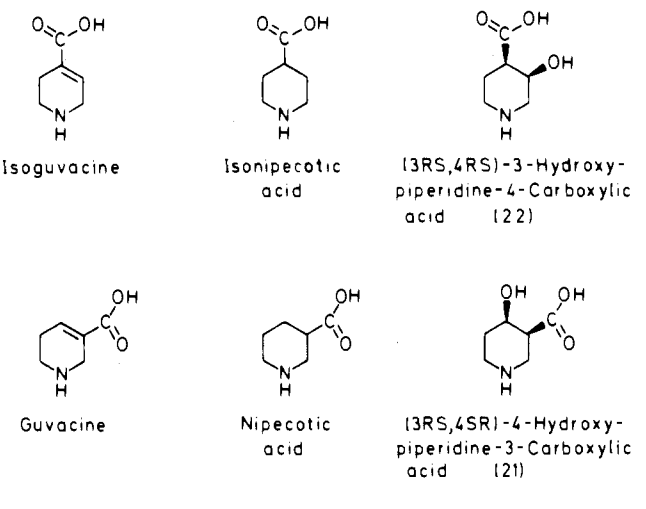
Malfunctions of the central GABA (γ-aminobutyric acid) neurotransmitter system seem to contribute to the development of certain psychiatric and neurological diseases.[1,2] Consequently, agonists at the postsynaptic GABA receptors and inhibitors of the GABA uptake systems have considerable pharmacological interest.

Structure–activity studies on a variety of conformationally restrained GABA analogues have demonstrated that GABA interacts with its transport carriers in conformations that are different from its "receptor-active conformation(s)".[3-5] Thus, isoguvacine and piperidine-4-carboxylic acid (isonipecotic acid) are specific and very effective GABA agonists for the postsynaptic GABA receptors,[6,7] whereas the isomeric compounds, guvacine and (*RS*)-piperidine-3-carboxylic acid (nipecotic acid) (Chart I), are potent inhibitors of neuronal and glial GABA uptake, with no affinity for the GABA receptors.[8,9]

(3*RS*,4*SR*)-4-Hydroxypiperidine-3-carboxylic acid (the cis isomer, **21**) is equipotent with nipecotic acid as an inhibitor of GABA uptake.[4] While nipecotic acid inhibits equally effectively neuronal and glial GABA uptake, the former compound preferentially interacts with the glial uptake system. (3*RS*,4*RS*)-3-Hydroxypiperidine-4-carboxylic acid (the cis isomer, **22**; Chart I), on the other hand, is a specific but relatively weak inhibitor of the binding of GABA to its receptor sites as compared to isonipecotic acid and isoguvacine.[5]

A variety of cyclic amino acids related to nipecotic acid and **21**, including (3*RS*,4*SR*)- (the trans isomer) and (3*RS*,4*RS*)-4-aminopiperidine-3-carboxylic acid (the cis isomer), (3*RS*,4*SR*)- (the cis isomer) and (3*RS*,4*RS*)-4-mercaptopiperidine-3-carboxylic acid (the trans isomer), and (3*RS*,5*SR*)-5-aminopiperidine-3-carboxylic acid (the cis isomer), have been synthesized and tested as inhibitors of GABA uptake.[10,11] These studies have demonstrated that even minor structural variations of nipecotic acid and **21** (Chart I) result in compounds that are inactive or very weak inhibitors of GABA uptake, emphasizing the pronounced structural specificity of the GABA uptake processes.

Chart I. Structures of Some Heterocyclic GABA Agonists and GABA Uptake Inhibitors



In continuation of these conformation–activity studies, we here describe the syntheses and biological activity of

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(10) P. Krogsgaard-Larsen, K. Thyssen, and K. Schaumburg, *Acta Chem. Scand., Ser. B*, **32**, 327 (1978).
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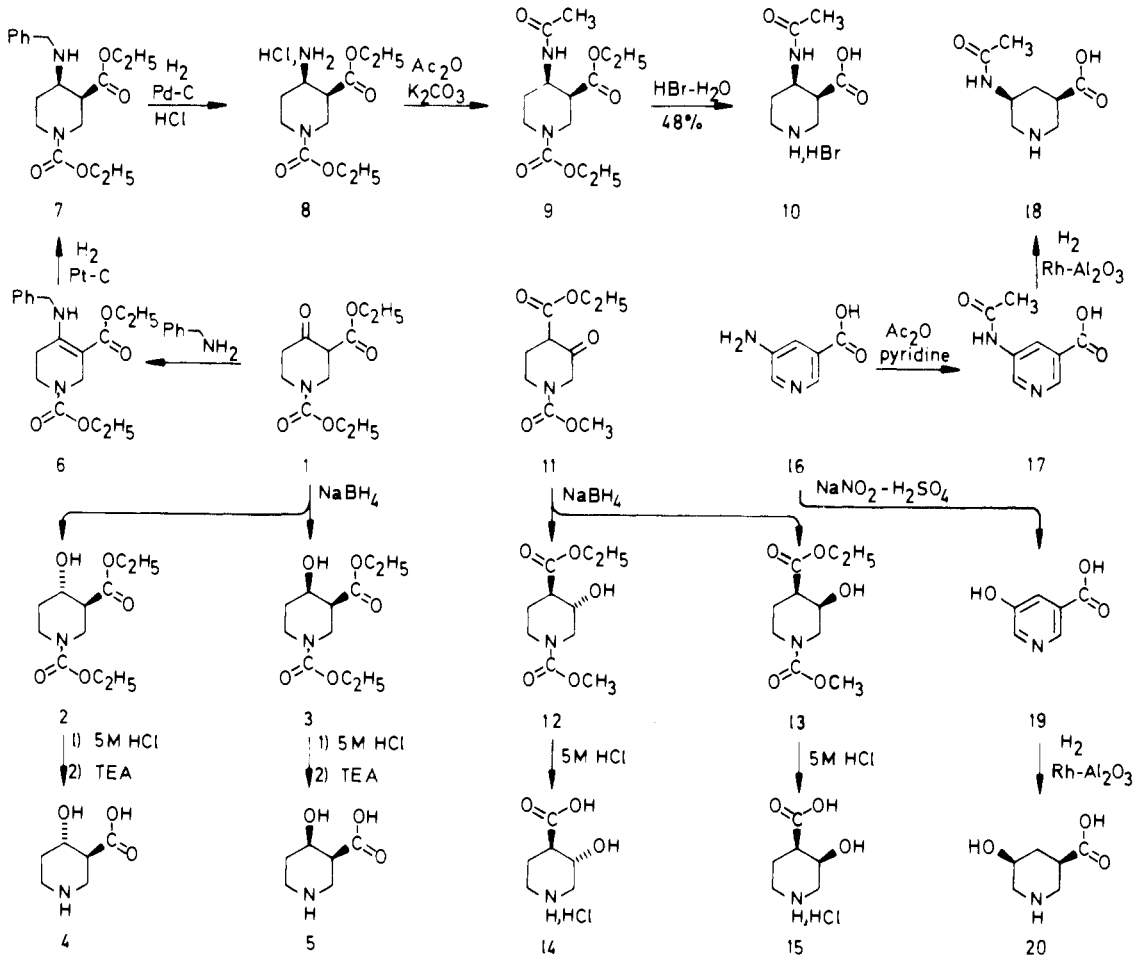
Scheme I



a series of substituted piperidinecarboxylic acids in order to obtain further information about the preferred orientations of substituents with regard to biological activity. A series of compounds related to nipecotic acid have been prepared, i.e., (3*RS*,4*RS*)-4-hydroxypiperidine-3-carboxylic acid (**4**, the trans isomer), (3*RS*,5*SR*)-5-hydroxypiperidine-3-carboxylic acid (**20**, the cis isomer), (3*RS*,4*SR*)-4-acetamidopiperidine-3-carboxylic acid (**10**, the cis isomer), and (3*RS*,5*SR*)-5-acetamidopiperidine-3-carboxylic acid (**18**, the cis isomer). We here describe the synthesis of these compounds and of the GABA agonist (3*RS*,4*SR*)-3-hydroxypiperidine-4-carboxylic acid (**14**, the trans isomer) and studies of their affinity for the GABA receptors and the neuronal (synaptosomal) GABA uptake system in vitro.

Chemistry and Spectroscopy. High-pressure hydrogenation of the β-oxo esters **1** and **11** has earlier been shown to proceed stereospecifically to give the respective *cis*-β-hydroxy compounds **3** and **13**.[10,15] Reduction of **1** and **11** with sodium borohydride gave separable mixtures of the corresponding *cis*- and *trans*-β-hydroxy derivatives (Scheme I), the cis-isomers **3** and **13** being the major products. Acid treatment of the hydroxy esters gave the deprotected β-hydroxy amino acids **4**, **5**, **14**, and **15**, the cis-isomers **5** and **15** being identical with those earlier described.[10,15]

Condensation of the β-oxo ester **1** with benzylamine gave the enamine **6** (Scheme I), which by stepwise hydrogenation yielded the *cis*-4-amino derivative **8** via **7** by analogy with a method previously described.[11] Acetylation of **8**, followed by appropriate acid treatment, gave the *cis*-4-acetamido compound **10**. Acetylation of 5-aminonicotinic acid (**16**), followed by catalytic hydrogenation of the acetylated derivative **17** with rhodium–Al_2O_3 as a catalyst, gave **18** as the only product.

Treatment of **16** with sodium nitrite in aqueous sulfuric acid, followed by treatment of the intermediate diazo compound with boiling aqueous sulfuric acid, gave 5-hydroxynicotinic acid (**19**). Hydrogenation of **19** gave the *cis*-5-hydroxy amino acid **20** as the only product. Compound **20** was identical with that previously described[12] as a byproduct after hydrogenation of 5-aminonicotinic acid, with PtO_2 as a catalyst.

Using low temperature ^1H NMR, it has been established[16-19] that the chemical shift difference ($\Delta\delta$) between the geminal protons at C(2) and C(6) in piperidines is 0.5–1.0 ppm, depending on the solvent,[20] with the axial

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(13) P. Krogsgaard-Larsen, P. Jacobsen, L. Brehm, J.-J. Larsen, and K. Schaumburg, *Eur. J. Med. Chem.*, **15**, 529 (1980).
(14) L. Brehm, P. Krogsgaard-Larsen, and P. Jacobsen, in ref 2, p 247.
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Table I. Some Values Pertinent to the Conformational State of a Number of Cyclic GABA Analogues Calculated from ^{1}H NMR Data

	18	10	20	14	4	21	22
$\frac{\delta_{2a} + \delta_{2e}}{2}$	3.24	3.32	3.23	3.17	3.32	3.32	3.28
$\frac{\delta_{6a} + \delta_{6e}}{2}$	3.14	3.15	3.29	3.19	3.28	3.23	3.23
$\Delta\delta_2$	0.60	−0.15	0.39	0.52	0.32	0.02	0.24
$\Delta\delta_6$	0.77	0.0	0.60	0.31	0.28	0.0	0.41
α^a	0.92	0.81	0.79	0.76	0.60	0.82	
α^b	0.85		0.76	0.63	0.65		0.98
α	1.0[c]		0.83[c]	0.76[d]	0.76[c]		
R^e				1.94	2.17		1.87

[a] Calculated on the basis of the coupling between the axial protons at C(2) and C(3): $\alpha = (J_{exp} - 2.5)/10.5$. [b] Calculated on the basis of the coupling between the axial protons at C(5) and C(6). [c] Calculated on the basis of $\Delta\delta_2$ using $\alpha = 0.5 + (\Delta\delta_2/1.2)$. [d] Calculated on the basis of $\Delta\delta_6$ using $\alpha = 0.5 + (\Delta\delta_6/1.2)$. [e] See ref 24.

proton shifted upfield. In positions 3–5 the corresponding shift difference is 0.4–0.5 ppm.[20] The enhancement of $\Delta\delta$ in positions 2 and 6 is caused by the configuration having an axial lone pair. The compounds studied are present as zwitterions (**4**, **18**, and **20**) or as acidic salts (**10** and **14**) in solution. The effect of protonation is a uniform downfield chemical shift of the protons in positions 2 and 6.[22] Based on ^{13}C NMR spectroscopic studies on piperidines, there is a preference for axial orientation of the lone pair (ΔG = 1255.2 J/mol).[19,21]

At room temperature, nitrogen inversion and ring inversion are both rapid processes, and the observed chemical shifts and coupling constants are weighted average values. The averages $(\delta_{2a} + \delta_{2e})/2$ and $(\delta_{6a} + \delta_{6e})/2$ should remain constant, if the substituents do not produce large anisotropic contributions to the shielding or modify the preference for the lone-pair orientation. The average shifts based on the ^{1}H NMR data are given in Table I. Data for two 3,4-cis-configurated compounds **21**[10] and **22**[22] have been included in the table for comparison. The very similar average values lead to the conclusion that the substituted piperidines in this study have a similar proportion of free nitrogen lone-pair orientations.

Like the relative configuration of **21**[10] and **22**,[22] those of **4**, **10**, **14**, **18**, and **20** were established by analysis of the 270-MHz ^{1}H NMR spectra. The vicinal coupling constants provide the conclusive information. In **4** the magnitude of the coupling constants between protons on C(2) and C(3) are typical for axial–axial and equatorial–axial configuration of the protons. The coupling constants between the C(4) proton and the C(5) protons likewise indicate an axial orientation of the C(4) proton. The 3,4-trans configuration of **4** is hereby established. The coupling constants found in the analysis of the spectrum of **14** are very similar to those of **4**, indicating a 3,4-trans configuration of **14**. In **18** the coupling constants identify the protons at C(3) and C(5) as located preferentially in the diaxial position, consistent with a 3,5-cis configuration in **18**. The same conclusion is reached for **20**. In **10** the coupling constants between the C(2) protons and the C(3) proton point out the preferred axial orientation of the proton at C(3). The mutual coupling between the protons at C(3) and C(4) and between C(4) and C(5) are consistent with a preferred equatorial orientation of the proton at C(4) corresponding to a 3,4-cis configuration of **10**.

The biased ring conformation is of a certain interest for the series of compounds studied. Information is available from the chemical-shift differences, $\delta_{eq} - \delta_{ax} = \Delta\delta$, as well as from the vicinal coupling constants. The $\Delta\delta_2$ or $\Delta\delta_6$ may be used when the substituents are primarily in an equatorial position,[20] whereas axial substituents influence the $\Delta\delta$ values so strongly that the estimate is unreliable. In Table I the fraction (α) of the dominating conformation is calculated with $(\delta_{eq} - \delta_{ax})$ = 0.6 ppm, as observed in positions 2 and 6 of piperidine[20] at low temperature. The estimates of α based on the vicinal coupling constants are assuming the axial–axial coupling constant between C(2) and C(3) to be 13 Hz and the equatorial–equatorial coupling constant to be 2.5 Hz.[23]

The three estimates are based on simplification and should be used with caution. The trends within the series are, however, valid. It is seen that **18** and **22** are almost exclusively present in one conformation, permitting COOH to occupy the preferred equatorial position and OH to occupy the preferred axial position. In **20**, **14**, and **4**, the conflict between the orientational preference of the two substituents leads to a less biased average but still with the COOH group primarily in the equatorial position.

In order to check the tacit assumption of an undisturbed chair, we calculated the R value,[24] where the data allowed this to be done. The obtained values correspond to slight distortions of the ring. Similar distortions have previously been found by the R value method and X-ray crystallographic investigation of substituted piperidines.[22]

Biological Results and Discussion

The procedure of Enna and Snyder[25] with the modifications earlier described[6] was used for studies of the compounds as inhibitors of the binding of [^{3}H]GABA to a synaptosomal membrane fraction isolated from rat brain. The procedures used for the isolation of a crude synaptosomal fraction (synaptosomes) and for the measurement of the inhibition of the uptake of [^{3}H]GABA are described elsewhere in detail.[26]

Compound **14** interacts with the GABA receptors without affecting GABA uptake (Table II) like the structurally related compounds isonipecotic acid[6,7] and (3*RS*,4*RS*)-3-hydroxypiperidine-4-carboxylic acid.[5] The effect of **14** is, however, much weaker than that of isonipecotic acid and somewhat weaker than that of the cis-isomeric compound **22**.

The nipecotic acid analogues **4**, **18**, and **20** interact with the GABA uptake system without affecting the GABA

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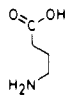
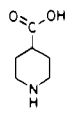
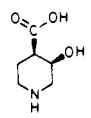
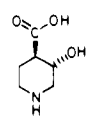
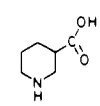
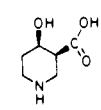
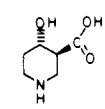
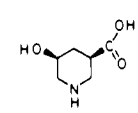
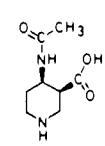
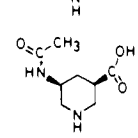
Table II. Inhibition of GABA Receptor Binding and GABA Uptake in Synaptosomes

COMPOUND	FORMULA	pK_A VALUES	INHIBITION OF GABA RECEPTOR BINDING IC_{50} VALUES (μM)	INHIBITION OF GABA UPTAKE IN SYNAPTOSOMES IC_{50} VALUES (μM)
GABA		4.0,10.7	0.033	2
Isonipecotic acid		3.8,10.5	0.33	>300
22		3.7,10.0	12	>300
14		3.6,9.3	43	>300
Nipecotic acid		3.9,10.3	>100	6
21		3.4,10.0	>100	11
4		3.1,9.4	>100	203
20		3.5,8.9	>100	173
10		3.2,9.7	>100	>300
18		3.0,8.6	>100	240

receptors, whereas compound **10** is inactive in both test systems (Table II).

The piperidinium rings of **21**[27] and nipecotic acid[28] adopt chair conformations in the crystalline state and in aqueous solution, and in both compounds the carboxylate groups have predominantly equatorial orientation. Consequently, the hydroxy group in the former compound is in an axial position.[27] The ^1H NMR spectroscopic analyses reveal that **20** and **4** have predominantly equatorial orientation of both the hydroxy and the carboxylate groups. Both compounds have only weak effects on the GABA uptake system, **4** being 20 times less potent than the cis-isomeric compound **21** (Table II). These studies suggest that equatorial orientation of the hydroxy group hinders these compounds from binding to the GABA transport carrier. It must, however, be emphasized that the present studies have not disclosed the conformations, in which these compounds are transported through the synaptic membrane.

The acetylated amino compounds **10** and **18** have very weak or no effects on GABA uptake (Table II), as previously found for the corresponding compounds with free amino groups.[11,12] In compound **10** the preferred orientation of the substituents are the same as in the *cis*-4-hydroxy compound **21** with the 4-substituent in a preferentially axial orientation. These results, together with previous structure–activity studies on GABA uptake inhibitors,[11,12] further underline that nipecotic acid substituted with amino or protected amino groups in the 3-, 4-, or 5-position in general have little or no effect on the GABA processes.

Experimental Section

Melting points, determined in capillary tubes, are corrected. Analyses, indicated by elemental symbols, were within ±0.4% of the theroetical values and were performed by G. Cornali, Micoranalytical Laboratory, Leo Pharmaceutical Products, DK-2750 Ballerup, Denmark. TLC and column chromatography (CC) were accomplished with silica gel F_{254} plates (Merck) and silica gel (Woelm 0.063–0.100 mm), respectively. Columns were developed by stepwise gradient elution. The pK_a values were determined as described earlier,[29] the numbers in parentheses being the estimated standard deviation on the last significant digit.

A Perkin-Elmer grating infrared spectrophotometer (Model 247), a Perkin-Elmer ultraviolet–visible spectrophotometer (Model 402), and a JEOL JMN-C-60HL (60 MHz) ^1H NMR instrument were used. ^1H NMR spectra were recorded with Me_4Si as an internal standard. Compounds dissolved in D_2O were referred to TSP. In some cases the analysis of the spectrum could only be completed using Me_2SO-d_6 as a solvent. The 270-MHz ^1H NMR spectra were obtained on a Bruker HX-270 S instrument operating at 193 K for aqueous solutions and at 333 K for Me_2SO-d_6 solutions. We used Fourier transform mode to obtain the spectra with a width of 5000 Hz, using 16K data points and quadrature detection. Spectra were transformed with 16K zerofilling. Homodecoupling was used to verify the interpretations of the spectra and provide starting parameters for the analysis of the spectra. ^1H NMR spectra were simulated with the SIMEQ program[30] and a Varian 620/i computer. The iterative refinement was obtained with the MIMER program[31] on a UNIVAC 1100/82 at the Regional Computing Centre at the University of Copenhagen (RECKU).

^{13}C NMR spectra were obtained at 67 889 MHz on the same spectrometer with the same samples. The Fourier transform mode was used to obtain the proton decoupled and coupled spectra, the latter with gated decoupling. Sample temperature was 305 K, and 5K transients were accumulated in 32K data points and an acquisition time of 1 s. The pulse width used was 6 μs corresponding to 60 °C. Chemical-shift data are referred to TSP.

(3*RS*,4*RS*)- and (3*RS*,4*SR*)-Ethyl 1-(Ethoxycarbonyl)-4-hydroxypiperidine-3-carboxylate (2 and 3). To a solution of **1**[32] (10 g, 43.7 mmol) in ethanol (150 mL) was added gradually sodium borohydride (1 g; 26 mmol). After stirring at room temperature for 3 h, the mixture was evaporated in vacuo. Aqueous hydrochloric acid (75 mL, 4 M) was added, and the mixture was extracted with ethyl acetate (4 × 100 mL). The combined and dried ($MgSO_4$) ethyl acetate phases were evaporated in vacuo. Column chromatography [silica gel, 500 g; eluents: cyclohexane containing ethyl acetate (25–66%)] gave **2** (1 g, 10%) and **3** (6 g, 60%) and a mixture of **2** and **3** (1 g, 10%). An analytical sample of **2** was purified by ball-tube distillation at 100 Pa (oven tem-

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perature 220 °C): IR (film) 3445 (s), 3030–2830 (m, several bands), 1740–1640 (s), 1470 (s), 1430 (s) cm^{-1}; ^1H NMR (60 MHz, $CDCl_3$) δ 4.39–3.45 (4 H, m), 3.12 (1 H, d), 2.94–2.8 (2 H, m), 2.63–2.35 (2 H, m), 2.2 (1 H, s), 2.15–1.66 (2 H, m), 1.40–1.20 (6 H, m). Anal. ($C_{11}H_{19}NO_5$) C, H, N. An analytical sample of **3** was purified by recrystallization (ethyl acetate–cyclohexane–light petroleum): mp 71–72 °C; IR (KBr) 3450 (s), 3030–2830 (m, several bands), 1720–1640 (s), 1470 (s), 1430 (s) cm^{-1}; ^1H NMR (60 MHz, $CDCl_3$) δ 4.49–3.83 (4 H, m), 3.8–3.2 (5 H, m), 3.16–3.03 (1 H, m), 2.83–2.43 (2 H, m), 2.23–1.49 (2 H, m), 1.46–1.06 (6 H, m). Anal. ($C_{11}H_{19}NO_5$) C, H, N.

(3*RS*,4*RS*)- and (3*RS*,4*SR*)-4-Hydroxypiperidine-3-carboxylic Acid (4 and 5). A solution of **2** (1.0 g, 4.1 mmol) in hydrochloric acid (40 mL, 5 M) was refluxed for 2 h. Evaporation in vacuo, followed by subsequent addition of ethanol (5 mL) and a solution of TEA (412 mg, 4.1 mmol) in ethanol (3 mL), yielded **4** (280 mg, 45%): mp 230–232 °C dec; IR (KBr) 3500–3200 (m, several bands), 3200–2200 (m, several bands), 1630 (s), 1580 (s), 1480 (s) cm^{-1}; ^{13}C NMR δ 46.3 [C(2), t], 51.6 [C(3) d], 69.0 [C(4) d], 31.3 [C(5) t], 44.2 [C(6) t], 180.4 (COOH); ^1H NMR (270 MHz, D_2O) δ_{2a} 3.16, δ_{2e} 3.48, δ_{3a} 2.60, δ_{4a} 4.10, δ_{5a} 1.77, δ_{5e} 2.14, δ_{6a} 3.14, δ_{6e} 3.42 ($J_{2a,2e}$ = −12.7, $J_{2a,3a}$ = 8.85, $J_{2e,3a}$ = 3.82, $J_{3a,4a}$ = 8.29, $J_{4a,5a}$ = 8.85, J_{4a5e} = 3.67, $J_{5a,5e}$ = −14.10, $J_{5a,6a}$ = 9.30, $J_{5a,6e}$ = 3.66, $J_{5e,6a}$ = 3.66, $J_{5e,6e}$ = 6.65, $J_{6a,6e}$ = −12.51 Hz). Anal. Calcd for $C_6H_{11}NO_3$: C, 49.64; H, 7.64; N, 9.56. Found: C, 48.85; H, 7.70; N, 9.17. pK_a values (H_2O, 24 °C): 3.10 ± 0.07; 9.41 ± 0.02. In a similar manner using **3** (1.0 g, 4.1 mmol) as the starting materials, we obtained **5** (370 mg, 60%). The spectral data were identical with an authentic sample prepared as previously reported.[10]

Ethyl 1-(Ethoxycarbonyl)-4-(benzylamino)-1,2,5,6-tetrahydropyridine-3-carboxylate (6). To a solution of 1[32] (10.0 g, 41.2 mmol) in toluene (150 mL) was added benzylamine (5.0 g, 46.7 mmol) and molecular sieve 3A, Union Carbide (10 g). The mixture was refluxed for 20 h with a Dean–Stark water separator. Evaporation in vacuo, followed by column chromatography [silica gel, 200 g; eluents: dichloromethane containing ethyl acetate (60–80%)] gave **6** (9.3 g, 68%): IR (film) 3300 (w), 3100–2800 (m, several bands), 1700 (s), 1650 (s), 1600 (s), 1440 (s), 1240 (s) cm^{-1}; UV (methanol) 296 nm (log ϵ 4.15); ^1H NMR (60 MHz, $CDCl_3$) δ 9.4 (1 H, broad signal), 7.25 (5 H, s), 4.5–3.9 (8 H, m), 3.40 (2 H, t), 2.4 (2 H, m), 1.25 (6 H, t).

(3*RS*,4*SR*)-Ethyl 1-(Ethoxycarbonyl)-4-(benzylamino)-piperidine-3-carboxylate (7). A solution of **6** (8.0 g, 24.1 mmol) in ethanol (500 mL) was hydrogenated (ca. 6 MPa) with a 5% Pt/C catalyst (4 g) for 72 h. Evaporation in vacuo, followed by column chromatography [silica gel, 250 g; eluents: dichloromethane containing ethyl acetate (60–90%)] gave **7** (6.0 g, 77%). An analytical sample was purified by ball-tube distillation at 50 Pa (oven temperature 250 °C): IR (film) 3500 (w), 3100–2900 (m, several bands), 1720 (s), 1700 (s), 1480 (s), 1460 (s), 1410 (m), 1240 (s), 1190 (s) cm^{-1}; ^1H NMR (60 MHz, $CDCl_3$) δ 7.25 (5 H, s), 4.13 (4 H, q), 3.75 (2 H, d), 3.7–3.3 (4 H, m), 3.2–2.9 (1 H, m), 2.9–2.5 (1 H, m), 2.36 (1 H, s), 1.9–1.5 (2 H, m), 1.23 (6 H, t). Anal. ($C_{18}H_{26}N_2O_4$) C, H, N.

(3*RS*,4*SR*)-Ethyl 1-(Ethoxycarbonyl)-4-acetamidopiperidine-3-carboxylate (9). A solution of **7** (4.0 g, 12.0 mmol) and aqueous hydrochloric acid (24.0 mL, 0.5 M) in aqueous ethanol (400 mL, 50%) was hydrogenated (ca. 300 kPa) in a PARR low-pressure hydrogenation apparatus with a 5% Pd/C catalyst (1 g) for 24 h. Evaporation in vacuo gave crude **8**, characterized by TLC [R_f 0.43; eluent: butanol–acetic acid–water (4:1:1)]. To an ice-cooled solution of crude **8** in water (7 mL) was added an iced solution of potassium carbonate (4.1 g, 29 mmol) in water (5 mL) with stirring, followed by addition of acetic anhydride (2.5 g, 24 mmol). Stirring was continued at 0 °C for 45 min. The solution was acidified with 4 M aqueous hydrochloric acid and then extracted with ethyl acetate (3 × 50 mL). The combined and dried ($MgSO_4$) ethyl acetate phases were evaporated in vacuo. Column chromatography [silica gel, 200 g; eluents: ethyl acetate containing acetic acid (2%)] gave **9** (2.0 g, 59%): IR (film) 3300 (m), 2970 (m), 1710–1660 (s, several bands), 1540 (s), 1430 (s), 1240 (s) cm^{-1}; ^1H NMR (60 MHz, $CDCl_3$) δ 4.10 (2 H, q), 4.05 (2 H, q), 4.5–3.7 (3 H, m), 3.3–2.6 (3 H, m), 2.03 (1 H, s), 1.97 (3 H, s), 1.9–1.5 (2 H, m), 1.25 (3 H, t), 1.20 (3 H, t). Anal. ($C_{13}H_{22}N_2O_5$) C, H, N.

(3*RS*,4*SR*)-4-Acetamido-3-carboxypiperidinium Bromide (10). A solution of **9** (1.0 g, 3.5 mmol) in aqueous hydrobromic acid (20 mL, 48%) was refluxed for 10 min. Evaporation in vacuo and recrystallization (water–ethanol) gave **10** (0.5 g, 53%): mp ~250 °C dec; IR (KBr) 3300 (s), 3100–2400 (m–s, several bands), 1710 (s), 1640 (s), 1540 (s), 1190 (s) cm^{-1}; ^1H NMR (60 MHz, D_2O) δ 4.4 (2 H, m), 3.5–3.2 (4 H, m), 2.0 (3 H, s), 2.2–1.8 (2 H, m); ^{13}C NMR (Me_2SO-d_6) δ 41.36 [C(2)], 41.47 [C(3)], 43.89 [C(4)], 27.17 [C(5)], 40.41 [C(6)], 23.27 (CH_3), 171.86 (COOH), 172.71 (NHCO); ^1H NMR (270 MHz, D_2O) δ_{2a} 3.42, δ_{2e} 3.26, δ_{3a} 3.05, δ_{4e} 4.49, δ_{5a} 1.90, δ_{5e} 1.77, δ_{6a} 3.15, δ_{6e} 3.15 ($J_{2a,2e}$ = −13.23, $J_{2a,3a}$ = 11.03, $J_{2e,3a}$ = 4.05, $J_{3a,4e}$ = 4.0, $J_{4e,5a}$ = 4.1, $J_{4e,5e}$ = 4.3, $J_{5a,5e}$ = −14.5, $J_{5a,6a}$ + $J_{5a,6e}$ = 16.0, $J_{5e,6a}$ = 4.3, $J_{5e,6e}$ = 4.3 Hz). Anal. ($C_8H_{15}N_2O_3Br$) C, H, N, Br. pK_a values (H_2O, 24 °C): 3.15 ± 0.10; 9.73 ± 0.05.

(3*RS*,4*SR*)- and (3*RS*,4*RS*)-Ethyl 1-(Methoxycarbonyl)-3-hydroxypiperidine-4-carboxylate (12 and 13). Compounds **12** and **13** were synthesized from 11[33] (10.0 g, 43.7 mmol) as described above for **2** and **3**. Column chromatography [silica gel, 500 g; eluents: dichloromethane containing ethyl acetate (50–75%)] gave **12** (1.0 g, 10%) and **13** (6.0 g, 60%). An analytical sample of **12** was purified by ball-tube distillation at 100 Pa (oven temperature 250 °C): IR (film) 3700 (s, broad signal), 2950 (w), 1720–1680 (s, several bands), 1480 (s), 1450 (s), 1260 (m), 1230 (m), 1180 (s) cm^{-1}; ^1H NMR (60 MHz, $CDCl_3$) δ 4.17 (2 H, q), 4.3–3.8 (3 H, m), 3.63 (3 H, s), 3.3 (1 H, broad signal), 3.1–2.1 (3 H, m), 2.0–1.6 (2 H, m), 1.25 (3 H, t). Anal. ($C_{10}H_{17}NO_3$) C, H, N. An analytical sample of **13** was recrystallized (toluene–light petroleum): mp 84.0–85.0 °C; IR (KBr) 3450 (s), 2950 (w), 1720–1680 (s, several bands), 1480 (s), 1360 (w), 1260 (w), 1170 (s) cm^{-1}; ^1H NMR (60 MHz, $CDCl_3$) δ 4.4–3.9 (5 H, m), 3.68 (3 H, s), 3.25 (1 H, d), 3.1 (1 H, m), 2.9 (1 H, m), 2.5–1.7 (3 H, m), 1.28 (3 H, t). Anal. ($C_{10}H_{17}NO_5$) C, H, N.

(3*RS*,4*SR*)- and (3*RS*,4*RS*)-3-Hydroxy-4-carboxypiperidinium Chloride (15). A solution of **12** (0.5 g, 2.3 mmol) in aqueous hydrochloric acid (5 mL, 6 M) was refluxed for 2 h. Evaporation in vacuo and recrystallization (ethanol–ether) gave **14** (0.2 g, 50%): mp 196–198 °C dec; IR (KBr) 3400 (s), 3000 (s), 1700 (s), 1580 (m), 1400 (m), 1370 (m), 1200 (s) cm^{-1}; ^{13}C NMR δ 52.8 [C(2)], 67.4 [C(3)], 48.9 [C(4)], 24.2 [C(5)], 47.4 [C(6)], 184.1 (COOH); ^1H NMR (270 MHz, D_2O) δ_{2a} 2.91, δ_{2e} 3.43, δ_{3a} 4.12, δ_{4a} 2.66, δ_{5a} 1.89, δ_{5e} 2.22, δ_{6a} 3.03, δ_{6e} 3.34 ($J_{2a,2e}$ = −12.54, $J_{2a,3a}$ = 9.50, $J_{2e,3a}$ = 3.82, $J_{3a,4a}$ = 10.05, $J_{4a,5a}$ = 9.11, $J_{4a,5e}$ = 4.10, $J_{5a,5e}$ = −14.75, $J_{5a,6a}$ = 10.05, $J_{5a,6e}$ = 4.67, $J_{5e,6a}$ = 3.0, $J_{5e,6e}$ = 4.37, $J_{6a,6e}$ = −13.26 Hz). Anal. ($C_6H_{12}NO_3Cl$) C, H, N, Cl. pK_a values (H_2O, 24 °C): 3.62 ± 0.04; 9.26 ± 0.01. In a similar manner with **13** (2.0 g, 9.2 mmol) as the starting material, **15** (0.75 g, 47%) was obtained. The spectral data were identical with those of an authentic sample prepared when the previously reported method was used.[15]

5-Acetamidonicotinic Acid (17). A solution of 16[34] (1.08 g, 7.8 mmol) in acetic anhydride (4.5 mL, 47 mmol) and pyridine (3.2 mL, 39 mmol) was heated at 120 °C for 22 h. The precipitated product was filtered and washed with two 20-mL portions of water. Recrystallization (ethanol) gave **17** (750 mg, 54%): mp 282–283 °C dec; IR (KBr) 3600–3350 (m), 3200–2250 (m, several bands), 1715 (s), 1650 (s), 1600 (s), 1580–1500 (s), 1450 (s), 1430 (s), 1420 (s), 1370 (m) cm^{-1}; ^1H NMR (60 MHz, Me_2SO-d_6) δ 12.45 (1 H, s), 9.0–8.5 (3 H, m), 5.9–4.5 (1 H, broad signal), 2.13 (3 H, s). Anal. ($C_8H_8N_2O_3$) C, H, N.

(3*RS*,5*SR*)-5-Acetamidopiperidine-3-carboxylic Acid (18). A solution of **17** (650 mg, 3.4 mmol) in water (90 mL) and aqueous ammonia (2 mL , 25%) was hydrogenated (ca. 300 kPa) for 72 h in a Parr low-pressure hydrogenation apparatus with a 5% rhodium–Al_2O_3 (300 mg) catalyst. Evaporation in vacuo and recrystallization (water–ethanol) gave **18** (380 mg, 44%): mp 238–240 °C dec; IR (KBr) 3600–3350 (s, several bands), 3200–2250 (m, several bands), 1660–1600 (s, broad band), 1600–1520 (s, broad band), 1395 (s) cm^{-1}; ^{13}C NMR δ 46.3 [C(2)], 43.7 [C(3)], 34.6 [C(4)], 48.0 [C(5)], 48.6 [C(6)]; ^1H NMR (270 MHz, D_2O) δ_{2a} 2.94, δ_{2e} 3.53, δ_{3a} 2.73, δ_{4a} 1.61, δ_{4e} 2.32, δ_{5a} 4.09, δ_{6a} 2.76, δ_{6e} 3.53 ($J_{2a,2e}$ = −12.7, $J_{2a,3a}$ = 12.2, $J_{2e,3a}$ = 3.9, $J_{3a,4a}$ = 12.3, $J_{3a,4e}$ = 3.9, $J_{4a,4e}$ = −12.3, $J_{4a,5a}$ = 12.3, $J_{4e,5a}$ = 4.0, $J_{5a,6a}$ = 11.4, $J_{5a,6e}$ = 4.0, $J_{6a,6e}$ = −12.9 Hz). Anal. ($C_8H_{14}N_2O_3$·0.5H_2O) C, H, N. pK_a values

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(H_2O, 25 °C): 3.00 ± 0.03; 8.56 ± 0.03.

5-Hydroxynicotinic Acid (19). To an ice-cooled solution of **16**[34] (5 g, 36.2 mmol) in sulfuric acid (37 mL, 20%) was added a solution of sodium nitrite (2.75 g, 39.8 mmol) in water (3 mL). The mixture was stirred for 10 min at 0 °C and then added over a period of 10 min to a boiling solution of sulfuric acid (35 mL, 60%). Boiling was continued until the evolution of nitrogen had ceased. The mixture was concentrated in vacuo to approximately one-half volume. The precipitated product was filtered and washed with two 50-mL portions of water. Recrystallization (ethanol) gave **19** (2.1 g, 42%): mp 293–295 °C dec; IR (KBr) 3600–3250 (s), 3000–2400 (m, several bands), 1680–1640 (s), 1540 (s), 1390 (s) cm^{-1}; ^1H NMR (60 MHz; Me_2SO-d_6) δ 7.69–7.63 (1 H, m), 8.41–8.35 (1 H, m), 8.63 (1 H, s). Anal. ($C_6H_5NO_3$) C, H, N.

(3*RS*,5*SR*)-5-Hydroxypiperidine-3-carboxylic Acid (20). Compound **20** was synthesized as described above for **18** with **19** (700 mg, 5.1 mmol) as a starting material and 5% rhodium–Al_2O_3 (700 mg) as a catalyst. Recrystallization (water–ethanol) gave **20** (270 mg, 35%): mp 249–250 °C dec; IR (KBr) 3500–3225 (s), 3200–2500 (m, several bands), 1680–1515 (s, several bands), 1380 (s) cm^{-1}; ^{13}C NMR δ 47.94 [C(2)], 42.43 [C(3)], 36.96 [C(4)], 66.05 [C(5)], 50.77 [C(6)], 181.74 (COOH); ^1H NMR (270 MHz, D_2O) δ_{2a} 3.04, δ_{2e} 3.42, δ_{3a} 2.66, δ_{4a} 1.68, δ_{4e} 2.34, δ_{5a} 4.02, δ_{6a} 3.44 (δ_{2e} and δ_{6a} overlapping) ($J_{2a,2e}$ = −12.8, $J_{2a,3a}$ = 10.8, $J_{2e,3a}$ = 4.1, $J_{3a,4a}$ = 10.2, $J_{3a,4e}$ = 4.0, $J_{4a,4e}$ = −13.12, $J_{4a,5a}$ = 10.2, $J_{4e,5a}$ = 4.0, $J_{5a,6a}$ = 9.5, $J_{5a,6e}$ = 4.1, $J_{6a,6e}$ = −12.4 Hz). Anal. ($C_6H_{11}NO_3 \cdot 0.5H_2O$) C, H, N. p$K_a$ values (H_2O, 24 °C): 3.45 ± 0.06; 8.78 ± 0.01.

Inhibition of GABA Receptor Binding. The [^{3}H]GABA binding assay was performed with rat brain synaptic membranes as described earlier in detail.[6] For the standard [^{3}H]GABA binding assay procedure, aliquots of synaptic membranes (0.8–1.2 mg of protein) were incubated in triplicate at 4 °C for 5 min in 2 mL of 0.05 M Tris–citrate buffer (pH 7.1), which was 5 nM with respect to [^{3}H]GABA and 1 mM with respect to GABA or 100 μM with respect to the indicated compounds (Table II): If [^{3}H]GABA binding was inhibited by more than 50% at this concentration, we estimated the concentration of inhibitor producing 50% inhibition (IC_{50}) by examining the effects of at least four different concentrations of inhibitor and performing a log-probit analysis of the results. Protein was measured by the method of Lowry et al.[35]

Inhibition of Neuronal (Synaptosomal) GABA Uptake. The rat brain synaptosomes were prepared as described elsewhere.[26] The whole brains were homogenized in 10 volumes of ice-cold 0.32 M sucrose, and the homogenate was centrifuged at 600*g* at 4 °C for 10 min. The pellet was discarded, and the supernatant was centrifuged at 25000*g* at 4 °C for 55 min. The pellet fraction was resuspended in 100 volumes of oxygenated phosphate medium at 0 °C, and 500 μL 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 an additional 10 min. The synaptosomes were isolated by rapid filtration through Whatman GF/C glass fiber filters, and the filters were washed with phosphate medium (10 mL). The filters were transferred to scintillation vials, and the radioactivity was measured by liquid scintillation counting after addition of 3 mL of Liposolve/Lipolume/water (1:10:0.2) (Lumac, Basel). The IC_{50} values for inhibition of high-affinity neuronal (synaptosomal) GABA uptake at 50 nM GABA with preincubation of the tissue for 10 min in the presence of inhibitor were determined as described elsewhere in detail.[36]

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