β-Carbolines as Benzodiazepine Receptor Ligands II: Synthesis and Benzodiazepine Receptor Affinity of β-Carboline-3-carboxylic Acid Amides

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Abstract □ Numerous β-carboline-3-carboxamides were synthesized by amidation of β-carboline-3-carboxylic acid, with various amino acids and amino acid esters serving as amine components, and tested in respect to their affinity for the benzodiazepine receptor in mouse brain membranes. The title compounds have affinities in the low micromolar range. The results are discussed with respect to their relevance for a possible β-carboline structure containing the endogenous ligand of the benzodiazepine receptor.

The initial observations that β-carboline derivatives, like harmane and norharmane and esters of β-carboline-3-carboxylic acid, bind with moderate or even high affinity to the benzodiazepine receptor[1,2] have activated a vigorous search for structurally related compounds.[3-6] Interest was further stimulated by the findings that the interaction of β-carbolines with the benzodiazepine receptor can cause quite different pharmacological effects. These range from benzodiazepine-opposite, CNS-exciting properties to benzodiazepine-like, CNS-depressing properties. Subsequent investigations indicated that all these quite different effects can be explained by benzodiazepine receptor mediated modulations of GABAergic transmission, with a reduction of GABAergic inhibition on the one side and an enhancement of GABAergic transmission on the other side. These observations have finally led to the concept of benzodiazepine receptor ligands with negative and positive efficacy (inverse agonists and agonists).[7,8]

Scientific interest has also been focused on the question of whether or not β-carbolines can be related to an endogenous ligand of the benzodiazepine receptor.[1,4,9] None of the presently known β-carboline derivatives can be finally considered in this respect because of the low affinity relative to the brain concentration (harmane, norharmane) or because of their absence in the brain (β-carboline-3-carboxylates).[1,2] Some authors generally question the presence of such an endogenous compound since the benzodiazepine receptor may represent only an allosteric site modulating GABAergic transmission and not an independent neuronal system.[10,11] However, recent experimental evidence points again at the presence of an endogenous ligand with β-carboline-like properties.[12,13]

Accordingly, our interest is directed to the search for β-carboline derivatives which on the one hand exhibit a high affinity for the benzodiazepine receptor and on the other hand possess a structure that can be formed by biochemical pathways. Relatively high receptor affinities have been reported for several β-carboline-3-carboxamides including a glycine derivative.[3] Contrary to the β-carboline-3-carboxylates, a biochemical pathway is feasible for β-carboline-3-carboxamides if physiological amino acids are considered as amine components. Based on this approach, the present communication describes the synthesis of numerous amides of β-carboline-3-carboxylic acid, with amino acids serving as amine components. In addition to various physiological L-amino acids (*S*-configuration), two D-amino acids (*R*-configuration) were incorporated in order to observe possible stereoselectivity in binding. Structure–affinity relationships for the benzodiazepine receptor and the relevance of the findings in the search for the still unknown endogenous ligand of the benzodiazepine receptor are discussed.

Results

Chemistry—The amides of β-carboline-3-carboxylic acid, compounds **4–7** were prepared as outlined in Schemes I–III. Methyl 1,2,3,4-tetrahydro-β-carboline-3-carboxylate (**1**) was synthesized as reported previously.[4] The dehydrogenation of **1** with lead tetraacetate[4] was modified, resulting in methyl β-carboline-3-carboxylate hydrogen oxalate (**2**). Alkaline hydrolysis of **2** gave β-carboline-3-carboxylic acid (**3**). Amides **4** were prepared in high yield by reaction of **3** with 1,1′-carbonyldiimidazole and subsequent addition of an amine.

Catalytic hydrogenation of *S*-(+)-N_α-(β-carboline-3-carbonyl)-N_ϵ-(benzyloxycarbonyl)lysine methyl ester (**4f**) readily yielded *S*-(+)-N_α-(β-carboline-3-carbonyl)lysine methyl ester (**5**). The *N*-(β-carboline-3-carbonyl)amino acids **6** and **7** were obtained by partial saponification of the appropriate *N*-(β-carboline-3-carbonyl)amino acid esters **4** and **5**.

The physical and pharmacological properties of these compounds are given in Table I. The $[\alpha]_D^{20}$ is −9.5° (c 2.0, methanol) for **4i**, +9.6° (c 2, methanol) for **4j**, −45.7° (c 0.4, Me_2SO) for **7i**, and +45.5° (c = 0.4, Me_2SO) for **7j**.

Binding to the Benzodiazepine Receptor—All newly synthesized β-carboline derivatives interact with the benzodiazepine receptor at micromolar concentrations as indicated by the half-maximal inhibitory concentrations (IC_{50}) in Table I. The relatively high affinity of some simple carboxamides was confirmed: *N*-methyl β-carboline-3-carboxamide, IC_{50} = 360 nM (lit.[3] IC_{50} = 444 nM); *N*-ethyl β-carboline-3-carboxamide, IC_{50} = 160 nM (lit.[3] IC_{50} = 460 nM). However, out of all new compounds summarized in Table I, the glycine methyl ester derivative **4a** was the only one with an IC_{50} below 1 μM. The glycine derivative **7a** was about four times less active with an IC_{50} of 1.3 μM (Table I).

Similar observations were made for most other amino acid derivatives where usually the esters exhibit higher affinities than the free amino acid derivatives (Table I). This agrees with previous findings about the relatively low affinity of β-carboline-3-carboxylic acid[4] and indicates that the anionic charge interferes with the binding to the benzodiazepine receptor. The differences between the affinities of the esterified and free amino acid derivatives tend to decrease in accordance with the affinity of the mother compound; moderately potent derivatives have large differences [(*S*)-histidine, **4j/7j**: 16-fold; (*S*)-N_ϵ-

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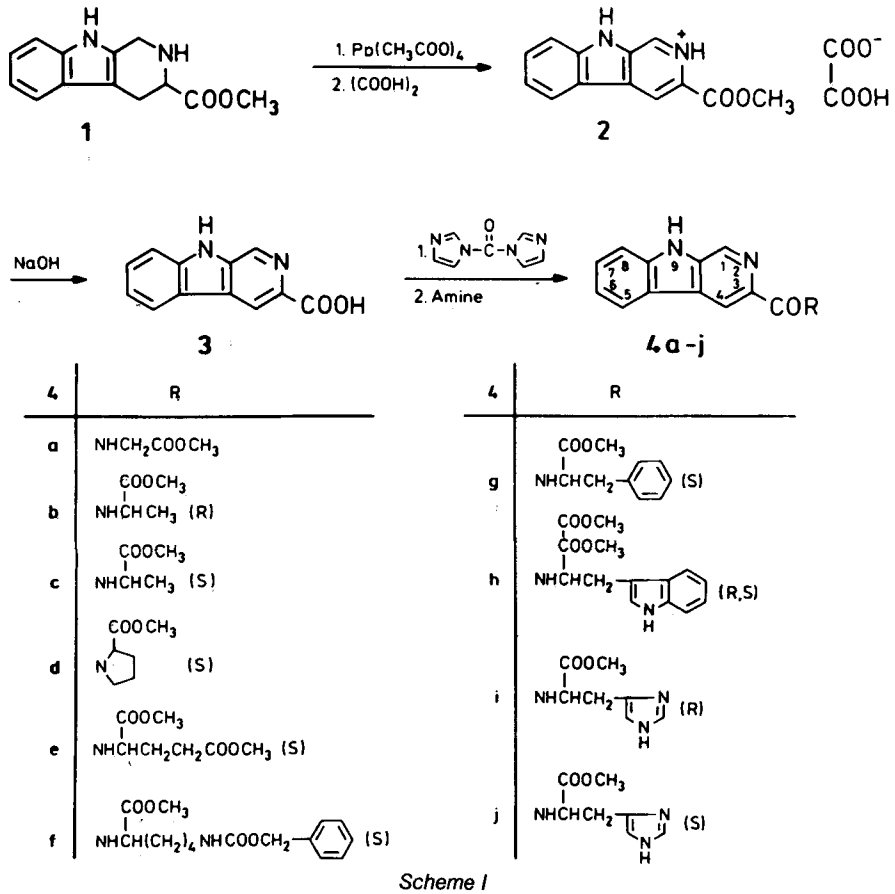
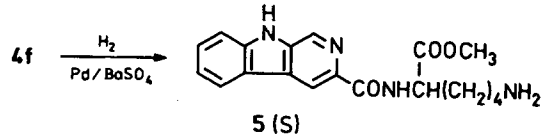
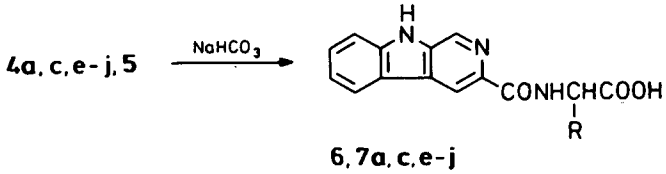
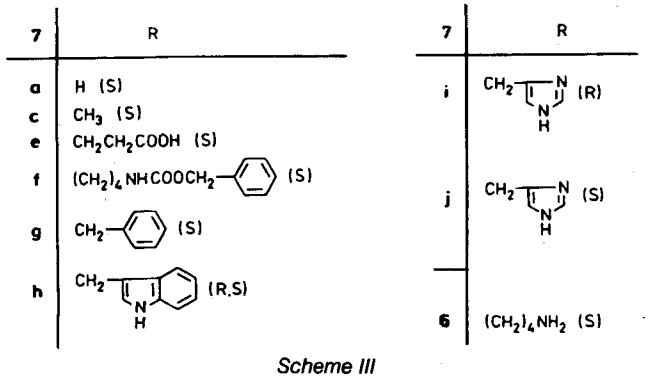


Scheme I



Scheme II





Scheme III

CBZ-lysine, **4f/7f**: 10-fold; (*S*)-alanine, **4c/7c**: 7-fold], while less active compounds have only small differences [(*R,S*)-tryptophan, **4h/7h**; (*S*)-glutamic acid, **4e/7e**; (*S*)-lysine, **5/6**: ~2-fold]. In the case of the (*S*)-phenylalanine derivative **4g** the partial saponification brings a boost in affinity.

There are only small differences between the affinities of all amino acid ester derivatives with IC_{50} values ranging from 1 to 3 μM (Table I). Exceptions are the already mentioned glycine methyl ester derivative **4a** with an IC_{50} of 0.3 μM, the (*S*)-glutamic acid dimethyl ester derivative **4e** with an IC_{50} of 9.8 μM, and the (*S*)-phenylalanine methyl ester derivative **4g** with an IC_{50} of 23 μM. Similarly, the range of the IC_{50} values of the free amino acid derivatives is also relatively small, from 7 μM [(*R,S*)-tryptophan, **7h**] to ~20 μM [(*S*)-glutamic acid, **7e**; (*S*)-histidine, **7j**; Table I]. Here also the glycine derivative **7a**, with an IC_{50} of 1.3 μM, was somewhat more active.

For some of the compounds the inhibition of specific [^{3}H]-flunitrazepam binding in the mouse brain in vivo after intravenous administration was also investigated. However, these experiments were limited by the relatively low affinities of the compounds combined with their low solubility in the injection buffer. Accordingly, only *N*-ethyl β-carboline-3-carboxamide showed a significant inhibition of specific [^{3}H]flunitrazepam binding in vivo 10 min after intravenous administration of 15 μmol/kg (percent inhibition in vivo: 20.4 ± 4.7). This agrees with the relatively high in vitro affinity of this compound. In any case, our data do not indicate higher in vivo activities relative to the in vitro affinities for the few compounds tested (Table I).

Discussion

Considering the pronounced differences in the affinities for the benzodiazepine receptor we had found previously within a group of ester derivatives of β-carboline-3-carboxylic acid,[4] only small variations of the affinities were apparent within the group of amino acid β-carboline-3-carboxamides reported in this communication (Table I). Moreover, compared with the high affinities of the methyl, ethyl, and propyl β-carboline-3-carboxylates with IC_{50} values in the low nanomolar range,[4] all the compounds reported in the present study have only moderate to low affinities for the benzodiazepine receptor (Table I). While the β-carboline structure itself is important, the addition of a small ester group at position 3 obviously adds a further point of attachment and has a tremendous effect on the affinity (β-

Table I—Amides of β-Carboline-3-carboxylic Acid

Compound	Amine Component	mp, °C	Formula[d]	[^{3}H]Flunitrazepam Binding IC_{50}, nM[e]	Inhibition In Vivo, %[f]
4a	Glycine methyl ester	248–249[a]	$C_{15}H_{13}N_3O_3$	370 ± 90	—
4b	*(R)*-(−)-Alanine methyl ester	248–249[a]	$C_{16}H_{15}N_3O_3$	1900 ± 900	—
4c	*(S)*-(+)-Alanine methyl ester	249–250[a]	$C_{16}H_{15}N_3O_3$	1400 ± 50	<10
4d	*(S)*-(+)-Proline methyl ester	205–206[a]	$C_{18}H_{17}N_2O_3$	1600 ± 200	—
4e	*(S)*-(+)-Glutamic acid dimethyl ester	149–150[b]	$C_{19}H_{19}N_3O_5$	9800 ± 600	—
4f	*(S)*-(+)-N_ϵ-(Benzyloxycarbonyl)lysine methyl ester	103–104[b]	$C_{27}H_{28}N_4O_5$	1500 ± 200	—
4g	*(S)*-(+)-Phenylalanine methyl ester	162–163[b]	$C_{22}H_{19}N_3O_3$	23400 ± 3400	—
4h	(±)-Tryptophan methyl ester	153–154[b]	$C_{24}H_{20}N_4O_3$	3000 ± 400	—
4i	*(R)*-(−)-Histidine methyl ester	182–184[b]	$C_{19}H_{17}N_5O_3$	2000 ± 300	—
4j	*(S)*-(+)-Histidine methyl ester	184–186[b]	$C_{19}H_{17}N_5O_3$	1200 ± 100	<10
5	*(S)*-(+)-Lysine methyl ester	115–116	$C_{19}H_{22}N_4O_3 \cdot 0.25\ H_2O$	3400 ± 800	—
6	*(S)*-(+)-Lysine	240–241	$C_{18}H_{22}Cl_2N_4O_3 \cdot H_2O$	8200 ± 2100	—
7a	Glycine	302–303[c]	$C_{14}H_{11}N_3O_3$	1300 ± 200	—
7c	*(S)*-(+)-Alanine	222–223	$C_{15}H_{13}N_3O_3 \cdot 0.25\ H_2O$	10100 ± 2300	—
7e	*(S)*-(+)-Glutamic acid	225–226	$C_{17}H_{15}N_3O_5$	19000 ± 2600	—
7f	*(S)*-(+)-N_ϵ-(Benzyloxycarbonyl)lysine	168–170	$C_{26}H_{26}N_4O_5 \cdot H_2O$	14100 ± 1700	—
7g	*(S)*-(+)-Phenylalanine	210–212	$C_{21}H_{17}N_3O_3 \cdot 0.5\ H_2O$	8500 ± 2400	—
7h	(±)-Tryptophan	169–172	$C_{23}H_{18}N_4O_3$	6500 ± 2400	<10
7i	*(R)*-(−)-Histidine	266–268	$C_{18}H_{15}N_5O_3 \cdot 0.25\ H_2O$	8900 ± 1400	—
7j	*(S)*-(+)-Histidine	266–268	$C_{18}H_{15}N_5O_3 \cdot 0.25\ H_2O$	20300 ± 3800	—

[a] Recrystallization solvent: methanol. [b] Recrystallization solvent: acetonitrile. [c] mp >320°C (ref. 3). [d] Satisfactory analytical data (±0.4% for C, H, N, Cl) were reported for all compounds listed in the table. [e] Concentrations necessary for 50% inhibition (IC_{50}) are mean ± SEM of four to six determinations. [f] Inhibition of specific [^{3}H]flunitrazempam binding in the mouse brain in vivo 10 min after intravenous administration of 15 μmol/kg, according to the literature (ref. 15).

carboline, IC_{50} = 3 μM; methyl β-carboline-3-carboxylate, IC_{50} = 3 nM).[4,14] Taking into account that all the compounds summarized in Table I possess amide substituents which are considerably larger than the three ester groups of the β-carboline-3-carboxylates just mentioned, the present data support our previous hypothesis that the size of the substituent at the 3-carboxylate group plays a crucial role for an optimal fit of the β-carboline molecule into the benzodiazepine receptor.[4] Increased substituent size, as in the case of the amino acid derivatives (Table I), presumably hinders the molecule from interacting with the additional point of attachment. Accordingly, all these compounds are more or less as active as the β-carboline nucleus itself. The somewhat higher affinity of the glycine methyl ester derivative **4a**, where also a relatively small substituent is present, is in accord with this assumption.

The relatively small glycine methyl ester derivative **4a** is 60-fold more active than the relatively large (*S*)-phenylalanine methyl ester derivative **4g** and 30-fold more active than the also relatively large (*S*)-glutamic acid dimethyl ester derivative **4e.** Appropriately, the medium-sized alanine methyl ester derivatives **4b**, **4c** and the (*S*)-proline methyl ester derivative **4d** show a five-fold loss in affinity. The three enantiomeric pairs tested (**4b/4c**, **4i/4j**, **7i/7j**) showed only small differences in binding. The *S*-forms containing the methyl ester structure (**4c**, **4j**) were bound slightly better than the corresponding *R*-forms (**4b**, **4i**). In contrast, the less active free acids (**7i**, **7j**) showed inverted action; the reason for the generally lower affinities of the free acid derivatives in comparison to the parent methyl esters has already been discussed. Here, the *S*-form **7j** is less potent than the *R*-form **7i.** This could mean that the free acid structure interferes with binding to such an extent that the molecule is repelled from the receptor and, if binding is stereoselective, the *S*-form should be repelled relatively more than the *R*-form. However, although stereoselectivity is suggested, these compounds do not seem to be structurally specific enough to clearly substantiate stereoselective binding to the benzodiazepine receptor.

The results in Table I show that substituent size is not the only parameter influencing binding to the benzodiazepine receptor. Although the histidine methyl ester derivatives **4i** and **4j** are as large, the (*R,S*)-tryptophan methyl ester derivative **4h** is larger, and the (*S*)-N_ϵ-CBZ-lysine methyl ester derivative **4f** is much larger than the (*S*)-phenylalanine methyl ester derivative **4g**; these compounds are all approximately one order of magnitude more potent.

Similarly, the (*S*)-N_ϵ-CBZ-lysine methyl ester derivative **4f** is much larger than the (*S*)-alanine methyl ester derivative **4c**, but equally potent. Furthermore, the (*S*)-lysine methyl ester derivative **5** has the same size as the (*S*)-glutamic acid dimethyl ester derivative **4e** but is threefold more potent. Critical scrutiny of the lysine, histidine, and tryptophan containing β-carboline-3-carboxamides reveals that these compounds all have an NH-group located approximately five to six atoms away from the carboxamide nitrogen. The presence of this NH-group positively influences binding to the benzodiazepine receptor and seems to play a more important role than substituent size. The possibility that this NH-group takes part in hydrogen bonding to the receptor is to be considered. The potent activity of those compounds containing this NH-group does not seem to be primarily determined or even influenced by hydrophilicity/hydrophobicity. The hydrophilic histidine methyl ester derivatives **4i** and **4j** are approximately equipotent to the hydrophobic (*S*)-N_ϵ-CBZ-lysine methyl ester derivative **4f.** In contrast, the hydrophobic (*S*)-phenylalanine methyl ester derivative **4g** is 12- to 20-fold less potent.

These results taken together suggest that, for good receptor binding, an NH-group must be located in the carboxamide side chain. This NH-group should be positioned at a distance of five to six atoms from the carboxamide nitrogen. The latter point is important in respect to our working hypothesis of β-carboline-containing peptides as possible links to an endogenous ligand of the benzodiazepine receptor. Every third position in a peptide chain is occupied by an NH-group. The observation that large substituents containing an NH-group distanced approximately six atoms from the β-carboline-3-carboxamide nitrogen do not interfere with the binding of the compounds to the benzodiazepine receptor opens the possibility to synthesize peptide derivatives which could interact with a further point of attachment located outside the recognition site for the β-carboline nucleus. In such a case the affinity would increase again. Future studies will be directed to the search for such a β-carboline-containing peptide with high affinity for the benzodiazepine receptor which certainly could represent a link to a possible endogenous ligand of the benzodiazepine receptor.

Experimental Section

Melting points were determined in open glass capillary tubes, with a Büchi-Tottoli melting point apparatus, and are uncorrected. Elemental analyses (C, H, N, and Cl) were performed by the Mikroanalytisches Laboratorium, Universität Mainz, and were within ±0.4% of the theoretical values. The structures of all compounds were confirmed by IR spectra (Beckman IR 4220 spectrophotometer; potassium bromide pellets), ^{1}H NMR spectra [Bruker WH90, 90 MHz; sample temperature 30°C with tetramethylsilane (1%) as internal standard], and MS (Varian-MAT CH7A). Representative NMR data are given. Optical rotations were measured using a Perkin-Elmer 241 MC automatic polarimeter. TLC was carried out on Merck 60 F_{254} silica gel plates. Many of the compounds in this series had a strong tendency to hold water within their solid structure. Heating these compounds under reduced pressure (0.1 mm Hg, 100°C, 24 h) would not completely remove the last traces of solvent. For these compounds, elemental analyses have been reported with solvent.

Tritiated flunitrazepam was obtained from New England Nuclear (Dreieich, F.R.G.) and had a specific activity of 3.256 TBq/mmol and a radiochemical purity >99%. Diazepam, clonazepam, and chlordiazepoxide were gifts of Hoffman-La Roche (Basel, Switzerland). All other chemicals were of reagent grade and obtained from commercial suppliers. The preparation of *N*-(β-carboline-3-carbonyl)glycine (**7a**) has been reported elsewhere (lit.[3] mp >320°C).

Methyl β-Carboline-3-carboxylate Hydrogen Oxalate (2)—Lead tetraacetate (44.5 g, 100 mmol) was added to a cooled, rapidly stirring solution of **1** (11.5 g, 50 mmol) in 200 mL of glacial acetic acid. The resulting mixture was stirred for 15 min. Oxalic acid (45 g, 500 mmol) was added and stirring was continued for 1 h. The pale-yellow precipitate was collected, washed sparingly with methanol, and then extensively extracted with hot methanol. The methanol extract was evaporated to dryness under reduced pressure and the residue was recrystallized from methanol to yield **2** (10.3 g, 65%), mp 232–233°C. ^{1}H NMR (Me_2SO-d_6): δ 12.03 (br s, 1, NH), 8.98 (s, 1, C*H*=N̊H), 8.92 (s, 1, CH=C′COO), 8.40 (d, 1, *J* = 7.6 Hz, ArH), 7.76–7.22 (m, 3, ArH), and 3.92 ppm (s, 3, $COOCH_3$).

Anal.—Calc. ($C_{15}H_{12}N_2O_6$) C, H, N.

β-Carboline-3-carboxylic Acid (3)—A mixture of **2** (15.8 g, 50 mmol), sodium hydroxide (12.0 g, 300 mmol), ethanol (100 mL), and water (200 mL) was refluxed for 30 min, and the ethanol was removed under reduced pressure. The clear solution was neutralized with dilute hydrochloric acid and cooled. The precipitate was collected, washed with water and with methanol, and dried under reduced pressure (20 mm Hg, 100°C, 12 h) to give **3** (10.2 g, 96%), mp 310°C (lit.[15] mp 309–310°C).

Preparation of *N*-(β-Carboline-3-carbonyl)-α-Amino Acid Methyl Esters (4)—A mixture of **3** (1.06 g, 5 mmol) and 1,1′-carbonyldiimidazole (1.62 g, 10 mmol) in 50 mL of anhydrous dimethylformamide was stirred, under exclusion of moisture, at room temperature for 2 h. A clear solution resulted. The methyl ester of an appropriate amino acid hydrochloride (10 mmol) was added with vigorous stirring. The reaction was monitored by TLC (2-butanone). Generally, the product formed quantitatively within 30 min. The solvent was evaporated under reduced pressure. The oily residue was diluted first with 10 mL of methanol and then with 200 mL of water. The precipitate was collected and recrystallized to give **4** (60–75%); **4a** ^{1}H NMR (Me_2SO-d_6): δ 11.93 (br s, 1, NH), 8.96 (t, 1, *J* = 6 Hz, CONH), 8.93 (s, 1, CH=N), 8.84 (s, 1, C*H*=C′CONH), 8.39 (d, 1, *J* = 7.8 Hz, ArH), 7.73–7.20 (m, 3, ArH), 4.15 (d, 2, *J* = 6 Hz, CH_2COO), and 3.68 ppm (s, 3, $COOCH_3$).

(*S*)-(+)-N_α-(β-Carboline-3-carbonyl)lysine Methyl Ester (5)—A solution of (*S*)-(+)-N_α-(β-carboline-3-carbonyl)-N_ϵ-(benzyloxycarbonyl)lysine methyl ester (**4f**; 976 mg, 2 mmol) in methanol (80 mL) and water (20 mL) was hydrogenated in the presence of palladium-on-barium sulfate (100 mg, 10%) at atmospheric pressure for 6 h. The mixture was filtered. The clear filtrate was evaporated to one-third its volume and then diluted with water (300 mL). The precipitated product was collected and dried under reduced pressure (0.1 mm Hg, 80°C, 24 h) to give **5** (650 mg, 90%); ^{1}H NMR (Me_2SO-d_6): δ 8.94 (s, 1, CH=N), 8.84 (s, 1, C*H*=C′CONH), 8.73 (d, 1, *J* = 6.7 Hz, CONH), 8.38 (d, 1, *J* = 7.6 Hz, ArH), 7.78–7.11 (m, 3, ArH), 4.61 (m, 1, C′HCOO), 3.69 (s, 3, $COOCH_3$), 2.64 (m, 2, CH_2NH_2), 1.91 (m, 2, $CHCH_2$), and 1.40 ppm (m, 4, $CH_2CH_2CH_2CH_2NH_2$).

Preparation of *N*-(β-Carboline-3-carbonyl)-α-amino Acids (6, 7a, c, e–j)—A suspension of the appropriate ester **4**, **5** (2 mmol), and sodium bicarbonate (1.68 g, 20 mmol) in ethanol (15 mL) and water (30 mL) was refluxed for 1 h. The resulting clear solution was evaporated to dryness under reduced pressure. The solid residue was dissolved in hot water (50 mL). The solution was filtered and acidified to pH 5 with acetic acid. The precipitate was collected, washed well with water, and dried under reduced pressure (0.1 mm Hg, 100°C, 24 h) to yield **7a**, **c**, **e–j** (75–85%). The TLC mobile phase used here was 1-butanol:acetic acid:water (40:10:10); **7a** ^{1}H NMR (Me_2SO-d_6): δ 11.91 (br s, 1, NH), 8.92 (s, 1, CH=N), 8.84 (s, 1, C*H*=C′CONH), 8.83 (t, 1, *J* = 6 Hz, CONH), 8.39 (d, 1, *J* = 7.9 Hz, ArH), 7.73–7.18 (m, 3, ArH), and 4.07 ppm (d, 2, *J* = 6 Hz, CH_2COOH).

In the case of (*S*)-(+)-N_α-(β-carboline-3-carbonyl)lysine dihydrochloride (**6**) this precipitate was dissolved in 20 mL of dimethylformamide and acidified with 2 mL of concentrated hydrochloric acid. The solution was filtered and evaporated to dryness under reduced pressure. The solid residue was dissolved in 5 mL of methanol and then 100 mL of ether was added. The precipitate which formed was collected and dried under reduced pressure (0.1 mm Hg, 100°C, 24 h) to give **6** (78%); ^{1}H NMR (Me_2SO-d_6): δ 12.54 (br s, 1, NH), 9.26 (s, 1, C*H*=N̊H), 9.08 (d, 1, *J* = 6.6 Hz, CONH), 9.04 (s, 1, C*H*=C′CONH), 8.42 (d, 1, *J* = 7.7 Hz, ArH), 7.98 (m, 3, $\dot{N}H_3$), 7.82–7.22 (m, 3, ArH), 4.54 (m, 1, C′*H*COOH), 2.78 (m, 2, $CH_2\dot{N}H_3$), 1.94 (m, 2, $CHCH_2$), and 1.57 ppm (m, 4, $CH_2CH_2CH_2CH_2\dot{N}H_3$).

Receptor Binding Assay—Male NMRI mice (18–20 g body weight) were killed by decapitation. The brains were quickly removed and homogenized in ice-cold Tris-HCl buffer (100 mM, pH 7.4) and centrifuged for 10 min at 48,000×*g*. The pellet was resuspended to a final volume of 80 mL of the same buffer per mouse brain (approximately 0.4 g). Aliquots (900 μL) of this homogenate were incubated (4°C) for 30 min together with [^{3}H]flunitrazepam (4 nM, 50 μL) and 50 μL of buffer containing blank (diazepam) or different concentrations of the displacers. The incubation was terminated by rapid filtration through Whatman GF-B filters. The filters were washed three times with 3 mL of ice-cold incubation buffer, placed in minivials, and dried for 30 min at 60°C. Radioactivity on the filters was determined by liquid scintillation spectrophotometry in 4 mL Quickszint 402 (Zinsser, Frankfurt, F.R.G.). Nonspecific binding was determined by parallel experiments containing diazepam (10 μM) and accounted for <10% of total binding. The concentrations of the β-carboline derivatives that inhibit specific [^{3}H]flunitrazepam binding by 50% (IC_{50}) were determined by log-probit analysis with four to six concentrations of the displacers, each performed in triplicate. Under these experimental conditions, the benzodiazepine derivatives clonazepam, diazepam, and chlordiazepoxide had IC_{50} values of 2.5, 16, and 700 nM, respectively. All IC_{50} values given are means ± SEM of four to six individual determinations. Inhibition of specific [^{3}H]flunitrazepam binding in vivo after intravenous administration of the β-carbolines to mice was determined as previously described.[16]

References and Notes

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