**Method I. Circular Dichroism Studies.** Compounds (+)-2 and (+)-AG were dissolved in Uvasol acetonitrile (c = 80.3 and 163.5 μmol/L, respectively. The CD spectra were measured from 220 to 350 nm at a temperature of 23 °C. The $\Delta\epsilon$ scales given previously[20,21] show values which are lower because of erroneous calibrations of the dichrograph.

**Biological Methods.** The preparation of aromatase and desmolase as well as the enzyme assays were performed as described previously.[13]

**Acknowledgment.** Thanks are due to Verband der Chemischen Industrie, Fonds der Chemischen Industrie, and to Deutsche Forschungsgemeinschaft, who supported this work by grants (SFB 234). The technical assistance of Lydia Gottswinter, Nikola Pustet, Martina Palzer, and Doris Schuster is gratefully acknowledged. Some of the experiments were performed at the Institute of Pharmacy (Lehrstuhl Pharmazeutische Chemie II, Prof. Dr. Schönenberger) at the University of Regensburg. Thanks are due to Prof. Schönenberger.

(20) Hartmann, R. W.; Batzl, C.; Mannschreck, A.; Seydel, J. K. New Aromatase Inhibitors. QSAR and Evaluation of Mammary Tumor Inhibiting Activity. In *Trends in Medicinal Chemistry, '88*; van der Goot, H., Domány, G., Pallos, L., Timmerman, H., Eds.; Elsevier: Amsterdam, 1989; pp 821-838.

(21) Hartmann, R. W.; Batzl, C.; Mannschreck, A.; Pongratz, T. M. Stereoselective Aromatase Inhibition by the Enantiomers of 3-Cyclohexyl-3-(4-Aminophenyl)-2,6-Piperidinedione. In *Chirality and Biological Activity*; Frank, H., Holmstedt, B., Testa, B., Eds.; Alan R. Liss, Inc.: Hitchin, England, 1990; pp 185-189.

**Registry No.** (±)-1, 140926-09-6; (±)-1a, 140926-10-9; (±)-1b, 140926-11-0; (±)-1c, 140926-12-1; (±)-1d, 140926-13-2; 1e, 140-29-4; (±)-2, 137548-41-5; (+)-2, 137623-89-3; (-)-2, 137623-91-7; (±)-2a, 140926-14-3; (±)-2b, 140926-15-4; (±)-2c, 140926-16-5; (±)-2d, 140926-17-6; (±)-3, 140926-18-7; 4A, 141042-79-7; 4A·brucine, 141042-80-0; 4B, 141042-81-1; 4B·brucine, 141042-82-2; (±)-AG, 55511-45-0; (+)-AG, 55511-44-9; (+)-AG·(+)-tartrate, 57344-88-4; (-)-AG, 57288-03-6; (-)-AG·(-)-tartrate, 57288-04-7; cyclopentyl iodide, 1556-18-9; cyclohexyl bromide, 108-85-0; aromatase, 9039-48-9; desmolase, 37292-81-2.

**Supplementary Material Available:** $^1$H-NMR data (Tables II and III) of the synthesized compounds (2 pages). Ordering information is given on any current masthead page.

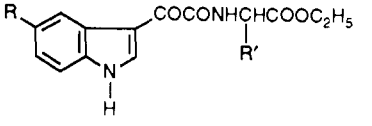
# Benzodiazepine Receptor Affinity and Interaction of Some *N*-(Indol-3-ylglyoxylyl)amine Derivatives

Anna Maria Bianucci,† Antonio Da Settimo,† Federico Da Settimo,† Giampaolo Primofiore,*,† Claudia Martini,‡ Gino Giannaccini,‡ and Antonio Lucacchini‡

*Istituto di Chimica Farmaceutica and Istituto Policattedra di Discipline Biologiche, Università di Pisa, 56126 Pisa, Italy.*
*Received August 8, 1991*

Several derivatives, in which tryptamine, tyramine, and dopamine moieties are linked to the indole nucleus by an oxalyl bridge, were tested for their ability to displace the specific binding of [$^3$H]flunitrazepam from bovine brain membranes. GABA ratio and in vivo tests for the most potent compounds showed they behave as inverse agonists at the benzodiazepine receptor (BzR). To better define the structure-activity relationship (SAR) of this kind of ligand, several phenylethylamine derivatives were synthesized to evaluate their affinity to BzR. Some of these derivatives (**17**, **21**, **24**, **26**, and **30**) were found to exhibit high affinity ($K_i$ = 0.51-0.085 μM) for BzR and possessed a partial agonist activity, although their chemical structure is closely related to tryptamine **2-6**, tyramine **7-11**, and dopamine **12-16** derivatives. A different interaction of these ligands to the receptor site is hypothesized. Moreover, all the prepared 1-methyl derivatives exhibited very low binding affinity to BzR.

The identification of high-affinity, saturable, and stereospecific binding sites for benzodiazepines in the central nervous system[1,2] has prompted an intensive search for an endogenous ligand that physiologically acts on these receptors. Some β-carboline derivatives as esters of the β-carboline-3-carboxylic acid,[3] which exhibited high affinity to the benzodiazepine receptor (BzR), gave rise to a noteworthy interest[4-13] as they could have represented the endogenous ligand. However, the endogenous compound has not yet been isolated. Although the complete biosynthetic pathway of β-carbolines in animals or in man is not known, it seems evident that these compounds are derivatives of tryptophan, tryptamine, or more generally of a (2-aminoethyl)indole structure. With this in mind, in the past we prepared some *N*-(indol-3-ylglyoxylyl)amino acid derivatives **1** containing a more flexible structure but analogous to that of β-carboline.[14]




**1**

Since there are many suggestions[12,15-17] indicating that

† Istituto di Chimica Farmaceutica.
‡ Istituto Policattedra di Discipline Biologiche.

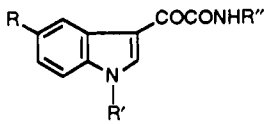
(1) Squires, R. F.; Braestrup, C. Benzodiazepine Receptors in Rat Brain. *Nature (London)* **1977**, *266*, 732-734.

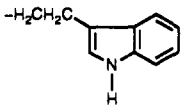
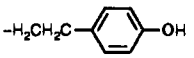
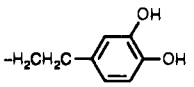
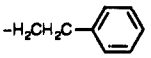
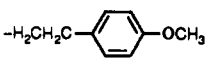
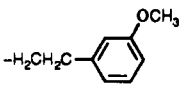
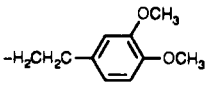
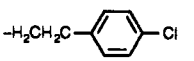
(2) Mohler, H.; Okada, T. Benzodiazepine Receptor: Demonstration in the Central Nervous System. *Science* **1977**, *198*, 849-851.

(3) Braestrup, C.; Nielsen, M.; Olsen, C. E. Urinary and Brain β-Carboline-3-carboxylates as Potent Inhibitors of Brain Benzodiazepine Receptors. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 2288-2292.

0022-2623/92/1835-2214$03.00/0 

**Table I.** Physical Properties of *N*-[(Substituted indol-3-yl)glyoxylyl]amine Derivatives

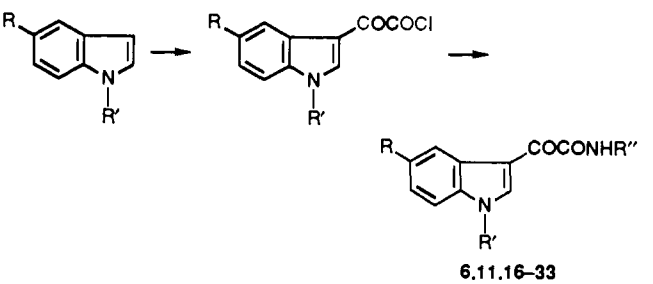


| no. | R | R′ | R″ | yield, % | cryst solvent | mp, °C | formula[a] |
|---|---|---|---|---|---|---|---|
| **6** | H | $CH_3$ |  | 45 | benzene | 193–195 | $C_{21}H_{19}N_3O_2$ |
| 11 | H | $CH_3$ |  | 53 | benzene | 178–179 | $C_{19}H_{18}N_2O_3$ |
| 16 | H | $CH_3$ |  | 34 | benzene | 174–175 | $C_{19}H_{18}N_2O_4$ |
| 17 | H | H |  | 74 | benzene | 208–210 | $C_{18}H_{16}N_2O_2$[b] |
| 18 | Cl | H | | 59 | methanol | 238–239 | $C_{18}H_{15}ClN_2O_2$ |
| **19** | $NO_2$ | H | | 57 | ethanol | >300 | $C_{18}H_{15}N_3O_4$ |
| **20** | H | $CH_3$ | | 32 | benzene–petroleum ether | 131–132 | $C_{19}H_{18}N_2O_2$ |
| 21 | H | H |  | 58 | methanol | 203–205 | $C_{19}H_{18}N_2O_3$ |
| **22** | $NO_2$ | H | | 41 | ethanol | 277–279 | $C_{19}H_{17}N_3O_5$ |
| **23** | H | $CH_3$ | | 26 | benzene–petroleum ether | 166–167 | $C_{20}H_{20}N_2O_3$ |
| 24 | H | H |  | 77 | benzene | 177–178 | $C_{19}H_{18}N_2O_3$ |
| **25** | $NO_2$ | H | | 49 | ethanol | 239–241 | $C_{19}H_{17}N_3O_5$ |
| **26** | H | H |  | 64 | benzene | 158–159 | $C_{20}H_{20}N_2O_4$ |
| 27 | Cl | H | | 69 | benzene | 156–157 | $C_{20}H_{19}ClN_2O_4$ |
| **28** | $NO_2$ | H | | 68 | methanol | 232–234 | $C_{20}H_{19}N_3O_6$ |
| **29** | H | $CH_3$ | | 41 | benzene–petroleum ether | 127–129 | $C_{21}H_{22}N_2O_4$ |
| **30** | H | H |  | 55 | benzene | 222–223 | $C_{18}H_{15}ClN_2O_2$[c] |
| 31 | Cl | H | | 63 | methanol | 252–253 | $C_{18}H_{14}Cl_2N_2O_2$ |
| **32** | $NO_2$ | H | | 59 | DMF–water | >300 | $C_{18}H_{14}ClN_3O_4$ |
| **33** | H | $CH_3$ | | 54 | benzene–petroleum ether | 160–161 | $C_{19}H_{17}ClN_2O_2$ |

[a] Elemental analyses for C, H, N were within ±0.4% of the calculated values. [b] Previously described in ref 42. [c] This product is described in ref 43 with a mp of 90 °C, very different from the one reported here. The IR and $^1H$ NMR data for our product are fully consistent with the assigned structure.

the inverse agonist/antagonist binding site is constituted by a large lipophilic region at the base of a narrow cleft,

**Scheme I**



**6.11.16–33**

we have hypothesized[18] that compounds **1** interact at the receptor site in a planar or pseudoplanar conformation in


(4) Cain, M.; Weber, R. W.; Guzman, F.; Cook, J. M.; Barker, S. A.; Rice, K. C.; Crawley, J. N.; Paul, S. M.; Skolnick, P. β-Carbolines: Synthesis and Neurochemical and Pharmacological Actions on Brain Benzodiazepine Receptors. *J. Med. Chem.* **1982**, *25*, 1081–1091.

(5) Lippke, K. P.; Schunack, W. G.; Wenning, W.; Müller, W. E. β-Carbolines as Benzodiazepine Receptor Ligands. 1. Synthesis and Benzodiazepine Receptor Interaction of Esters of β-Carboline-3-carboxylic Acid. *J. Med. Chem.* **1983**, *26*, 499–503.

(6) Guzman, F.; Cain, M.; Larscheid, P.; Hagen, T.; Cook, J. M.; Schweri, M.; Skolnick, P.; Paul, S. M. Biomimetic Approach to Potential Benzodiazepine Receptor Agonists and Antagonists. *J. Med. Chem.* **1984**, *27*, 564–570.

(7) Lippke, K. P.; Müller, W. E.; Schunack, W. G. β-Carbolines as Benzodiazepine Receptor Ligands II: Synthesis and Benzodiazepine Receptor Affinity of β-Carboline-3-carboxylic Acid Amides. *J. Pharm. Sci.* **1985**, *74*, 676–680.

(8) Hagen, T. J.; Guzman, F.; Schultz, C.; Cook, J. M.; Skolnick, P.; Shannon, H. E. Synthesis of 3,6-Disubstituted β-Carbolines Which Possess Either Benzodiazepine Antagonist or Agonist Activity. *Heterocycles* **1986**, *24*, 2845–2855.

(9) Hagen, T. J.; Skolnick, P.; Cook, J. M. Synthesis of 6-Substituted β-Carbolines That Behave as Benzodiazepine Receptor Antagonists or Inverse Agonists. *J. Med. Chem.* **1987**, *30*, 750–753.

(10) Dodd, R. H.; Ouannès, C.; Potier, M. C.; Prado de Carvalho, L.; Rossier, J.; Potier, P. Synthesis of β-Carboline–Benzodiazepine Hybrid Molecules: Use of the Known Structural Requirements for Benzodiazepine and β-Carboline Binding in Designing a Novel, High-Affinity Ligand for the Benzodiazepine Receptor. *J. Med. Chem.* **1987**, *30*, 1248–1254.

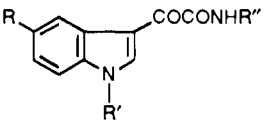
(11) Potier, M. C.; Prado de Carvalho, L.; Dodd, R. H.; Besselievre, R.; Rossier, J. "In Vivo" Binding of β-Carbolines in Mice: Regional Differences and Correlation of Occupancy to Pharmacological Effects. *J. Mol. Pharmacol.* **1988**, *34*, 124–128.

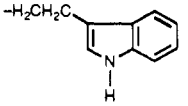
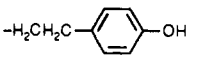
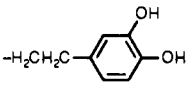
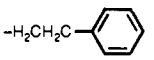
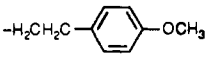
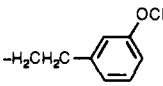
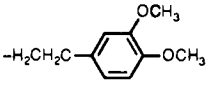
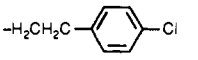
(12) Allen, M. S.; Hagen, T. J.; Trudell, M. L.; Codding, P. W.; Skolnick, P.; Cook, J. M. Synthesis of Novel 3-Substituted β-Carbolines as Benzodiazepine Receptor Ligands: Probing the Benzodiazepine Receptor Pharmacophore. *J. Med. Chem.* **1988**, *31*, 1854–1861.

(13) Settimj, G.; Del Giudice, M. R.; Ferretti, R.; Gatta, F. β-Carbolines as Agonistic or Antagonistic Benzodiazepine Receptor Ligands. 1. Synthesis of some 5-, 6- and 7-Amino Derivatives of 3-Methoxycarbonyl-β-carboline (β-CCM) and of 3-Ethoxycarbonyl-β-carboline (β-CCE). *J. Heterocycl. Chem.* **1988**, *25*, 1391–1397.

(14) Martini, C.; Gervasio, T.; Lucacchini, A.; Da Settimo, A.; Primofiore, G.; Marini, A. M. Specific Inhibition of Benzodiazepine Receptor Binding by Some *N*-(Indol-3-ylglyoxylyl)-amino Acid Derivatives. *J. Med. Chem.* **1985**, *28*, 506–509.

Table II. In Vitro Data of *N*-[(Substituted indol-3-yl)glyoxylyl]amine Derivatives



| no. | R | R′ | R″ | inhibn,[a] % (10 μM) | $K_i$[b] (μM) | GABA ratio[c] |
|---|---|---|---|---|---|---|
| 2 | H | H |  | 83 ± 8 | 1.5 ± 0.2 | 1 |
| 3 | Cl | H | | 70 ± 5 | – | – |
| 4 | Br | H | | 62 ± 6 | – | – |
| 5 | $NO_2$ | H | | 90 ± 6 | 0.35 ± 0.05 | 0.65 |
| 6 | H | $CH_3$ | | 39 ± 5 | – | – |
| 7 | H | H |  | 82 ± 8 | 2.4 ± 0.2 | 0.7 |
| 8 | Cl | H | | 83 ± 9 | 2.2 ± 0.2 | 0.8 |
| 9 | Br | H | | 75 ± 6 | 2.3 ± 0.2 | 0.8 |
| 10 | $NO_2$ | H | | 100 ± 2 | 0.35 ± 0.04 | 0.7 |
| 11 | H | $CH_3$ | | 58 ± 6 | – | – |
| 12 | H | H |  | 68 ± 6 | 4.2 ± 0.4 | 0.92 |
| 13 | Cl | H | | 90 ± 5 | 1.0 ± 0.8 | 0.52 |
| 14 | Br | H | | 85 ± 7 | 1.2 ± 0.1 | 0.7 |
| 15 | $NO_2$ | H | | 100 ± 2 | 0.28 ± 0.03 | 0.64 |
| 16 | H | $CH_3$ | | 17 ± 5 | – | – |
| 17 | H | H |  | 94 ± 2 | 0.38 ± 0.02 | 1.3 |
| 18 | Cl | H | | 25 ± 3 | – | – |
| 19 | $NO_2$ | H | | 39 ± 4[d] | – | – |
| 20 | H | $CH_3$ | | 42 ± 2 | – | – |
| 21 | H | H |  | 97 ± 4 | 0.09 ± 0.01 | 1.3 |
| 22 | $NO_2$ | H | | 32 ± 3 | – | – |
| 23 | H | $CH_3$ | | 67 ± 8 | – | – |
| 24 | H | H |  | 97 ± 4 | 0.085 ± 0.005 | 1.2 |
| 25 | $NO_2$ | H | | 25 ± 3 | – | – |
| 26 | H | H |  | 96 ± 4 | 0.51 ± 0.03 | 1.3 |
| 27 | Cl | H | | 94 ± 3 | 0.7 ± 0.02 | 1.3 |
| 28 | $NO_2$ | H | | ND[e] | 0.18 ± 0.009 | 1.0 |
| 29 | H | $CH_3$ | | 51 ± 5 | – | – |
| 30 | H | H |  | ND[e] | 0.34 ± 0.02 | 1.2 |
| 31 | Cl | H | | 4 ± 0.3 | – | – |
| 32 | $NO_2$ | H | | 0 | – | – |
| 33 | H | $CH_3$ | | 25 ± 0.2 | – | – |
| diazepam | | | | | 0.010 ± 0.003 | 1.7 |
| β-CCE | | | | | 0.0012 ± 0.0001 | 0.75 |
| Ro 15-1788 | | | | | 0.00038 ± 0.00002 | 1 |

[a] Percents of inhibition of specific [$^3$H]flunitrazepam binding at 10 μM compound concentration are means ± SEM of five determinations. [b] $K_i$ are means ± SEM of three determinations. [c] GABA ratio = $K_i$ without GABA/$K_i$ with GABA. [d] Determined at 1 μM concentration of the compound for its insolubility at 10 μM. [e] Not determined for insolubility of the compound at 10 μM.

which the aromatic indole system and the glyoxylyl amide moiety lie approximately in the same plane. In this case, the interaction at the receptor site should occur via a hydrogen bond of the indole NH with a receptor hydrogen bond acceptor site, and of the oxygen atom, C=O(2), of the oxalyl bridge with a hydrogen bond donor site. This oxygen atom should play the same role as the β-carboline N(2) for a hydrogen bond with the donor site. The glyoxylyl amino acid chain in the 3 position of the indole nucleus could serve as a further point of attachment to the receptor.

In the present paper we report affinity data at BzR and the activity profile of several derivatives **2–16** in which the 3-glyoxylyl indole moiety has been linked to a biogenic amine such as tryptamine, tyramine, and dopamine. It is well known that these amines possess potent biological activities in the central nervous system. Since they physiologically derive from the amino acids tryptophan, tyrosine, and dioxyphenylalanine, our new compounds are related to amino acid derivatives **1**, and they could represent a link with the still unknown endogenous ligand at BzR. Moreover, for a better and wider definition of structure–activity relationships (SAR), several phenylethylamine derivatives **17–33** variously substituted on the phenyl ring have been synthesized and tested for their affinity at BzR.

## Results

Compounds **2–5**, **7–10**, and **12–15**, previously synthesized by us,[19,20] were tested for their ability to displace the

(15) Hollinshead, S. P.; Trudell, M. L.; Skolnick, P.; Cook, J. M. Structural Requirements for Agonist Actions at the Benzodiazepine Receptor: Studies with Analogues of 6-(Benzyloxy)-4-(methoxymethyl)-β-carboline-3-carboxylic Acid Ethyl Ester. *J. Med. Chem.* **1990**, *33*, 1062–1069.

(16) Allen, M. S.; Tan, Y. C.; Trudell, M. L.; Narayanan, K.; Schindler, L. R.; Martin, M. J.; Schultz, C.; Hagen, T. J.; Koehler, K. F.; Codding, P. W.; Skolnick, P.; Cook, J. M. Synthetic and Computer-Assisted Analyses of the Pharmacophore for the Benzodiazepine Receptor Inverse Agonist Site. *J. Med. Chem.* **1990**, *33*, 2343–2357.

(17) Trudell, M. L.; Lifer, S. L.; Tan, Y. C.; Martin, M. J.; Deng, L.; Skolnick, P.; Cook, J. M. Synthesis of Substituted 7,12-Dihydropyrido[3,2-*b*:5,4-*b*′]diindoles: Rigid Planar Benzodiazepine Receptor Ligands with Inverse Agonist/Antagonist Properties. *J. Med. Chem.* **1990**, *33*, 2412–2420.

(18) Primofiore, G.; Marini, A. M.; Da Settimo, F.; Martini, C.; Bardellini, A.; Giannaccini, G.; Lucacchini, A. Specific Inhibition of Benzodiazepine Receptor Binding by Some *N*-(Indol-3-ylglyoxylyl)amino Acid Derivatives: Stereoselective Interactions. *J. Med. Chem.* **1989**, *32*, 2514–2518.

Table III. Biological Activity of Some N-[(substituted indol-3-yl)glyoxylyl]amine Derivatives

| no. | anticonvulsant action[a] | proconvulsant action:[b] $ED_{50}$, mg/kg | diazepam antagonism:[c] $ED_{50}$, mg/kg |
|---|---|---|---|
| 10 | no effect[d] | 75[e] | 50[e] |
| 15 | no effect[d] | 100[e] | 75[e] |
| 17 | no effect[d] | no effect[d] | no effect[d] |
| 21 | no effect[d] | no effect[d] | no effect[d] |

[a] Test drug administered intraperitoneally before 80 mg/kg PTZ. [b] Dose necessary to induce convulsions in 50% of the mice that had been previously given a subconvulsant dose of PTZ (40 mg/kg). [c] Dose necessary to antagonize the anticonvulsant effects of diazepam (2.5 mg/kg) in mice that had been given a convulsant dose of PTZ (80 mg/kg). [d] The highest concentration of the tested compounds was 250 mg/kg. [e] Values represent the mean of at least three determinations (≤20% differences among experiments).

specific binding of [$^3$H]flunitrazepam from bovine cortical membranes and exhibited moderate to high affinities with $K_i$ values ranging from 4.2 to 0.28 μM (Table II). The affinity is enhanced when an electron withdrawing group is present at the 5-position of the indole nucleus (**5**, R = $NO_2$, $K_i$ = 0.35 μM; **10**, R = $NO_2$, $K_i$ = 0.35 μM; **15**, R = $NO_2$, $K_i$ = 0.28 μM compared with the 5-unsubstituted derivatives showing $K_i$ values of 1.5, 2.4, and 4.2 μM, respectively).

The 1-methyl analogues **6**, **11**, and **16** were prepared by condensation of (1-methylindol-3-yl)glyoxylyl chloride[21] with the appropriate amine in the presence of triethylamine in benzene solution for **6**, and in THF solution for **11** and **16** (Scheme I, Table I). These compounds demonstrated a very low affinity at BzR.

To better define the SAR of this type of ligand the O-methyl analogues of tyramine (**21–23**) and dopamine (**26–29**) have been prepared, as well as the phenylethylamine derivatives with no substituent on the phenyl ring (**17–20**), with a *m*-methoxy group (**24–25**), and with a *p*-chloro group (**30–33**). The general synthetic procedure used in the preparation of compounds **17–33** involved the acylation of the appropriate indole with oxalyl chloride according to a published procedure.[22] The resulting indolylglyoxylyl chlorides were allowed to react in mild conditions in benzene solution with the appropriate amine in the presence of triethylamine (Scheme I). All products were purified by crystallization (Table I). Some of these compounds showed high affinity at BzR with $K_i$ values ranging from 0.70 to 0.085 μM, demonstrating a class of ligands more potent than hydroxy analogues **7–10** and **12–15**. However, in this case, the presence of an electron withdrawing group in the 5-position of the indole nucleus, which enhances the polarization of the indole N–H bond, does not increase the affinity. Nevertheless, in this case, too, methylation in the 1-position (compounds **20**, **23**, **29**, **33**) drastically lowers the affinity similarly to what occurred for compounds **6**, **11**, and **16**.

Surprisingly, although a similar chemical structure, compounds **2–16** and **17–33** exhibited a different pharmacological profile. Indeed, using an exhaustively washed membrane preparation, the GABA ratio values were evaluated as in vitro indicators of the agonist, inverse agonist, or antagonist properties, according to the suggestions of different authors.[23,24] These values showed a pharmacological behavior of inverse agonist for compounds **2–16** and of partial agonist for compounds **17–33** (Table II), since they were lower and greater than unity, respectively.

Moreover, the efficacy profile of the most potent compounds **10**, **15**, **17**, and **21** (Table III), was checked by the in vivo tests, which confirmed inverse agonist properties for **10** and **15**, and partial agonist properties for **17** and **21**. In fact, these compounds were tested in mice for anticonvulsant properties by using the convulsant pentylenetetrazole (PTZ, 80 mg/kg) and for proconvulsant action by employing a subthreshold dose of PTZ (40 mg/kg). They were also tested as benzodiazepine antagonists by assessing their ability to disrupt the anticonvulsant action of diazepam (2.5 mg/kg) against PTZ (80 mg/kg). None of the compounds displayed anticonvulsant activity, since they did not antagonize the convulsant action of PTZ (80 mg/kg). In fact, PTZ (80 mg/kg) produced seizures in all animals even at the highest doses of the tested compounds (250 mg/kg). Compounds **17** and **21** were neither anticonvulsant or proconvulsant, nor did they antagonize the anti-PTZ action of diazepam even at highest doses (250 mg/kg). Compounds **10** and **15** showed proconvulsant activity with an $ED_{50}$ of 75 and 100 mg/kg, respectively. They also antagonize the anticonvulsant action of diazepam with an $ED_{50}$ of 50 and 75 mg/kg, respectively.

## Discussion

Compounds **2–33**, tested for their affinity at BzR, constitute a new class of ligands, some of which bind to the receptor site with high affinity similar to that exhibited by the *N*-[(5-substituted indol-3-yl)glyoxylyl]amino acid esters.[18] On the basis of GABA ratio values and in vivo tests, the examined products can be classified in two groups; the former includes compounds **2–16** showing an inverse agonist activity, while the latter, compounds **17–33**, exhibited a partial agonist profile. This is quite surprising as all products have a very similar chemical structure. However, compounds **2–16** can be differentiated by the occurrence of a protic moiety such as an indole NH for the tryptamine derivatives **2–6** or a hydroxy group for **7–16**. The compounds possessing aprotic moieties such as the unsubstituted and chloro- or methoxy-substituted phenyl rings (**17–33**) behave as partial agonists. This cannot be due to the steric hindrance of substituents, since the bulky tryptamine derivatives **2–6** demonstrated the same efficacy as the hydroxy compounds **7–16**. In addition, the unsubstituted phenyl compounds **17–20** showed the same pharmacological behavior as the chloro- and methoxy-substituted derivatives **21–33**.

In the first group of compounds (**2–16**), having an inverse agonist activity, the most potent derivatives were

(19) Da Settimo, A.; Primofiore, G.; Marini, A. M.; Mori, C.; Franzone, J. S.; Cirillo, R.; Reboani, C. Synthesis and Antiinflammatory Activity of Some N-(5-Substituted Indol-3-ylglyoxylyl)amine Derivatives. *Farmaco, Ed. Sci.* **1987**, *42*, 17–26.

(20) Primofiore, G.; Marini, A. M.; Da Settimo, F.; Franzone, J. S.; Mason, U.; Reboani, C.; Cirillo, R. N-(Indol-3-ylglyoxylyl)-dopamine Derivatives: Preparation and Anti-Depressant Activity. *Eur. J. Med. Chem.* **1988**, *23*, 397–401.

(21) Alemany, A.; Alvarez, E. F.; Lopez, O. N.; Herraez, M. E. R. Enzyme Inhibitors. XII. Preparation of 3-(2-propargylaminoethyl)indoles. *Bull. Soc. Chim. Fr.* **1974**, 2883–2888. Davis, P. D.; Bit, R. A.; Hurst, S. A. A convenient Synthesis of Bisindolyl- and Indolylaryl-maleic Anhydrides. *Tetrahedron Lett.* **1990**, *31*, 2353–2356.

(22) Da Settimo, A.; Primofiore, G.; Marini, A. M.; Ferrarini, P. L.; Franzone, J. S.; Cirillo, R.; Reboani, M. C. N-(Indol-3-ylglyoxylyl)methionine Derivatives: Preparation and Gastric Anti-Secretory Activity. *Eur. J. Med. Chem.* **1988**, *23*, 21–24.

(23) Braestrup, C.; Nielsen, M.; Honorè, T.; Jensen, L. H.; Petersen, E. M. Benzodiazepine Receptor Ligands With Positive and Negative Efficacy. *Neuropharmacology* **1983**, *22*, 1451–1457.

(24) Braestrup, C.; Nielsen, M. Benzodiazepine Receptors. In *Handbook of Psychopharmacology*; Iversen, L. L., Iversen, S. D. Snyder, S. H., Eds.; Plenum Press: New York, 1983; Vol. 17, pp 285–384.

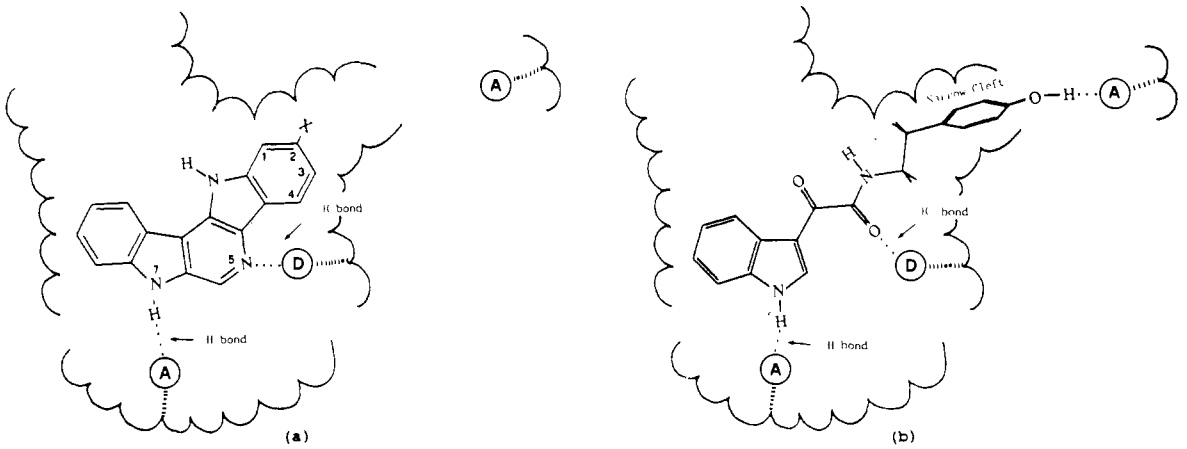



**Figure 1.** Binding interactions of pyridodiindoles **34** (a)[16,17] and phenylethylamide compounds **2–16** (b)[44] to the proposed pharmacophore model for the benzodiazepine receptor inverse agonist/antagonist site. A and D represent the acceptor and the donor hydrogen bond sites, respectively.

those bearing an electron withdrawing group at the 5-position, especially a nitro group. This fact is in accordance with our previous findings,[18] that an electron withdrawing substituent para to the indole NH function enhances the ability of this group to interact with a hydrogen bond acceptor site on the receptor via polarization of the indole NH bond, one of the primary points of interaction with the receptor site. Methylation at the 1-position of the indole nucleus gave compounds **6**, **11**, and **16** with very low affinity, confirming the importance of the NH group in the interaction at the receptor, as already evidenced by us and other authors.[12,15,16,25]

It is generally believed that BzR ligands elicit their pharmacological effects through modulation of the activity of GABA-gated chloride channels by interactions in different but partially overlapping domains of the same receptor site. Several pharmacophore models[26–31] have been proposed by the studies of SAR and molecular geometry of many ligands, both agonists and inverse agonists/antagonists. The recent report[32] that the BzR may be constituted by at least three homologous but distinct proteins has added a further degree of complexity to the problem. It is not surprising, therefore, that at present the nature of the binding sites necessary to accommodate inverse agonist/antagonist and agonist ligands has not been elucidated. For all these reasons and for an easier approach to the problem, the inverse agonist/antagonist[12,16,17,33] and agonist[15,34] binding sites are treated by some authors as two separate entities of the same receptor area. Thus, from the study of several β-carboline, pyridodiindole, azapyridodiindole, and 2-arylpyrazoloquinoline ligands, an inverse agonist/antagonist pharmacophore model has been proposed.[16,17] The essential features of this model include a narrow cleft at the base of which there should be a lipophilic region bearing two binding sites, one acceptor and one hydrogen bond donor. For this reason, only planar molecules or molecules capable of assuming a pseudoplanar conformation may sufficiently penetrate into the cleft and interact with the two binding sites. In the case of pyridodiindole derivatives **34**[17] the interacting groups should be the pyridinyl nitrogen atom in the 5-position, which binds to the hydrogen bond donor site on the receptor, and the NH group in the 7-position which is capable of forming a hydrogen bond with the acceptor site of the receptor. Moreover, the affinity values of several 1-, 2-, 3-, and 4-substituted pyridodiindole derivatives[17] evidenced the presence of a small lipophilic pocket that can accommodate only ligands bearing a 2-substituent.

We already have pointed out[18] that indolylglyoxylyl amino acid derivatives **1**, inverse agonists, although with a more flexible structure than β-carbolines or pyridodiindoles, should assume in the receptor interaction a planar or pseudoplanar conformation enabling these ligands to penetrate into the cleft to interact with the same bonding sites via the indole NH and the oxygen atom of the glyoxylyl C=O(2).

It is plausible to hypothesize that indolylglyoxylyl amine derivatives **2–16**, showing an inverse agonist pharmacological profile similar to the pyridodiindoles **34**, interact with the same receptor model[16,17] by the indole NH and

(25) Primofiore, G.; Marini, A. M.; Da Settimo, F.; Salvadori, C.; Martini, C.; Lucacchini, A.; Giannaccini, G. Specific Inhibition of Benzodiazepine Receptor Binding by Some N-(2-Methyl- or 1,2-Dimethylindol-3-ylglyoxylyl)amino Acid Derivatives. *Farmaco* **1990**, *45*, 341–351.
(26) Crippen, G. M. Distance Geometry Analysis of the Benzodiazepine Binding Site. *Mol. Pharmacol.* **1982**, *22*, 11–19.
(27) Loew, G. H.; Nienow, J. R.; Poulsen, M. Theoretical Structure–Activity Studies of Benzodiazepine Analogues. Requirements for Receptor Affinity and Activity. *Mol. Pharmacol.* **1984**, *26*, 19–34.
(28) Codding, P. W.; Muir, A. K. S. Molecular Structure of Ro15-1788 and a Model for the Binding of Benzodiazepine Receptor Ligands. Structural Identification of Common Features in Antagonists. *Mol. Pharmacol.* **1985**, *28*, 178–184.
(29) Fryer, R. I.; Cook, C.; Gilman, N. W.; Walser, A. Conformational Shifts at the Benzodiazepine Receptor Related to the Binding of Agonists Antagonists and Inverse Agonists. *Life Sci.* **1986**, *39*, 1947–1957.
(30) Borea, P. A.; Gilli, G.; Bertolasi, V.; Ferretti, V. Stereochemical Features Controlling Binding and Intrinsic Activity Properties of Benzodiazepine-Receptor Ligands. *Mol. Pharmacol.* **1987**, *31*, 334–344.
(31) Tebib, S.; Bourguignon, J. J.; Wermuth, C. G. The Active Analog Approach Applied to the Pharmacophore Identification of Benzodiazepine Receptor Ligands. *J. Comput.-Aided Mol. Des.* **1987**, *1*, 153–170.
(32) Pritchett, D. B.; Sontheimer, H.; Shivers, B. D.; Ymer, S.; Kettenmann, H.; Schofield, P. R.; Seeburg, P. H. Importance of a Novel $GABA_a$ Receptor Subunit for Benzodiazepine Pharmacology. *Nature (London)* **1989**, *338*, 582–585.
(33) Narayanan, K.; Cook, J. M. Molecular Yardsticks: Synthesis of Higher Homologs of 7,12-Dihydropyrido[3,4-*b*:5,4-*b*']diindole. Probing the Dimensions of the Benzodiazepine Receptor Inverse Agonist Site. *Heterocycles* **1990**, *31*, 203–209.
(34) Diaz-Arauzo, H.; Evoniuk, G. E.; Skolnick, P.; Cook, J. M. The Agonist Pharmacophore of the Benzodiazepine Receptor. Synthesis of a Selective Anticonvulsant/Anxiolytic. *J. Med. Chem.* **1991**, *34*, 1754–1756.

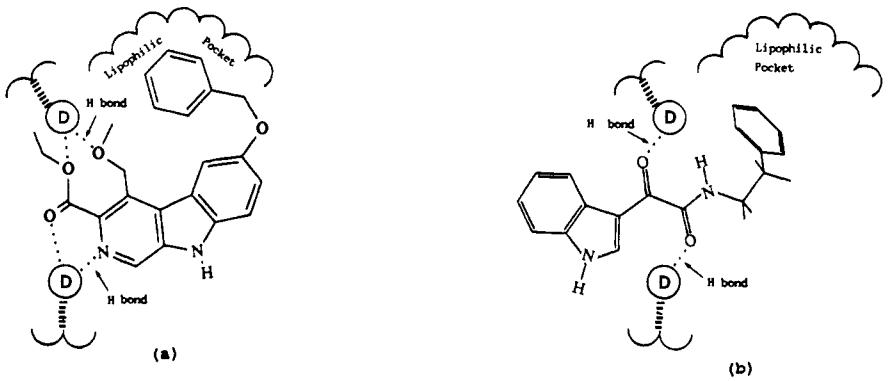



**Figure 2.** Binding interactions of **35** (a) and phenylethylamide compounds **17–33** (b)[44] to the pharmacophore model[15,34] for the benzodiazepine receptor agonist site. D represents the donor hydrogen bond site.

the C=O(2) of oxalyl bridge. However, a simple matching of the molecular size of pyridodiindoles **34**[17] and indolylglyoxylyl amine derivatives **2–16** indicated that the small lipophilic pocket, capable of accommodating only 2-substituted pyridodiindoles (the 1-, 3-, and 4-substituted ones show lower affinity values), does not have sufficient size to receive the indole or phenolic side chains. In order to rationalize these results, we propose a narrow cleft at the end of the small lipophilic pocket in which the planar phenyl or indole group of the side chain can be accommodated. Furthermore, we propose that the protic moieties of the indole NH or the hydroxy group on the phenyl ring of the side chain interact with a hydrogen bond acceptor site inside this narrow cleft to direct the molecules into the inverse agonist/antagonist pharmacophore. The molecules **17–33**, lacking this directing protic moiety, must be prohibited from interacting with the receptor site in the inverse agonist orientation. In Figure 1 we report, side-by-side, the interactions of the pyridodiindoles **34** (a) and of the compounds **2–16** (b) with the proposed receptor site.

In an agonist pharmacophore model[15,34] it is supposed that ligands should interact with two hydrogen bond donor sites, distanced 5.0–7.5 Å, and with a lipophilic region whose occupation leads to full agonist efficacy. In the case of partial agonists **17–33**, this interaction could be mediated by the two oxygen atoms of the oxalyl bridge, distanced 3.5 Å,[35] forming hydrogen bonds with the two donor sites on the receptor, and by the phenyl ring of the phenylethylamide chain, partially folded, in order to interact with the proposed lipophilic area of the receptor. By comparing the molecular model of a full agonist, such as the 6-(benzyloxy)-4-(methoxymethyl)-β-carboline-3-carboxylic acid ethyl ester **35**,[36,15] and our ligands **17–33**, it appears that the phenyl ring on the side chain of the latter compounds does not fully occupy this lipophilic area; consequently, only a partial agonist response is elicited. The binding interactions of the compound **35** (a) and the ligands **17–33** (b) with the agonist pharmacophore model are depicted in Figure 2. The NH indole group should not constitute an active bonding site, and electron withdrawing substituents in the 5-position of the indole ring decrease affinity, probably due to their steric hindrance or to the decreased lipophilic character of the ligands. However, compound **28** is an exception to this statement, being the most active in the series of compounds **26–29**. Moreover, the agonist model does not explain the drastic affinity decrease after methylation of indole NH group[15] (**20, 23, 29,** and **33**). Even if this information should be useful in understanding the correlation between the inverse agonist/antagonist and agonist models, it needs more probing before this is resolved.

Further work is now in progress in our laboratories to better define structural requirements necessary for a agonist or inverse agonist activity for this type of ligand.

## Experimental Section

**Chemistry.** Melting points were determined on a Köfler hotstage apparatus and are uncorrected. IR spectra were recorded with a Pye Unicam Infracord Model PU 9516 in Nujol mulls. $^1$H NMR spectra were determined in DMSO-$d_6$ with TMS as an internal standard on a Varian EM 360 A spectrometer and were also consistent with assigned structures. Magnesium sulfate was always used as drying agent. Evaporations were made in vacuo (rotating evaporator). Analytical TLC was carried out on Merck 0.2-mm precoated silica gel glass plates (60 F-254). Elemental analyses were performed by our analytical laboratory and agreed with theoretical values to within ±0.4%.

**General Procedure for the Synthesis of *N*-[(Substituted indol-3-yl)glyoxylyl]amine Derivatives 6, 11, 16–33.** To a stirred suspension of 2.5 mmol of indolylglyoxylyl chloride and 2.75 mmol of the appropriate amine hydrochloride in 50 mL of dry benzene (THF for compounds 11 and 16) cooled at 0 °C was added dropwise 6.0 mmol of triethylamine. The reaction mixture was left to warm to room temperature, stirred 24 h, refluxed 2 h, and then filtered. The collected precipitate was triturated with a saturated $NaHCO_3$ aqueous solution, washed with water, and collected again to give a first portion of crude product. The benzene (or THF) solution was evaporated to dryness, and the residue was treated with saturated $NaHCO_3$ aqueous solution, washed with water, and collected to yield an additional amount of crude product. The quantities of the amine derivatives obtained from the initial precipitate or from benzene (or THF) solution were variable depending upon the solubility of the various compounds. Yields, crystallization solvents, and melting points are listed in Table I.

**Binding Studies.** Tritiated flunitrazepam was obtained from E. I. du Pont de Nemours and Co., Inc., New England Nuclear Division (Dreieichenhaim, West Germany) and had a specific activity of 83.5 Ci/mol and a radiochemical purity > 99%. All other chemicals were of reagent grade and obtained from commercial suppliers.

[$^3$H]Flunitrazepam binding assay to bovine cerebral cortex membrane was carried out essentially as previously described.[18] Cortices were rapidly isolated and homogenized in 10 vol of ice-cold 0.32 M sucrose containing protease inhibitors.[37] The homogenate was centrifuged at 1000*g* for 10 min at 4 °C. The resulting pellet was discarded, and the supernatant was recentrifuged at 50000*g* for 15 min at 4 °C. The resulting membranes were frozen and washed using a procedure previously described

(35) Bianucci, A. M. Unpublished results.
(36) Stephens, D. N.; Shearman, G. T.; Kehr, W. Discriminative Stimulus Properties of β-Carbolines Characterized as Agonists and Inverse Agonists at Central Benzodiazepine Receptors. *Psychopharmacology* **1984**, *83*, 233–239.
(37) Martini, C.; Lucacchini, A.; Ronca, G.; Hrelia, S.; Rossi, C. A. Isolation of Putative Benzodiazepine Receptors From Rat Brain Membranes by Affinity Chromatography. *J. Neurochem.* **1982**, *38*, 15–19.

for removing endogenous GABA from rat cerebral cortex.[38] Finally, the pellet was suspended in 10 vol of 50 mM Tris-HCl buffer at pH 7.4 and used in the binding assay.

Protein concentration was assayed by the method of Lowry et al.[39]

Routine [$^3$H]flunitrazepam binding assays were run by incubating 0.4 mg of protein of crude bovine brain membrane suspensions at 0 °C for 90 min with [$^3$H]flunitrazepam (0.2 nM) in a total volume of 0.5 mL of Tris-HCl buffer. After incubation, the samples were diluted at 0 °C with 5 mL of the assay buffer and were immediately filtered under reduced pressure through glass fiber filter disks (Whatman GF/B). The filters were washed with 5 mL of the buffer, dried, and added to 8 mL of Ready Protein Beckman scintillation cocktail, and the radioactivity was counted in a LS 1800 scintillation counter. Nonspecific binding was determined by the radioactivity bound in the presence of 10 $\mu$M nonradioactive diazepam in parallel assays. Water-insoluble derivatives were dissolved in EtOH and added to the assay mixture. Blank experiments were carried out to determine the effect of EtOH (2%) on the binding. The inhibition of the specific binding was determined in the presence of various concentrations of unlabeled competing drugs. The affinity of drugs for the specific binding sites was expressed as the molar concentration inhibiting the specific binding by 50% ($IC_{50}$). These values were calculated from the displacement curves by log probit analysis with six to eight concentrations of the displacers each performed in triplicate.

$IC_{50}$ determinations for GABA ratio values were carried out in the absence and in the presence of 10 $\mu$M GABA. The dissociation constant ($K_i$) was derived according to the equation of Cheng and Prusoff.[40] The ligand affinity ($K_d$) of [$^3$H]flunitrazepam was 1.8 nM.

**In Vivo Studies.** Groups of 10 mice were injected intraperitoneally (0.1 mL) with graded doses of the compounds suspended in 1% (carboxymethyl)cellulose (vehicle) or an equal volume of vehicle, followed 30 min later by PTZ at 40 or 80 mg/kg to assess the proconvulsant and anticonvulsant actions, respectively, as described by Trudell et al.[41]

Antagonism of the anticonvulsant effects of diazepam was carried out as described by Cain et al.[4] Groups of 10 mice were injected with diazepam (2.5 mg/kg ip) followed 10 min later by administration of graded doses of agents or vehicle. Fifteen minutes after injection of the compound, animals were injected with PTZ (80 mg/kg).

**Acknowledgment.** This work was supported by grants from the Ministero della Pubblica Istruzione (research fund 60%) and the CNR Target Project on "Chimica Fine".

**Registry No.** **2**, 107634-85-5; **3**, 107610-05-9; **4**, 107610-04-8; **5**, 107610-06-0; **6**, 140677-06-1; **7**, 107610-00-4; **8**, 107610-02-6; **9**, 107610-01-5; **10**, 107610-03-7; **11**, 140677-07-2; **12**, 119284-49-0; **13**, 119284-51-4; **14**, 119284-50-3; **15**, 119284-53-6; **16**, 140677-08-3; **17**, 94209-12-8; **18**, 140677-09-4; **19**, 140677-10-7; **20**, 140677-11-8; **21**, 140677-12-9; **22**, 140677-13-0; **23**, 140677-14-1; **24**, 140677-15-2; **25**, 140677-16-3; **26**, 140677-17-4; **27**, 140677-18-5; **28**, 140677-19-6; **29**, 140677-20-9; **30**, 80554-83-2; **31**, 140677-21-0; **32**, 140677-22-1; **33**, 140677-23-2; indol-3-ylglyoxylyl chloride, 22980-09-2; (1-methylindol-3-yl)glyoxylyl chloride, 16382-38-0; (5-chloroindol-3-yl)glyoxylyl chloride, 883-55-6; (5-bromoindol-3-yl)glyoxylyl chloride, 63843-81-2; (5-nitroindol-3-yl)glyoxylyl chloride, 6953-35-1; 3-indoleethanamine, 61-54-1; tyramine, 51-67-2; dopamine, 51-61-6; phenylethylamine, 64-04-0; 4-methoxyphenethylamine, 55-81-2; 3-methoxyphenethylamine, 2039-67-0; 3,4-dimethoxyphenethylamine, 120-20-7; 4-chlorophenethylamine, 156-41-2; diazepam, 439-14-5.

(38) Martini, C.; Rigacci, T.; Lucacchini, A. $^3$H Muscimol Binding Site on Purified Benzodiazepine Receptor. *J. Neurochem.* **1983**, *41*, 1183–1185.

(39) Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein Measurement With the Folin Phenol Reagent. *J. Biol. Chem.* **1951**, *193*, 265–275.

(40) Cheng, Y. C.; Prusoff, W. H. Relation Between the Inhibition Constant ($K_i$) and the Concentration of Inhibitor Which Causes Fifty Per Cent Inhibition ($I_{50}$) of an Enzymic Reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.

(41) Trudell, M. L.; Basile, A. S.; Shannon, H. E.; Skolnick, P.; Cook, J. M. Synthesis of 7,12-Dihydropyrido[3,4-*b*:5,4-*b'*] diindoles. A Novel Class of Rigid, Planar Benzodiazepine Receptor Ligands. *J. Med. Chem.* **1987**, *30*, 456–458.

(42) Brimblecombe, R. W.; Downing, D. F.; Green, D. M.; Hunt, R. R. Some Pharmacological Effects of a Series of Tryptamine Derivatives. *Br. J. Pharmacol.* **1964**, *23*, 43–54.

(43) Kumar, A.; Agarwal, J. C.; Nath, C.; Gurtu, S.; Sinha, J. N.; Bhargava, K. P.; Shanker, K. Synthesis and Biological Activity of 2-Substituted-3-ethyl-N-alkyl/arylindoles. *J. Heterocycl. Chem.* **1981**, *18*, 1269–1271.

(44) The figure has been obtained as hard copies of the screen in a Workstation Silicon Graphics 4D20G using the Insight II software from Biosym Technologies, San Diego, CA. The indole glyoxamide moiety has been fully optimized using Monstergauss.