Pergamon

Bioorganic & Medicinal Chemistry, Vol. 5, No. 11, pp 2089–2094, 1997

PII: S0968-0896(97)00143-0
0968-0896/97 $17.00 + 0.00

A Novel Class of Potent γ-Aminobutyric Acid Aminotransferase Inhibitor, 3-(Hydroxyamino)propylamine and Analogues

Shinji Fushiya,* Toshiyuki Kanazawa and Shigeo Nozoe
Faculty of Pharmaceutical Sciences, Tohoku University, Aobayama, Aoba-ku, Sendai 980-77, Japan

Abstract—Hydroxyamino analogues of γ-aminobutyric acid (GABA) were synthesized and evaluated for inhibitory activity toward γ-aminobutyric acid aminotransferase (GABA-T). The title compound, 3-(hydroxyamino)propylamine (HPA), showed a potent inhibitory activity. The inhibition is competitive with respect to GABA and the K_i value of GABA-T for HPA is 0.4 mmol. The activity of inhibition is comparable to those of aminoxyacetic acid and valproic acid. 3-(Hydroxyaminomethyl)piperidine (3HMP), a cyclic analogue of HPA, also showed a potent inhibitory activity, whereas 3-(methoxyamino)propylamine (OMe-HPA), 3-(*N*-hydroxy-*N*-methylamino)propylamine (NMe-HPA) and 4-(hydroxyamino)piperidine (4HP) showed weak activity. © 1997 Elsevier Science Ltd.

Introduction

γ-Amino butyric acid (GABA) is a major inhibitory neurotransmitter in the mammalian central nervous system (CNS) and controls the neurotransmission together with a major excitatory neurotransmitter, L-glutamic acid. The brain concentration of GABA is principally controlled by the synthetic enzyme, glutamic acid decarboxylase (EC 4.1.1.15; GAD) and the catabolic enzyme, γ-aminobutyric acid amino transferase (EC 2.6.1.19; GABA-T). GABA concentrations in the brains of some cases such as Huntington's disease are markedly low because of imperfection in the metabolism,[1] and the increase of GABA-T activity is observed in Alzheimer's disease.[2] So, it is not only GABA receptor agonists but also GABA-T inhibitors, which keep the GABA concentration moderate, that are expected to be medicines for such brain diseases. In fact, 2-propylpentanoic acid (valproic acid),[3] a reversible inhibitor of GABA-T, and 4-amino-5-hexenoic acid (γ-vinyl GABA),[4,5,6] a mechanism-based inactivator of the enzyme, have been used as anticonvulsants and/or antiepileptic agents, which prompt us to find new GABA-T inhibitors.

We previously found a potent inhibitory action of 4-*N*-hydroxy-L-2,4-diaminobutyric acid (NH-DABA) (**1**) on glutamine synthetase (GS), and suggested that the hydroxyamino moiety of NH-DABA functions as a carboxylic acid in the enzyme reaction.[7] We therefore synthesized GABA analogues (**2–6**), which have hydroxyamino groups in the place of the carboxylic acid of GABA, and evaluated them for inhibitory action on GABA-T. Among the compounds tested, 3-(hydroxyamino)propylamine (HPA, **2**) and 3-(hydroxyaminomethyl)piperidine (3HMP, **5**), a cyclic analogue of HPA, showed potent inhibitory activity against GABA-T. The synthesis and the inhibitory properties of these compounds are described in this paper.

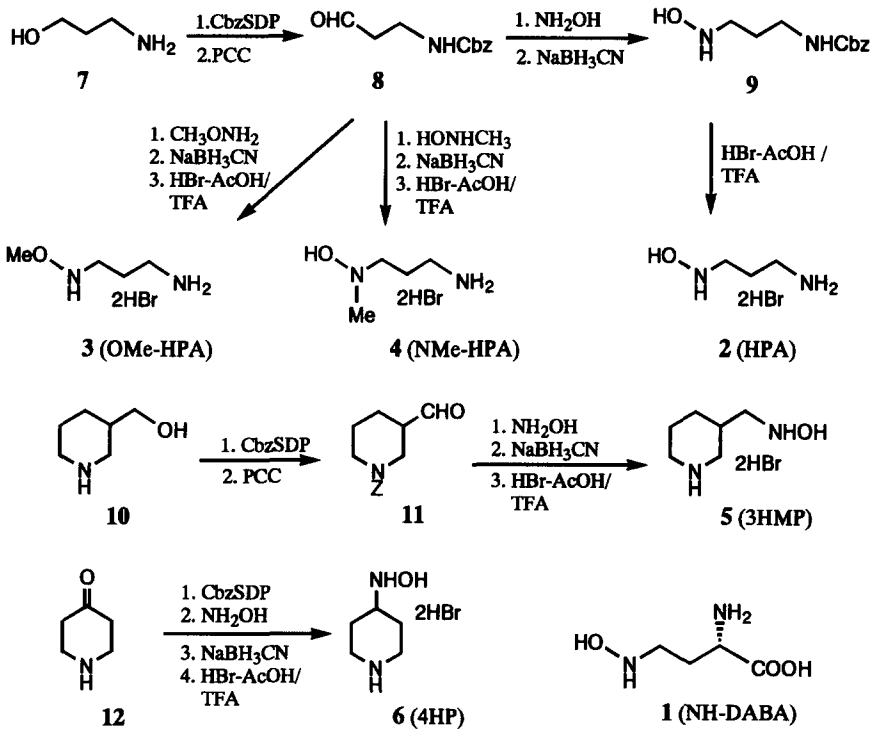
Results and Discussion

Chemistry

GABA analogues **2–6** were synthesized by reductive coupling of the corresponding carbonyl compounds and hydroxylamine derivatives with sodium cyanoborohydride ($NaBH_3CN$).[7] Thus, reduction of the stereoisomeric mixture of oximes obtained by the reaction of hydroxylamine hydrochloride and aldehyde **8**, prepared from 3-amino-1-propanol (**7**), yielded a hydroxylamine derivative **9**. Removal of carbobenzoxyl (Cbz) group of **9** by 25% hydrobromic acid in acetic acid/trifluoroacetic acid gave HPA dihydrobromide (**2**). OMe-HPA (**3**) and NMe-HPA (**4**) were prepared by the same sequence of reactions employing *N*-methylhydroxylamine hydrochloride and *O*-methylhydroxylamine hydrochloride in the place of hydroxylamine hydrochloride, respectively. 3HMP (**5**) was synthesized by reductive coupling of aldehyde **11**, derived from 3-(hydroxymethyl)piperidine (**10**), and hydroxylamine hydrochloride after deprotection. 4HP (**6**) was prepared from 4-piperidone (**12**) by the same sequence of operations.

Evaluation of inhibitory activity

GABA-T activity was measured in vitro by a coupled spectroscopic assay using Gabase from *Pseudomonas fluorescens* which contains both GABA-T and succinic semialdehyde dehydrogenase (SSADH).[8,9] In this assay, GABA is converted to succinic semialdehyde by GABA-T and then to succinate by SSADH with a stoichiometric reduction of NADP to NADPH which was recorded by the rate of decrease in absorption at



Scheme 1.

340 nm. The inhibitory activity of HPA on GABA-T was tested at various GABA and inhibitor concentrations (Fig. 1). At the concentrations tested in this experiment (0–3.0 mM), HPA is a competitive inhibitor of GABA with GABA-T, and the K_i value for HPA on GABA-T is 0.4 mM.

The inhibitory activity of HPA was compared with those of known inhibitors and the results are summarized in Table 1. Under our experimental conditions (6 mM GABA), HPA inhibited GABA-T activity by 74% at a 3.0 mM concentration, whereas aminooxyacetic acid (AOAA) and valproic acid (VA) inhibited the enzyme by 72% and 62% at the same concentrations, respectively. Further, OMe-HPA and NMe-HPA, *O*-methyl and *N*-methyl derivatives of hydroxyamino moiety of HPA, showed weaker inhibitory activity than HPA (39% and 29% inhibitions at the same concentrations, respectively).

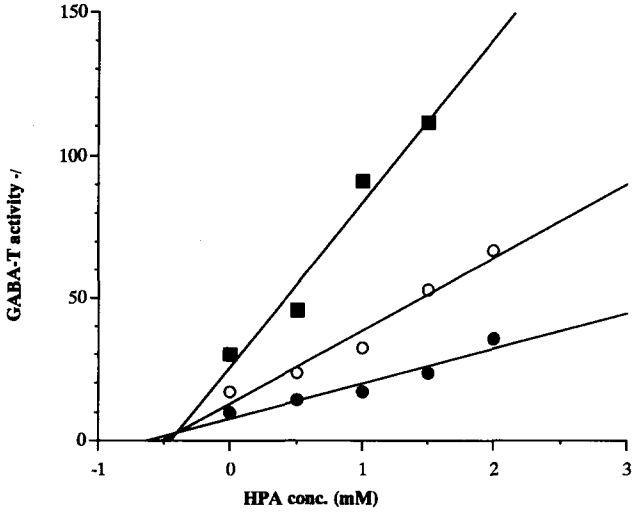


Figure 1. Inhibitory action of 3-(hydroxyamino)propylamine (HPA) on GABA-T. Plot of $1/v$ against inhibitor concentration at varying GABA concentrations (●: 4.0 mM, ○: 3.0 mM, ■: 2.0 mM).

From the present results, it is concluded that HPA is a strong inhibitor of GABA-T and the free hydroxyamino group plays an important role in the inhibition.

It is well known that hydroxylamine and AOAA inhibit GABA-T, which requires pyridoxalphosphate (PLP) as a cofactor, by forming Schiff's bases between PLP and the hydroxyamino moieties of the compounds. This indicates that the hydroxyamino moiety of HPA probably functions as an amine. To examine this idea, the effect of PLP on the inhibition was studied. Hydroxylamine inhibited GABA-T activity by 73% at

Table 1. Inhibitory action of compounds on GABA-T (percent inhibition)

Concn (mM)	HPA (2)	AOAA[a]	NH-DABA (1)	VA[b]
1.0	53	56	34	33
2.0	67	65	46	51
3.0	74	72	53	62

[a]AOAA: aminoxyacetic acid.
[b]VA: valproic acid.

Table 2. Inhibitory action of HPA analogues on GABA-T (percent inhibition)

Concn (mM)	OMe-HPA (3)	NMe-HPA (4)	3HMP (5)	4HP (6)
1.0	20	14	46	15
2.0	32	26	66	37
3.0	39	29	78	5

Table 3. Inhibitory action of HPA and hydroxylamine on GABA-T with or without 1 mM PLP (percent inhibition)

Concn (mM)	HPA (2)		Hydroxylamine	
	PLP[a] (−)	PLP (+)	PLP (−)	PLP (+)
1.0	53	53	73	56
2.0	67	67	81	70
3.0	74	73	87	77

[a]PLP: pyridoxal phosphate.

a 1.0 mM concentration, and the inhibition was decreased to 56% by addition of 1 mM PLP. On the other hand, the inhibition of HPA was not influenced by PLP addition (Table 3). Thus the inhibition of GABA-T by HPA is not result of Schiff's base formation between HPA and PLP.

In the previous work, we have demonstrated that NH-DABA inhibited glutamine synthetase (GS) and proposed the possibility of the hydroxyamino group of NH-DABA serving as a carboxylic acid mimic in the enzyme reaction. The hydroxyamino group of HPA might function as a carboxylic acid mimic, too.

Next we examined inhibitory activity of cyclic analogues of HPA, 3-(hydroxyaminomethyl)piperidine (3HMP) and 4-(hydroxyamino)piperidine (4HP) (Table 2). 3HMP showed the same activity with that of HPA (78% inhibition at a 3.0 mM concentration), whereas the activity of 4HP was weaker than that of HPA (51% inhibition at the same concentration). These findings indicate the necessity of conformational flexibility of the hydroxyamino moiety for inhibitory activity.

Conclusion

This study showed that HPA and 3HMP, hydyoxyamino analogues of GABA, strongly inhibited GABA-T and that a free hydroxyamino group and conformational flexibility of the hydroxyamino group are necessary for the strong inhibitory activity. These results imply the possibility of the hydroxyamino group being an effective mimic of carboxylic acid for designing enzyme inhibitors.

Experimental Section

General

Melting points were determined with a Yanagimoto micro melting point apparatus and are uncorrected. Elemental analyses were performed by A. Sato, Faculty of Pharmaceutical Sciences, Tohoku University, and were in agreement within ± 0.4% of the proposal structures. Mass spectra were recorded with Hitachi M-52G (EI) and a JEOL JMS-01 SG-2 spectrometer (FD). IR spectra were recorded on a JASCO infrared spectrometer (Model A-100S). ^{1}H NMR spectra were recorded on JEOL spectrometers (Model PMX 60 SI, 60 MHz, and FX 100, 100 MHz). The ^{1}H NMR spectra were recorded with Me_4Si as internal standard. IR and NMR spectral data were recorded for all numbered compounds and were judged to be consistent with the assigned structures.

3-Benzyloxycarbonylamino-1-propanal (8). To a solution of 3-amino-1-propanol (**7**) (3.76 g, 50 mmol) and triethylamine (14.0 mL, 100 mmol) in water (30 mL) was added benzyl *S*-(4,6-dimethylpyrimidin-2-yl)thiolcarbonate (CbzSDP) (16.5 g, 69 mmol) in dioxane (30 mL). After being stirred at room temperature for 2 h, the reaction mixture was extracted with ethyl acetate (30 mL × 3). The combined organic layer was washed with brine and dried over Na_2SO_4. After filtration, the extract was concentrated by evaporation. The residue was subjected to silica gel column chromatography eluting with hexane:ethyl acetate (3:1) to give 3-benzyloxycarbonylamino-1-propanol (**13**) (9.7 g, 93%). To a stirred suspension of pyridinium chlorochromate (6.59 g, 30.6 mmol, Aldrich) and Celite (6.5 g) in CH_2Cl_2 (40 mL) was added **13** (4.26 g, 20.4 mmol) in CH_2Cl_2 (25 mL) in one portion, and the mixture was stirred at room temperature for 4 h. Ether (70 mL) was added to the reaction mixture, and the mixture was subjected to a Florisil column eluting with ether (700 mL). After the solvent was removed, the remaining oily residue was chromatographed on a silica gel column eluting with hexane:ethyl acetate (4:1) to afford **8** (2.02 g, 48%), which was recrystallized from CH_2Cl_2:hexane. Mp 53–55 °C. IR ν_{max} ($CHCl_3$) cm^{-1}: 3460, 3000, 1725, 1520. ^{1}H NMR (100 MHz, $CDCl_3$): δ 2.71 (2H, t, J=7.7 Hz), 3.49 (2H, q, J=7.7 Hz), 5.06 (2H, s), 7.31 (5H, s), 9.77 (1H, s). Anal. calcd for $C_{11}H_{13}NO_3$: C, 63.75; H, 6.32; N, 6.76; found: C, 63.54; H, 6.32; N, 6.73.

3-(Hydroxyamino)propylamine (HPA) (2)

A mixture of **8** (887 mg, 4.2 mmol) and NH_2OH·HCl (450 mg, 6.6 mmol) in pyridine (10 mL) and ethanol (10 mL) was stirred at room temperature for 12 h. The reaction mixture was concentrated and the oily residue was chromatographed on a silica gel column eluting with hexane:ethyl acetate (2:1) to give a geometrically isomeric mixture of oximes **14** as white powder (882 mg, 95%), which was recrystallized from methanol. Mp 117–119 °C, IR ν_{max} ($CHCl_3$) cm^{-1}: 3340, 2980, 1720, 1530. ^{1}H NMR (100 MHz, $CDCl_3$): δ 2.37, 2.57 (each 1H, q, J=5.1 Hz), 3.37 (2H, q J=5.1 Hz), 5.09 (2H, s), 6.74,

7.40 (each 0.5H, t, J=5.1 Hz), 7.34 (5H, s). Anal. calcd for $C_{11}H_{14}N_2O_3$:C, 59.45; H, 6.35; N, 12.60 ; found: C, 59.42; H, 6.24; N, 12.61. To a solution of **14** (774 mg, 3.49 mmol) in methanol (25 mL) was added $NaBH_3CN$ (659 mg, 10.5 mmol, Aldrich), and the reaction mixture was stirred at room temperature for 1 h. The pH of the solution was kept at 2–3 by addition of methanolic HCl solution during the period of the reaction. The reaction mixture was neutralized with 1 N NaOH and concentrated by evaporation. Water (30 mL) was added to the residue, and the mixture was extracted with $CHCl_3$ (30 mL × 3). The combined organic layer was washed with brine and dried over Na_2SO_4. After filtration, the extract was concentrated by evaporation. The residue was subjected to silica gel column chromatography eluting with *n*-hexane:ethyl acetate (1:2) to give Cbz-HPA **9** (732 mg, 94%). Mp 98–100 °C, IR ν_{max} ($CHCl_3$) cm^{-1} : 3455, 2955, 1720, 1520. ^1H NMR (100 MHz, $CDCl_3$): δ 1.60 (2H, qui, J=5.8 Hz), 2.97 (2H, t, J=5.8 Hz), 3.26 (2H, q, J=5.8 Hz), 5.06 (2H, s), 7.31 (5H, s). Anal. calcd for $C_{11}H_{16}N_2O_3$: C, 58.91; H, 7.19; N, 12.49 ; found: C, 58.88; H, 7.13; N, 12.36. To a mixture of 25% HBr in acetic acid (1.2 mL) and trifluoroacetic acid (0.5 mL) was added **9** (312 mg, 1.25 mmol), and the mixture was stirred at room temperature for 70 min. Ether was added to the reaction mixture, and the resulting precipitate was collected by filtration and recrystallized from methanol–ether to give 3-(hydroxyamino)propylamine dihydrobromide (HPA) (**2**). Mp 175–178 °C. IR ν_{max} (KBr) cm^{-1}: 3395, 3020, 2560, 1935, 1600, 1510. ^1H NMR (100 MHz, D_2O): δ 2.00–2.20 (2H, m), 3.22 (2H, t, J=7.1 Hz), 3.44 (2H, t, J=7.18 Hz). Anal. calcd for $C_3H_{12}N_2OBr_2$: C, 14.30; H, 4.50; N, 11.12; Br, 63.43; found: C, 14.04; H, 4.50; N, 10.96; Br, 61.59.

3-(Methoxyamino)propylamine (OMe-HPA) (3). A mixture of **8** (1.30 g, 6.28 mmol) and *O*-methylhydroxylamine·HCl (0.63 g, 7.53 mmol) in pyridine (12 mL) and ethanol (12 mL) was stirred at room temperature for 2 h. The reaction mixture was concentrated and the oily residue was chromatographed on a silica gel column eluting with hexane:ethyl acetate (3:1) to give a geometrically isomeric mixture of oximes **15** as colorless oil (716 mg, 48%). IR ν_{max} ($CHCl_3$) cm^{-1} : 3445, 3000, 2960, 1725, 1520. ^1H NMR (100 MHz, $CDCl_3$): δ 2.24–2.65 (2H, m), 3.17–3.52 (2H, m), 3.81, 3.86 (each 3/2H, s), 5.10 (2H, s), 6.69 (1H, t, J=5.6 Hz), 7.34 (5H, s). EI-MS: 236 (M^+); HREI-MS: 236.1169 (Calcd for $C_{12}H_{16}N_2O_3$: 236.1160). To a solution of **15** (654 mg, 2.78 mmol) in methanol (30 mL) was added $NaBH_3CN$ (526 mg, 8.35 mmol), and the reaction mixture was stirred at room temperature for 75 min. The pH of the solution was kept at 2–3 by addition of methanolic HCl solution during the period of the reaction. The reaction mixture was neutralized with 1 N NaOH and concentrated by evaporation. Water (30 mL) was added to the residue, and the mixture was extracted with $CHCl_3$ (30 mL × 3). The combined organic layer was washed with brine and dried over Na_2SO_4. After filtration, the extract was concentrated by evaporation. The residue was subjected to silica gel column chromatography eluting with *n*-hexane:ethyl acetate (1:1) to give Cbz-OMe-HPA **16** (588 mg, 89%) as colorless oil. IR ν_{max} ($CHCl_3$) cm^{-1}: 3460, 3000, 2960, 1720, 1520. ^1H NMR (100 MHz, $CDCl_3$): δ 1.50–1.80 (2H, m), 2.96 (2H, t, J=5.6 Hz), 3.28 (2H, dd, J=13.5, 5.6 Hz), 3.51 (3H, s), 5.10 (2H, s), 7.32 (5H, s). EI-MS: 238 (M^+); HREI-MS: 238.1313 (Calcd for $C_{12}H_{18}N_2O_3$: 238.1316). To a mixture of 25% HBr in acetic acid (9.7 mL) and trifluoroacetic acid (5 mL) was added **16** (184 mg, 0.76 mmol), and the mixture was stirred at room temperature for 1 h. Ether was added to the reaction mixture, and the resulting precipitate was collected by filtration and recrystallized from methanol–ether to give 3-(methoxyamino)propylamine dihydrobromide (OMe-HPA) (**3**) (103 mg, 51%). Mp 175–178 °C. IR ν_{max} (KBr) cm^{-1}: 3005, 2680, 1600, 1565, 1515. ^1H NMR (100 MHz, D_2O): δ 2.01–2.38 (2H, m), 3.21 (2H, t, J=7.1 Hz), 3.53 (2H, t, J=7.1 Hz), 3.98 (3H, s). Anal. calcd for $C_4H_{14}N_2OBr_2$: C, 18.06; H, 4.55; N, 10.52; Br, 60.08; found: C, 18.12; H, 5.10; N, 10.34; Br, 60.37.

3-(*N*-Hydroxy-*N*-methylamino)propylamine (NMe-HPA) (4). To a solution of **8** (924 mg, 4.46 mmol) and *N*-methylhydroxylamine·HCl (745 mg, 8.92 mmol) in methanol (20 mL) was added $NaBH_3CN$ (561 mg, 8.93 mmol), and the reaction mixture was stirred at room temperature for 7 h. The pH of the solution was kept at 2–3 by addition of methanolic HCl solution during the period of the reaction. The reaction mixture was neutralized with 1 N NaOH and concentrated by evaporation. Water (30 mL) was added to the residue, and the mixture was extracted with ethyl acetate (30 mL × 3). The combined organic layer was washed with brine and dried over Na_2SO_4. After filtration, the extract was concentrated by evaporation. The residue was subjected to silica gel column chromatography eluting with hexane:ethyl acetate (1:1) to give Cbz-NMe-HPA **17** (184 mg, 17%) which was recrystallized from CH_2Cl_2–hexane. Mp 175–178 °C. IR ν_{max} ($CHCl_3$) cm^{-1}: 3460, 2950, 1720, 1520. ^1H NMR (100 MHz, $CDCl_3$): δ 1.55–1.97 (2H, m), 2.40–2.73 (2H, m), 2.62 (3H, s), 3.02–3.50 (2H, m), 5.09 (2H, s), 7.32 (5H, s), 7.51 (1H, s). Anal. calcd for $C_{12}H_{18}N_2O_3$: C, 60.48; H, 7.61; N, 11.76 ; found: C, 59.95; H, 7.50; N, 11.45. To a mixture of 25% HBr in acetic acid (1 mL) and trifluoroacetic acid (6 mL) was added **17** (111 mg, 0.47 mmol), and the mixture was stirred at room temperature for 1 h. Ether was added to the reaction mixture and the resulting precipitate was collected by filtration and recrystallized from methanol–ether to give 3-(*N*-hydroxy-*N*-methylamino)propylamine dihydrobromide (NMe-HPA) (**4**) (114 mg, 95%). Mp 111–114 °C. IR ν_{max} (KBr) cm^{-1}: 3440, 2980, 2620, 1940. ^1H NMR (500 MHz, D_2O): δ 1.83–1.97 (2H, m), 2.86 (2H, t, J=8 Hz), 2.93 (3H, s), 3.24 (2H, m). Anal. calcd for $C_4H_{14}N_2OBr_2$: C, 18.06; H, 4.55; N, 10.52; Br, 60.08; found: C, 18.06; H, 4.96; N, 10.59; Br, 59.81.

3-(Hydroxyaminomethyl)piperidine (3HMP, 5). To a solution of 3-(hydroxymethyl)piperidine (**10**) (4.45 g, 38.7 mmol) and triethylamine (5.58 g, 58.1 mmol) in water (70 mL) was added benzyl *S*-(4,6-dimethylpyrimidin-2-yl)thiolcarbonate (12.75 g, 46.4 mmol) in dioxane (70 mL). After being stirred at room temperature for 20 h, the reaction mixture was extracted with ethyl acetate (70 mL × 3). The combined organic layer was washed with brine and dried over Na_2SO_4. After filtration, the extract was concentrated by evaporation. The residue was subjected to silica gel column chromatography eluting with *n*-hexane:ethyl acetate (3:1) to give Cbz derivative **18** as colorless oil (8.62 g, 89%). To a stirred suspension of pyridinium chlorochromate (8.44 g, 39.2 mmol) and Celite (8 g) in CH_2Cl_2 (80 mL) was added **18** (6.50 g, 26.1 mmol) in CH_2Cl_2 (20 mL) in one portion, and the mixture was stirred at room temperature for 4 h. Ether (100 mL) was added to the reaction mixture and the mixture was subjected to a Florisil column eluting with ether (1.2 L). After the solvent was removed, the remaining oily residue was chromatographed on a silica gel column eluting with hexane:ethyl acetate (3:1) to afford 3-formyl derivative **11** as colorless oil (4.17 g, 65%). IR ν_{max} ($CHCl_3$) cm^{-1}: 3400, 2980, 2930, 2840, 2700. ^1H NMR (100 MHz, $CDCl_3$): δ 1.38–1.91 (4H, m), 2.27–2.59 (1H, m), 3.19–3.52 (2H, m), 3.73–4.19 (2H, m), 5.13 (2H, s), 7.34 (5H,s), 9.66 (1H, s). EI-MS: 247 (M^+); HREI-MS: 247.1210 (calcd for $C_{14}H_{17}NO_3$: 247.1208). A mixture of **11** (3.70 g, 14.5 mmol) and hydroxylamine·HCl (2.08 g, 29.9 mmol) in pyridine (15 mL) and ethanol (70 mL) was stirred at room temperature for 4 days. The reaction mixture was concentrated, and water (60 mL) was added to the residue and the mixture was extracted with ethyl acetate (60 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4 and evaporated to give a geometrically isomeric mixture of oximes **19** as colorless oil (0.99 g, 25%). To a solution of **19** (774 mg, 2.95 mmol) in methanol (20 mL) was added $NaBH_3CN$ (716 mg, 11.3 mmol), and the reaction mixture was stirred at room temperature for 1 h. The pH of the solution was kept at 2–3 by addition of methanolic HCl solution during the period of the reaction. The reaction mixture was neutralized with 1 N NaOH and concentrated by evaporation. Water (30 mL) was added to the residue and the mixture was extracted with ethyl acetate (30 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4 and concentrated to give crude Cbz-3HMP **20** (930 mg, quant). To a mixture of 25% HBr in acetic acid (9.7 mL) and trifluoroacetic acid (4.6 mL) was added **20** (930 mg) and the mixture was stirred at room temperature for 1 h. Ether was added to the reaction mixture, and the resulting precipitate was collected by filtration and recrystallized from methanol–ether to give 3-(hydroxyaminomethyl)piperidine dihydrobromide (3HMP) (**5**) (688 mg, 21% from **19**): Mp 175–178 °C. IR ν_{max} (KBr) cm^{-1}: 3380, 2860, 1570. ^1H NMR (100 MHz, D_2O): δ 1.26–1.84 (2H, m), 1.91–2.14 (2H, m), 2.23–2.63 (1H, m), 2.71–3.14 (2H, m), 3.31 (2H, d, J=5.1 Hz), 3.40–3.63 (2H, m). Anal. calcd for $C_6H_{16}N_2OBr_2$: C, 24.85; H, 5.56; N, 9.66; Br, 55.11; found: C, 24.94; H, 5.50; N, 9.80; Br, 55.08.

4-Hydroxyaminopiperidine (4HP, 6). To a solution of 4-piperidone (**12**) (2.07 g, 20.9 mmol) and triethylamine (3.17 g, 31.3 mmol) in water (50 mL) was added benzyl *S*-(4,6-dimethylpyrimidin-2-yl)thiolcarbonate (6.88 g, 25.1 mmol) in dioxane (50 mL). After being stirred at room temperature for 4 h, the reaction mixture was extracted with CH_2Cl_2 (50 mL × 3). The combined organic layer was washed with brine and dried over Na_2SO_4. After filtration, the extract was concentrated by evaporation. The residue was subjected to silica gel column chromatography eluting with *n*-hexane:ethyl acetate (4:1) to give Cbz derivative **21** as colorless oil (805 mg, 17%). A mixture of **21** (805 mg, 3.45 mmol) and hydroxylamine·HCl (686 mg, 9.58 mmol) in pyridine (10 mL) and ethanol (15 mL) was stirred at room temperature for 18 h. The reaction mixture was concentrated, and water (30 mL) was added to the residue, and the mixture was extracted with CH_2Cl_2 (30 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4 and evaporated to give a geometrically isomeric mixture of oximes **22** as colorless oil (786 mg, 92%). IR ν_{max} ($CHCl_3$) cm^{-1}: 3590, 3300, 3000, 1690. ^1H NMR (60 MHz, $CDCl_3$): δ 2.30, 2.60 (each 2H, t, J=6 Hz), 3.31, 3.53 (each 2H, t, J=6 Hz), 5.07 (2H, s), 7.20 (5H, s). EI-MS: 248 (M^+); HREI-MS: 248.1166 (calcd for $C_{13}H_{16}N_2O_3$: 248.1161). To a solution of **22** (749 mg, 3.02 mmol) in methanol (10 mL) was added $NaBH_3CN$ (571 mg, 9.06 mmol), and the reaction mixture was stirred at room temperature for 1 h. The pH of the solution was kept at 2–3 by addition of methanolic HCl solution during the period of the reaction. The reaction mixture was neutralized with 1 N NaOH and concentrated by evaporation. Water (30 mL) was added to the residue and the mixture was extracted with ethyl acetate (30 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4 and concentrated to give crude Cbz-4HP **23** (751 mg). To a mixture of 25% HBr in acetic acid (3.5 mL) and trifluoroacetic acid (8 mL) was added crude **23** (674 mg) and the mixture was stirred at room temperature for 1 h. Ether was added to the reaction mixture, and the resulting precipitate was collected by filtration and recrystallized from methanol to give 4-(hydroxyamino)piperidine dihydrobromide (4HP) (**6**) (388 mg, 51% from **22**). Mp 223–225 °C. IR ν_{max} (KBr) cm^{-1}: 2970, 2800, 2460, 1595, 1500. ^1H NMR (100 MHz, D_2O): δ 1.60–2.57 (4H, m), 2.66–3.74 (4H, m), 3.77–4.00 (1H, m). Anal. calcd for $C_5H_{14}N_2OBr_2$: C, 21.60; H, 5.08; N, 10.08; Br, 57.49 ; found: C, 21.73; H, 5.06; N, 10.28; Br, 57.23.

GABA-T activity

GABA-T activity was determined using a modified version of a literature procedure.[9] The reaction

mixtures contained Gabase (0.04 U, 0.02 mL), 2-mercaptoethanol (3.3 mM), α-ketoglutarate (5.0 mM), NADP (1.25 mM), GABA (6 mM), potassium phosphate buffer (pH 8.6), and inhibitor as indicated, final vol 3.0 mL. Enzyme activity was determined by observing the change in absorbance at 340 nm on a Hitachi-UV3200 spectrophotometer at 25 °C after several minutes of incubation and compared with a control in which no inhibitor was added.

Acknowledgements

This research was supported in part by Grant-in-Aid for General Scientific Research from the Ministry of Education, Science and Culture, Japan.

(*Received in Japan* 19 *May* 1997; *accepted* 30 *June* 1997)

References

1. Perry, T. L.; Hansen, S. *New Engl. J. Med.* **1973**, *288*, 337.
2. Aoyagi, T.; Wada, T.; Nagai, M.; Kojima, F.; Harada, S.; Takeuchi, T.; Takahashi, H.; Hirokawa, K.; Tsumita, T. *Chem. Pharm. Bull.* **1990**, *38*, 1748.
3. Simler, S.; Ciesielski, L.; Maitre, M.; Randrianarisoa, H.; Mandel, P. *Biochem. Pharmacol.* **1973**, *22*, 1701.
4. Lippert, B.; Metcalf, B. W.; Jumg, M. J.; Carasa, P. *Eur. J. Biochem.* **1977**, *74*, 441.
5. Larsen, P. K. *J. Med. Chem.* **1981**, *24*, 1377.
6. Gale, K.; Iadarola, M. J. *Science* **1980**, *208*, 288.
7. Fushiya, S.; Maeda, K.; Funayama, T.; Nozoe, S. *J. Med. Chem.* **1988**, *31*, 481.
8. Silverman, R. B.; Levy, M. A. *Biochemistry* **1981**, *20*, 1197.
9. Silverman, R. B.; Invergo, B. J.; Levy, M. A.; Andrew, C. R. *J. Med. Chem.* **1990**, *33*, 2944.