nation with isopropylamine. For analysis, 7 was purified by HPlC on a Whatman ODS-2 50-cm M-9 column eluting with 0.1 M PO_4^{3-} (pH 7.4)–CH_3CN (8.5:1.5) and then desalted on a XAD-2 column. The product was eluted with MeOH and then concentrated: ^1H NMR (Me_2SO-d_6) δ 2.7–3.0 (m, 2, $CHCH_2$), 3.3–3.7 (m, 2, $OCH_2CH\cdot{}^1/_2H_2O$), 3.99, 4.48 (dd, ^2J = 12 H, ^3J = 2 Hz, 2, OCH_2), 6.88 (d, J = 9 Hz, 1, H-2′), 7.33 (d, J = 9 Hz, 1, H-3′), 7.4–7.6 (m, 2, H-6′, H-7′), 8.0–8.3 (m, 2, H-5′, H-8′). Anal. ($C_{13}H_{11}KO_6S\cdot{}^1/_2H_2O$) C, H, S.

4′-Hydroxypropranolol Sulfate (8). A suspension of unpurified 7 (2.33 g) and phosphate salts from the pH 7.4 buffer (10.5 g) was stirred in isopropylamine (60 mL, 53.3 g, 902 mmol) for 19.5 h. The excess amine was removed on a rotary evaporator to yield 13.32 g of a tan solid. The residue was stirred with 100 mL of H_2O, filtered, and dried to yield 595 mg of a tan solid. The filtrate, which contained appreciable amounts of 8 in addition to several impurities, was lyophilized and chromatographed on the preparative ODS column eluting with pH 7.4 phosphate buffer–CH_3CN (92.5:7.5). The product fraction was collected, concentrated, and then desalted on the preparative ODS column eluting with MeOH to yield 230 mg of 8. The products were combined and recrystallized from H_2O (maximum H_2O temperature 80 °C) to yield 338 mg. The filtrates were concentrated to yield another 45 mg. Total yield was 383 mg (24.4% yield from **5a**) HPLC on an ODS-3 column [0.1 M PO_4^{3-} (pH 7.4)–CH_3CN (85:15) and 0.01 M NH_4OAc–CH_3CN (85:15)] showed the product to be free of impurities. 8: mp >210 °C dec; NMR (Me_2SO-d_6) δ 1.27 (d, J = 6 Hz, 6, $CH(CH_3)_2$, 3.7–2.9 (m, 3, CH_2NCH), 4.4–4.0 (m, 3, OCH_2CHO), 3.25, 5.89, 8.20 (br s, 5, OH, NH_2, H_2O), 6.83 (d, J = 9 Hz, 1, H-2′), 7.38 (d, J = 9 Hz, 1, H-3′), 7.3–7.6 (m, 2, H-6′, H-7′), 8.3–7.9 (m, 2, H-5′, H-6′); FAB/MS, m/z 356 (MH^+). Anal. ($C_{16}H_{21}NO_6S\cdot H_2O$) C, H, N, S.

Hydrolysis of 8 with aryl sulfatase from *Aerobacter aerogenes*[4] produced 4′-hydroxypropranolol in equimolar quantities. In addition, the HPLC retention volume for 8, using two mobile phases on a Spherisorb ODS column, was identical to the retention volume of the human and dog metabolite, demonstrating the metabolite to be 4′-hydroxypropranolol sulfate. Quantitative measurements of 8 in urine was also accomplished by HPLC. After single 80-mg oral doses of propranolol in normal healthy human subjects, 8 accounted for 21.8 ± 2.4% (mean ± SE, n = 6) of the dose.[3] After chronic oral doses, 80 mg every 6 h, 8 accounted for 24.8 ± 2.6% (n = 6) of the dose.

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Registry No. 1, 130-15-4; **2a**, 571-60-8; 3, 73661-06-0; 4, 95648-09-2; **5a**, 95648-10-5; 6, 95648-11-6; 7, 95648-12-7; 8, 95673-91-9; 4′-hydroxypropranolol, 10476-53-6; propranolol, 525-66-6.

3-Amino-β-carboline Derivatives and the Benzodiazepine Receptor. Synthesis of a Selective Antagonist of the Sedative Action of Diazepam

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Seven 3-N-substituted derivatives of 3-amino-β-carboline were synthesized and their affinities for the benzodiazepine receptor were assessed in vitro. Two compounds, 3-(ethylamino)-β-carboline and 3-[(methoxycarbonyl)amino]-β-carboline (β-CMC), showing IC_{50} values of 460 and 71 nM, respectively, were selected for in vivo studies. The former compound showed long-lasting proconvulsant activity in *Papio papio* baboons while β-CMC was shown in mice to selectively antagonize the sedative effects of diazepam without exhibiting convulsant, proconvulsant, or anxiogenic activity by itself.

The discovery of specific, high-affinity receptors in the central nervous system that apparently mediate the anticonvulsant, anxiolytic, and sedative actions of benzodiazepines has greatly facilitated the process of finding new agonists and antagonists structurally unrelated to this class of compounds.[1,2] Thus, binding assays using a radiolabeled benzodiazepine enabled Braestrup and co-workers[3] to isolate a potent benzodiazepine receptor antagonist, ethyl β-carboline-3-carboxylate (β-CCE, 1), from human urine.

Although now considered to be an artifact arising from the isolation procedure rather than a true endogenous ligand of the benzodiazepine receptor,[4] β-CCE and its methyl ester analogue β-CCM (2) are of great interest since not only do they block most of the pharmacological actions of benzodiazepines but they actually exhibit effects opposite to those of benzodiazepines in various animal behavior models: β-CCE and β-CCM are, respectively, proconvulsant and convulsant in photosensitive *Papio papio* baboons[5,6] and in mice,[9] both compounds are anxiogenic in mice,[7-9] and β-CCE significantly increases the wakefulness of cats.[10] The term "inverse agonist" has been applied to these compounds to distinguish them from classical antagonists.[11]

Inspired by these results, several recent reports deal with the synthesis of new β-carboline derivatives[12-19] and the

†ICSN/CNRS.
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(1) Möhler, H.; Okada, T. *Science* **1977**, *198*, 849.
(2) Squires, R.; Braestrup, C. *Nature (London)* **1977**, *266*, 732.
(3) Braestrup, C.; Nielsen, M.; Olsen, C. E. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 2288.
(4) Squires, R. "GABA and Benzodiazepine Receptors"; Costa, E., Dichiara, G., Gessa, G., Eds.; Raven Press: New York, 1981; p 129.
(5) Cêpeda, C.; Tanaka, T.; Besselièvre, R.; Potier, P.; Naquet, R.; Rossier, J. *Neurosci. Lett.* **1981**, *24*, 53.
(6) Valin, A.; Dodd, R. H.; Liston, D. R.; Potier, P.; Rossier, J. *Eur. J. Pharmacol.* **1982**, *85*, 93.
(7) Ninan, P. T.; Insel, T. M.; Cohen, R. M.; Cook, J. M.; Skolnick, P.; Paul, S. M. *Science* **1982**, *218*, 1332.
(8) Prado de Carvalho, L.; Grecksch, G.; Chapouthier, G.; Rossier, J. *Nature (London)* **1983**, *301*, 64.
(9) Prado de Carvalho, L.; Venault, P.; Cavalheiro, E.; Kaijima, M.; Valin, A.; Dodd, R. H.; Potier, P.; Rossier, J.; Chapouthier, G. *Adv. Biochem. Psychopharmacol.* **1983**, *38*, 175.
(10) Kaijima, M.; Da Costa-Rochette, L.; Dodd, R. H.; Rossier, J.; Naquet, R. *Electroenceph. Clin. Neurophysiol.* **1984**, *58*, 277.
(11) Polc, P.; Bonetti, E. P.; Schaffner, R.; Haefely, W. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1982**, *321*, 260.
(12) Fehske, K. J.; Müller, W. E.; Platt, K. L.; Stillbauer, A. E. *Biochem. Pharmacol.* **1981**, *30*, 3016.

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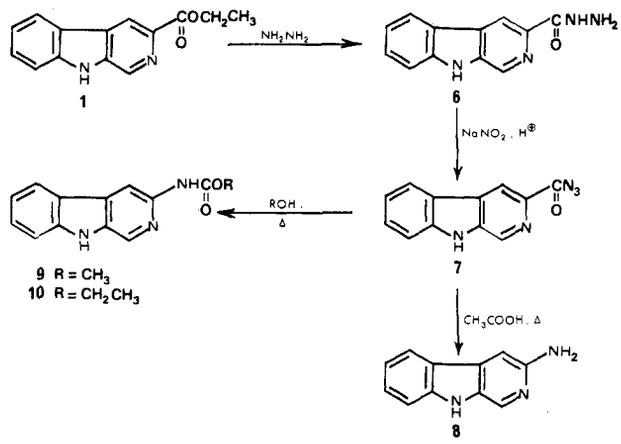
Scheme I



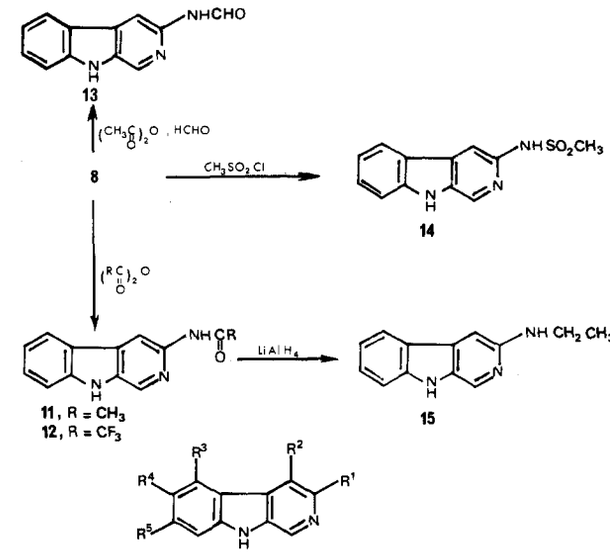
structure–activity relationships of the compounds with regard to the benzodiazepine receptor. It has been established that 1,2,3,4-tetrahydro-β-carbolines demonstrate considerably less affinity for the benzodiazepine receptor than their fully aromatic counterparts[13,17] and that substitutions at the 1- and 9-positions of β-CCE are unfavorable for affinity.[12,13,17] On the other hand, substitutions at the 4-, 5-, 6-, or 7-positions of β-CCM or β-CCE are not detrimental to the binding affinities of these compounds.[15,17] Thus, DMCM (3) retains the highly convulsive nature of β-CCM (2) in mice[20] while ZK91296 (4) represents the only known anticonvulsant β-carboline derivative.[21]

As far as the C-3 carboxyl function is concerned, its presence, in the form of an ester or an amide, seems essential for ensuring high receptor binding affinities of the β-carbolines.[17,22] For example, reduction of the ester group of β-CCE to a hydroxymethyl group (5) gives a β-carboline that still antagonizes the multiple actions of benzodiazepines but that inhibits diazepam binding in vitro at micromolar rather than nanomolar concentrations.[23,24]

The C-3 carboxyl group of β-carbolines could favor interactions of these compounds with the benzodiazepine receptor by virtue of a discrete receptor binding site for this group and/or by virtue of its electron-withdrawing effects on the heterocycle. A chelating effect between the carboxyl group and the nitrogen of the pyridino ring has also been suggested.[18] It thus seemed interesting to us to study the binding characteristics of 3-amino-β-carboline (8) and its derivatives. The amino group contrary to the carbonyl group would serve to increase the electron density on the aromatic heterocycle, and furthermore, these electron-donating properties of the amine could be modulated by appropriate substitutions on this atom.

In this report we present further evidence,[18] using derivatives of 3-amino-β-carboline, that the presence of a carbonyl group at the C-3 of β-carboline is not strictly necessary to maintain a good affinity of the molecule for the benzodiazepine receptor. Moreover, the methyl carbamate derivative **9** (β-CMC) was found to be a selective antagonist of only the sedative properties of diazepam, exhibiting no antagonism of the anticonvulsant and anxiolytic effects of this benzodiazepine.

Scheme II



1. R^1 = $CO_2CH_2CH_3$; R^2 = R^3 = R^4 = R^5 = H
2. R^1 = CO_2CH_3 ; R^2 = R^3 = R^4 = R^5 = H
3. R^1 = CO_2CH_3 ; R^2 = CH_2CH_3 ; R^3 = H ; R^4 = R^5 = OCH_3
4. R^1 = $CO_2CH_2CH_3$; R^2 = CH_2OCH_3 ; R^3 = $OCH_2C_6H_5$; R^4 = R^5 = H
5. R^1 = CH_2OH ; R^2 = R^3 = R^4 = R^5 = H

Chemistry. 3-Amino-β-carboline (8) was prepared by a Curtius-type reaction starting with β-CCE[14,17] (1, Scheme I). Refluxing of β-CCE with 85% hydrazine hydrate in ethanol gave the hydrazide **6**, which after treatment with sodium nitrite, yielded the acyl azide **7**. When **7** was refluxed for several minutes in acetic acid, rearrangement to the amine derivative **8** occurred in good yield. Alternatively, the azide **7** could be refluxed in anhydrous methanol or ethanol to give, respectively, the methyl and ethyl carbamates **9** and **10**.

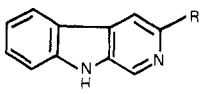
The amine **8** was used to prepare the remaining derivatives (Scheme II). Thus, treatment of **8** with acetic or trifluoroacetic anhydride produced the 3-*N*-acetyl or 3-*N*-trifluoroacetyl derivatives **11** and **12**, respectively, while heating **8** in acetic anhydride in the presence of formic acid yielded the N-formylated compound **13**. The amine **8** could be sulfonylated with mesyl chloride to afford **14**. Reduction of the carbonyl group of **11** with lithium aluminum hydride yielded the *N*-ethyl derivative **15**.

Results and Discussion

The affinities of 3-amino-β-carboline and its derivatives for the benzodiazepine receptor were assessed in vitro by use of competition experiments with [^{3}H]flunitrazepam on

(13) Robertson, H. A.; Baker, G. B.; Coutts, R. T.; Benderly, A.; Locock, R. A.; Martin, I. L. *Eur. J. Pharmacol.* **1981**, *76*, 281.
(14) Lippke, K. P.; Schunack, W. G.; Wenning, W.; Müller, W. E. *J. Med. Chem.* **1983**, *26*, 499.
(15) Neef, G.; Eder, U.; Huth, A.; Rahtz, D.; Schmiechen, R.; Seidelmann, D. *Heterocycles* **1983**, *20*, 1295.
(16) Cain, M.; Guzman, F.; Cook, J. M.; Rice, K. C.; Skolnick, P. *Heterocycles* **1982**, *19*, 1003.
(17) Cain, M.; Weber, R. W.; Guzman, F.; Cook, J. M.; Barker, S. A.; Rice, K. C.; Crawley, J. N.; Paul, S. M.; Skolnick, P. *J. Med. Chem.* **1982**, *25*, 1081.
(18) Guzman, F.; Cain, M.; Larscheid, P.; Hagen, T.; Cook, J. M.; Schweri, M.; Skolnick, P.; Paul, S. M. *J. Med. Chem.* **1984**, *27*, 564.
(19) Coutts, R. T.; Micetich, R. G.; Baker, G. B.; Benderly, A.; Dewhurst, T.; Hall, T. W.; Locock, A. R.; Pyrozko, J. *Heterocycles* **1984**, *22*, 131.
(20) Braestrup, C.; Schmiechen, R.; Neef, G.; Nielsen, M.; Petersen, E. N. *Science* **1982**, *216*, 1241.
(21) Meldrum, B. S.; Evans, M. C.; Braestrup, C. *Eur. J. Pharmacol.* **1983**, *91*, 255.
(22) Rossier, J.; Dodd, R. H.; Feldblum, S.; Valin, A.; Prado de Carvalho, L.; Potier, P.; Naquet, R. *Lancet* **1983**, Jan 1, 77.
(23) Skolnick, P.; Paul, S.; Crawley, J.; Rice, K.; Barker, S.; Weber, R.; Cain, M.; Cook, J. *Eur. J. Pharmacol.* **1981**, *69*, 525.
(24) Mendelson, W. B.; Cain, M.; Cook, J. M.; Paul, S. M.; Skolnick, P. *Science* **1983**, *219*, 414.

Table I. Benzodiazepine Receptor Affinities of Substituted 3-Amino-β-carbolines



compd	R	IC_{50}(nM)[a]
1	$CO_2CH_2CH_3$	2.0
8	NH_2	25,000
9	$NHCO_2CH_3$	71
10	$NHCO_2CH_2CH_3$	80
11	$NHCOCH_3$	4 000
12	$NHCOCF_3$	6 300
13	NHCHO	4 700
14	$NHSO_2CH_3$	62,000
15	$NHCH_2CH_3$	460

[a] Concentration of compound required to inhibit 50% of [^{3}H]flunitrazepam binding to in vitro preparations of rat cerebral cortex membranes at 0 °C; average of triplicate determinations.

rat brain membrane preparations by previously described methods.[25] The resulting IC_{50}'s are reported in Table I.

Although the binding affinity of 3-amino-β-carboline (**8**) itself was 4 orders of magnitude poorer than that of β-CCE, the 3-*N*-ethyl derivative **15** had a sufficiently low IC_{50} (460 nM) to warrant a preliminary in vivo study. Thus, with use of the photosensitive model of epilepsy in *Papio papio* baboons,[26] compound **15** (3.5 mg/kg, iv) demonstrated a potent, proconvulsant effect, provoking full tonicoclonic seizures in these animals in the same manner as β-CCE.[5] However, in contrast to β-CCE, whose duration of activity in baboons is ca. 15 min, compound **15** showed proconvulsant effects for over 1 h, as judged by its induction of photosensitivity in previously nonphotosensitive animals. The short duration of activity of β-CCE, as well as its proconvulsant rather than convulsant activity, has been attributed to pharmacokinetic factors.[27] The sensitive ester function is rapidly hydrolyzed in vivo to the inactive β-carboline-3-carboxylic acid, preventing its accumulation to convulsive levels in nerve synapses. The absence of such a function in **15** could explain its longer lasting proconvulsant effect.

In terms of favorable binding affinities, the most interesting compounds were the methyl and ethyl carbamates **9** and **10** of which the former was chosen for more extensive studies in vivo.

By itself, the methyl carbamate **9** (β-CMC) showed neither convulsant nor proconvulsant activity in mice (tested up to 20 mg/kg, sc). Proconvulsant or convulsant activity in photosensitive or nonphotosensitive *Papio papio* baboons, respectively (7.5 mg/kg), were also not observed. No abnormal EEG activity was recorded in the latter species. β-CMC did not block or potentiate convulsions induced by pentylenetetrazol in mice nor did it show anticonvulsant effects in the photosensitive baboons. The anticonvulsant activity of diazepam in pentylenetetrazol-treated mice was also unaffected by β-CMC. However, β-CMC did effectively block β-CCM (**2**) induced convulsions in both of these species (1 mg/kg of β-CMC for 5 mg/kg β-CCM in mice, 1.5 mg/kg for 150 μg/kg in nonphotosensitive baboons).

In a previously described conflict model in mice,[8] used to evaluate the anxiolytic–anxiogenic properties of a compound, β-CMC (20 mg/kg) demonstrated neither anxiolytic nor anxiogenic properties and, furthermore, did not antagonize the anxiolytic effect of diazepam. However, β-CMC completely reversed the sedative effects of diazepam in mice, both at the gross behavioral level and in the rotarod-deficit test. Thus, treatment of these animals with β-CMC (10 mg/kg) 10 min after administration of a sedative dose of diazepam (2 mg/kg) led to complete arousal as judged by increased muscle tone, alertness, and mobility. In addition, diazepam-induced rotarod deficits[28] were entirely eliminated by β-CMC: mice injected with sedative doses of diazepam cannot maintain themselves on the rotating rod whereas mice treated with diazepam and β-CMC behave as nontreated control animals, remaining on the cylinder without difficulty. These effects were comparable to those produced by the benzodiazepine receptor antagonist Ro 15-1788 (5 mg/kg) administered 10 min after diazepam (2 mg/kg) or by the inverse agonist β-CCM (**2**). Both of these compounds are known to block the multiple effects of benzodiazepines, including their sedative effects.[28,29] These findings thus indicate that, in addition to blocking the convulsive action of β-CCM, β-CMC is a partial antagonist of diazepam, blocking only the sedative effects but not the anticonvulsant or anxiolytic effects of the latter.

Of the remaining compounds synthesized, none had less than μM binding affinities and were thus not tested in vivo. In general, the IC_{50}'s of the 3-amino-β-carboline derivatives appear to increase as the electron-withdrawing power of the N-substituent increases. Correspondingly, an increase in the electron density of the heterocycle has beneficial effects on the binding affinity, as shown by N-ethylation of 3-amino-β-carboline (**15**).

In conclusion, the present study corroborates previous findings[17,18] in that, with respect to binding affinity for the benzodiazepine receptor, a high degree of modification of the C-3 side chain of β-carbolines can be tolerated, particularly as concerns the carbonyl group. Thus, elimination of the latter functionality, as in the 3-N-ethyl derivative **15** (and as in the 3-hydroxymethyl compound **5**[17]), maintains a reasonably high binding affinity of the β-carboline for the benzodiazepine receptor. However, in contrast to **5**, which has been shown[23,24] to only antagonize the various effects of benzodiazepines, compound **15** actually exhibits inverse agonist properties, being proconvulsant in our animal models of epilepsy. As has been pointed out by others[17,18] maximum binding affinity of the β-carbolines in vitro appears to be best achieved if the carbonyl group is attached directly to the heterocycle at C-3 since none of the compounds in this study equal the binding affinity of β-CCE (**1**, IC_{50} = 2 nM).

In the case of β-CMC, a change in the position of this carbonyl group has yielded a highly selective partial antagonist of the sedative properties of diazepam. Since these sedative effects of benzodiazepines are often a drawback in their use as antiepileptics and anxiolytics,

(25) Rehavi, M.; Skolnick, P.; Paul, S. M. *Eur. J. Pharmacol.* **1982**, *78*, 353.
(26) Killam, K. F.; Killam, E. K.; Naquet, R. *Electroenceph. Clin. Neurophysiol.* **1967**, *22*, 497.
(27) Schweri, M. M.; Martin, J. V.; Mendelson, W. B.; Barrett, J. E.; Paul, S. M.; Skolnick, P. *Life Sci.* **1983**, *33*, 1505.
(28) Prado de Carvalho, L.; Grecksch, G.; Cavalheiro, E. A.; Dodd, R. H.; Chapouthier, G.; Rossier, J. *Eur. J. Pharmacol.* **1984**, *103*, 287.
(29) Bonetti, E. P.; Pieri, L.; Cumin, R.; Schaffner, R.; Pieri, M.; Gamzu, E. R.; Müller, R. K. M.; Haefely, W. *Psychopharmacology* **1982**, *78*, 8.

compounds of the β-CMC type could have important therapeutic applications.

Experimental Section

Chemistry. Melting points were determined on a Büchi apparatus and are uncorrected. IR spectra of samples were obtained in Nujol or KBr pellets with a Perkin-Elmer 297 instrument. Proton NMR spectra were determined on Varian T-60 or Bruker 200-MHz instruments. Chemical shifts are given as δ values with reference to Me_4Si as internal standard. Thin-layer chromatography was performed on Merck silica gel 60 plates with fluorescent indicator generally with use of 9:1 toluene–ethanol as developer. The plates were visualized under UV light (254 and 366 nm). Merck silica gel 60 (230–400 mesh) was used for all column chromatography. Mass spectral measurements were done on an AEI MS-9 or an AEI MS-50 spectrometer. Elemental analyses were performed at the ICSN, CNRS, Gif-sur-Yvette, France.

β-Carboline-3-carbohydrazide (6). A solution of ethyl β-carboline-3-carboxylate[14,17] (2.0 g, 8.3 mmol) in ethanol (20 mL) containing 85% hydrazine hydrate (10 mL) was refluxed under an argon atmosphere for 6 h. The resulting precipitate was collected by filtration, washed with ethanol, and air-dried, yielding 1.5 g (80%) of **6**. An analytical sample was recrystallized from 90% ethanol, giving shiney, silver plates: mp 289–291 °C; IR (Nujol) 3225, 1650, 1620, 1600 cm^{-1}; EIMS, m/z 226 (M^+), 195 ($M^+ - NHNH_2$); ^1H NMR (Me_2SO-d_6) δ 4.25 (2 H, s, NH_2, D_2O exchangeable), 6.60–7.18 (3 H, m, Ar), 7.77 (1 H, d, Ar), 8.21 (1 H, s, H-4), 8.27 (1 H, s, H-1), 8.98 (1 H, s, NH, D_2O exchangeable), 11.18 (1 H, br s, NH indole, D_2O exchangeable). Anal. ($C_{12}H_{10}N_4O$) C, H, N.

3-(Azidocarbonyl)-β-carboline (7). A suspension of the hydrazide **6** (640 mg, 2.8 mmol) in water (20 mL) was dissolved by the dropwise addition of concentrated hydrochloric acid (0.5 mL). The pale yellow solution was cooled to 0 °C before the addition of a solution of sodium nitrite (200 mg, 2.9 mmol) in water (1 mL). After 20 min at 0 °C, the mixture was made basic with saturated aqueous sodium hydrogen carbonate, and the precipitate was collected by filtration, washed with water, and dried in a dessicator under vacuum, yielding 510 mg (77%) of **7** as a yellowish solid with a tendency to decompose. The material was used without further purification for the following steps.

3-Amino-β-carboline (8). A suspension of the azide **7** (600 mg, 2.5 mmol) in a mixture of 1:1 water–acetic acid (30 mL) was brought to reflux, during which carbon dioxide was evolved and the starting material disappeared. After 30 min of refluxing, the reaction mixture was cooled, the solvents were removed under vacuum, and the solid residue was crystallized from ethanol, yielding **8** (350 mg, 77%) as shiney yellow plates: mp 289–291 °C; EIMS, m/z 183 (M^+); NMR (Me_2SO-d_6) δ 5.30 (2 H, br s, NH_2, D_2O exchangeable), 7.11 (2 H, m + s, H-4, Ar), 7.46 (2 H, m, Ar), 8.06 (1 H, d, Ar), 8.33 (1 H, s, H-1), 10.92 (1 H, s, NH indole, D_2O exchangeable). Anal. ($C_{11}H_9N_3$) C, H, N.

3-[(Methoxycarbonyl)amino]-β-carboline (9, β-CMC). A suspension of the azide **7** (200 mg, 0.84 mmol) in methanol (10 mL) was refluxed for 6 h. The reaction mixture was cooled and concentrated in vacuo, whereupon the product **9** precipitated (120 mg, 60%). The material was recrystallized from ethanol: mp 180–182 °C and then 230 °C dec; EIMS, m/z 241 (M^+), 210 ($M^+ - OCH_3$), 182 ($M^+ - CO_2CH_3$), 167 ($M^+ - NHCO_2CH_3$); NMR (Me_2SO-d_6) δ 3.45 (3 H, s, Me), 6.60–7.35 (3 H, m, Ar), 7.80 (1 H, d, Ar), 8.05 (1 H, s, H-4), 8.23 (1 H, s, H-1), 9.50 (1 H, br s, NHCO, D_2O exchangeable), 11.00 (1 H, br s, NH indole, D_2O exchangeable). Anal. Calcd for $C_{13}H_{11}N_3O_2$: C, 64.73; H, 4.56; N, 17.43. Found: C, 64.88; H, 4.78; N, 17.00.

3-[(Ethoxycarbonyl)amino]-β-carboline (10). The same conditions as for the preparation of **9** were used for the preparation of **10** except that ethanol was the reaction solvent: yield 65%; mp 222–224 °C; EIMS, m/z 255 (M^+), 210 ($M^+ - OEt$); NMR (Me_2SO-d_6) δ 1.36 (3 H, t, J = 7.0 Hz, CH_2CH_3), 4.23 (4 H, q, J = 7.0 Hz, CH_2CH_3), 6.98–7.58 (3 H, m, Ar), 8.03 (1 H, d, Ar), 8.31 (1 H, s, H-4), 8.48 (1 H, s, H-1), 9.70 (1 H, br s, NHCO, D_2O exchangeable), 11.23 (1 H, br s, NH indole, D_2O exchangeable). Anal. ($C_{14}H_{13}N_3O_2$) C, H, N.

3-(Acetylamino)-β-carboline (11). A solution of the amine **8** (250 mg, 1.4 mmol) and acetic anhydride (20 mL) in anhydrous pyridine (2 mL) was stirred at room temperature for 1 h. The reaction mixture was then diluted with xylene and evaporated in vacuo to dryness. Addition and evaporation of xylene was repeated twice. The remaining reddish solid crystallized from ethanol–hexane to give **11** (230 mg, 60%) containing 1 equiv of acetic acid: mp 206 °C; EIMS, m/z 225 (M^+), 182 ($M^+ - Ac$); IR (KBr) 3440, 3200, 1683, 1650, 1600 cm^{-1}; NMR (Me_2SO-d_6) δ 1.85 (3 H, s, CH_3), 2.05 (3 H, s, CH_3), 6.90–7.50 (3 H, m, Ar), 8.00 (1 H, CH_3CO_2H), 8.45 (1 H, s, H-4), 8.55 (1 H, s, H-1), 10.15 (1 H, br s, NHCO, D_2O exchangeable), 11.20 (1 H, br s, NH indole, D_2O exchangeable). Anal. ($C_{13}H_{11}N_3O \cdot CH_3CO_2H$) C, H, N. Heating of a sample of **11** at 110 °C under vacuum for 8 h gave pure **11** without its solvent of crystallization as shown by the disappearance of the acetic acid peak in the NMR; mp 210 °C.

3-[(Trifluoroacetyl)amino]-β-carboline (12). A mixture of the amine **8** (55 mg, 0.3 mmol) and trifluoroacetic anhydride (63 mg, 0.3 mmol) in chloroform (4 mL) containing pyridine (1 mL) was stirred at room temperature for 4 days. The insoluble material was removed by filtration, and the filtrate was concentrated and purified by column chromatography on silica gel with 9:1 toluene–ethanol as developer, yielding **12**, which was crystallized from toluene (35 mg, 42%): mp 253 °C dec; EIMS, m/z 279 (M^+), 260 ($M^+ - F$), 210 ($M^+ - CF_3$), 182 ($M^+ - COCF_3$); NMR (Me_2SO-d_6) δ 7.17–7.52 (3 H, m, Ar), 8.16 (1 H, d, Ar), 8.49 (1 H, s, H-4), 8.64 (1 H, s, H-1), 11.52 (1 H, s, NH), 11.71 (1 H, s, NH). Anal. Calcd for $C_{13}H_8F_3N_3O \cdot 0.1CH_3C_6H_5$: C, 57.04; H, 3.05; N, 14.57. Found: C, 56.74; H, 3.40; N, 14.71.

3-(Formylamino)-β-carboline (13). A solution of acetic anhydride (2 mL) and formic acid (0.8 mL) was heated at 60 °C for 2 h. The solution was cooled in an ice bath and the amine **8** (50 mg, 0.24 mmol) was added. The reaction mixture was stirred for 1.5 h and then made basic with saturated aqueous sodium hydrogen carbonate and extracted twice with ethyl acetate. The combined organic extracts were washed twice with water and dried over sodium sulfate, and the solvent was evaporated in vacuo, yielding crude **13** (40 mg, 80%), which was crystallized from ethanol–hexane: mp 266 °C dec; EIMS, m/z 211 (M^+), 183 ($M^+ - CO$); NMR (Me_2SO-d_6) δ 7.12–7.55 (3 H, m, Ar), 8.05 (0.5 H, d, H-6), 7.99 (0.5 H, d, H-6 tautomer), 8.24 (1 H, s, H-4), 8.49 (0.5 H, s, H-1), 8.54 (0.5 H, s, H-1 tautomer), 8.59 (0.5 H, s, N=CH), 9.10 (0.5 H, d, CHO), 10.39 (1 H, s, NH indole), 11.28 (0.5 H, s, NHCHO), 11.34 (0.5 H, s, N=COH). Anal. ($C_{12}H_9N_3O$) C, H, N.

3-[(Methylsulfonyl)amino]-β-carboline (14). A solution of the amine **8** (50 mg, 0.3 mmol) and mesyl chloride (32 mg, 1 equiv) in anhydrous pyridine (1 mL) was stirred at room temperature for 1 h. The reaction mixture was then diluted with saturated aqueous sodium hydrogen carbonate (5 mL) and extracted twice with ethyl acetate. The combined organic extracts were washed twice with water and dried over sodium sulfate, and the solvent was removed under vacuum, leaving a crude solid, which was crystallized from ethanol, yielding **14** as a pale yellow powder (40 mg, 50%): mp 287 °C dec; EIMS, m/z 261 (M^+), 182 ($M^+ - SO_2Me$); NMR (Me_2SO-d_6) δ 3.23 (3 H, s, CH_3), 7.15–7.47 (3 H, m, Ar), 7.68 (1 H, s, H-4), 8.11 (1 H, d, Ar), 8.54 (1 H, s, H-1), 9.97 (1 H, br s, $NHSO_2$), 11.34 (1 H, br s, NH indole). Anal. ($C_{12}H_{11}N_3O_2S$) C, H, N.

3-(Ethylamino)-β-carboline (15). To a rapidly stirred suspension of the *N*-acetyl derivative **11** (940 mg, 4.2 mmol) in anhydrous THF was added lithium aluminum hydride (400 mg, 10 equiv) and the mixture was refluxed for 1.5 h. Saturated aqueous sodium sulfate (1 mL) was added, and the mixture was stirred at room temperature for 2 h and then filtered. The filtrate was concentrated in vacuo and the residue purified by column chromatography on silica gel with 10:10:1.5 toluene–ethyl acetate–ethanol as the developer. Compound **15** was obtained as a colored oil (360 mg, 40%), which could be crystallized from ethanol–hexane: mp 143–145 °C; EIMS, m/z 211 (M^+), 196 ($M^+ - CH_3$), 182 ($M^+ - CH_2CH_3$), 167 ($M^+ - NHEt$); NMR ($CDCl_3$) δ 1.25 (3 H, t, J = 7.0 Hz, CH_2CH_3), 3.27 (2 H, q, J = 7.0 Hz, CH_2CH_3), 3.45–3.95 (1 H, br s, NHEt, D_2O exchangeable), 7.00 (1 H, s, H-4), 7.10–7.45 (3 H, m, Ar), 8.00 (1 H, d, Ar), 8.35 (1 H, s, H-1). Anal. ($C_{13}H_{13}N_3$) C, H, N.

Biological Methods. In Vitro Benzodiazepine Receptor Binding Assays. Male Sprague–Dawley rats were decapitated, the brains were excised, and the cortex was dissected. Each cortex

was homogenized in 5 mL of ice-cold Tris-HCl (50 mM, pH 7.4) with a Polytron. The homogenate was centrifuged a first time at 460g for 3 min and the supernatant was recentrifuged at 22 400g for 20 min. The resulting supernatant was discarded, and each pellet was resuspended in 3 mL of buffer and centrifuged again at 22 400g for 20 min. The resulting pellet was again suspended in 3 mL of buffer, rehomogenized, divided into 0.5-mL fractions, and stored at -20 °C for at least 24 h before use. For the inhibition studies, the thawed membrane preparations were diluted with 20 volumes of ice-cold buffer, and 900-μL aliquots were incubated at 0 °C for 60 min with [^{3}H]flunitrazepam (76.9 Ci/mmol, NEN, final concentration of 0.4 nM) and varying concentrations of the test compound ranging from 10^{-5} to 5×10^{-10} M (final concentrations for a total volume of 1 mL). Nonspecific binding was measured in the presence of 10 μM nonradioactive flunitrazepam and represented 10–15% of the total binding. Incubations were terminated by adding 3 mL of cold buffer to each incubation tube, filtering through Whatman GF/B glass fiber filters, and washing each filter three times with 5 mL of cold buffer. Radioactivity retained on the filters was counted in 10 mL of Aquasol scintillation solution with an LKB Wallac 1215 Rackbeta 2 counter. Each value was determined in triplicate. IC_{50} values (the concentration of ligand inhibiting 50% of flunitrazepam binding) were determined by Hofstee analysis.

The acyl azide **7** was not tested since, under the conditions necessary to achieve and maintain solubilization (aqueous HCl, buffer), the molecule was not stable.

In Vivo Studies. Baboons. *Papio papio* baboons (5–10 kg) either naturally sensitive to intermittent light stimulation (ILS) (25 cps) of convulsions or nonsensitive to ILS were used.[26] EEG was registered by means of brain-implanted electrodes[26] connected to a Grass polygraph recorder. β-Carbolines (1–10 mg/kg) were suspended in 200–400 μL of saline and dissolved by the addition of the minimum amount of concentrated hydrochloric acid and the resulting solution was diluted to 1–2 mL with saline. All solutions were injected intravenously (iv). A compound was judged convulsant if it induced convulsions in nonphotosensitive baboons without the use of ILS. Proconvulsant compounds, without inducing convulsions in nonphotosensitive baboons, made these animals sensitive to ILS presented 1 min after drug administration and also induced convulsions in photosensitive animals without the use of ILS. A decrease in the latency time of the onset of a convulsion under ILS as well as enhanced proconvulsive symptoms (a higher score in the Killam scale of convulsant activity[26]) was also used as criterion for judging the proconvulsant character of a compound. Anticonvulsant compounds blocked the response to ILS in photosensitive animals, increased the latency time, and/or diminished the animal's score on the Killam scale.

Mice. Convulsion Studies with β-CMC (9). Male Swiss mice (25 g, 10 per group) were used to assess the pharmacological activity of β-CMC. Thus, β-CMC (solutions prepared as for baboons) was administered subcutaneously at doses up to 20 mg/kg alone, 10 min before pentylenetetrazol treatment (convulsive and subconvulsive doses) or 10 min before β-CCM treatment. For studies in conjunction with diazepam, β-CMC was injected 10 min after diazepam administration but 10 min before pentylenetetrazol.

Conflict Studies with β-CMC. The punishment–reward conflict experiments used in assessing the anxiolytic or anxiogenic properties of drugs have been previously described in detail.[8] Briefly, mice were deprived of food until they attained 80% of their free-feeding weight. They were then trained in a Skinner box to press a lever to receive a food pellet. After attainment of stable pressing rates of approximately eight presses/min, mice were submitted to 15-min daily sessions divided such that, during the first and last 5-min periods, each lever press resulted in a food pellet (nonconflict) while, during the central 5-min period, each lever press was similarly rewarded with a food pellet but also concomitantly punished with an electric foot shock (conflict). After 2 weeks of training on this 15-min paradigm, mice showed stable day-to-day performances. Anxiolytic drugs (diazepam) produce increased rates of lever pressing during the conflict period relative to control, while anxiogenic drugs (β-CCM) result in decreased rates of lever pressing during this period. β-CMC was injected alone (sc, up to 20 mg/kg) or 10 min after diazepam (1 mg/kg, sc). Groups of seven to ten mice were used for each experiment.

Rotarod Studies with β-CMC. The rotarod-deficit was evaluated on a 2.5-cm diameter wooden rod rotating at 4 rpm. Mice were placed backwards on the rotating rod in such a way that they had to turn around to face the movement. Mice were pretrained for a 5-min period 30 min before the beginning of the experiment. The ability of each mouse to stay on the rod for 1 min was then pretested just before the first drug treatment. The occasional mouse not succeeding this pretest was eliminated. Mice were then injected with diazepam (2 mg/kg, sc) followed 10 min later by β-CMC (up to 20 mg/kg, sc) or saline and tested every 10 min during the next hour. Each experiment was repeated on 10 mice.

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S-(Nitrocarbobenzoxy)glutathiones: Potent Competitive Inhibitors of Mammalian Glyoxalase II

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Three potent competitive inhibitors of mammalian liver glyoxalase II, the *S*-(*o*-, *m*-, and *p*-nitrocarbobenzoxy)-glutathiones, have been synthesized and studied. The K_i values of the ortho, meta, and para isomers, as inhibitors of rat liver glyoxalase II, were 15, 9, and 6.5 μM, respectively. While showing marked competitive inhibition of glyoxalase II, the glutathione derivatives were almost inactive as inhibitors of glyoxalase I. For example, with the para isomer, $[I]_{0.5}$ values for rat liver glyoxalase I and II were 925 and 12 μM, respectively. This is in marked contrast to other glyoxalase II competitive inhibitors, which in general are even more effective against glyoxalase I. The *S*-(*o*-, *m*-, and *p*-nitrocarbobenzoxy)glutathiones have found utility as affinity ligands for the purification of rat liver glyoxalase II and may well have use in the study of the glyoxalase enzymes in vivo.

The glyoxalase system[1-6] catalyzes the reactions given in eq 1 and 2. Throughout the study of the enzymes of the glyoxalase system, Glo-I and Glo-II (EC 4.4.1.5 and 3.1.2.6, respectively), several inhibitors of these enzymes

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