Synthesis, Structure−Activity Relationships, and Molecular Modeling Studies of *N*-(Indol-3-ylglyoxylyl)benzylamine Derivatives Acting at the Benzodiazepine Receptor[†,‡]

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A number of *N*-(indol-3-ylglyoxylyl)benzylamine derivatives were synthesized and tested for [^{3}H]flunitrazepam displacing activity in bovine brain membranes. Some of these derivatives (**9**, **12**, **14**, **15**, **17**, **27**, **34**, **35**, **38**, **41**, and **45**) exhibited high affinity for the benzodiazepine receptor (BzR) with K_i values ranging from 67 to 11 nM. The GABA ratio and [^{35}S]-*tert*-butylbicyclophosphorothionate binding data, determined for the most active compounds, showed that they elicit an efficacy profile at the BzR which depends on the kind of substituent present on the phenyl ring of the benzylamine moiety. Moreover, lengthening (propylamine derivatives **1**−**3**) and shortening (aniline derivatives **46**−**54**) of the distance between the phenyl ring and the amide group of the side chain gave compounds with a drastically lower binding potency. The biological results are discussed in the light of a recently proposed pharmacophore model and compared, by molecular modeling studies, with those obtained from effective BzR ligands.

Introduction

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian central nervous system (CNS), eliciting its physiological effects through interaction with two distinct classes of cell-surface receptors: $GABA_A$ and $GABA_B$ receptors.[1,2] The $GABA_A$ receptor is a member of the superfamily of ligand-gated ion channels. The interaction of GABA with this receptor determines the opening of the intrinsic chloride ion selective channel, which is followed by an increase in chloride flux, with the result of a hyperpolarization of the neuronal cell membrane and a concomitant decrease in neuronal transmission. The $GABA_A$ receptor complex also carries other high-affinity binding sites able to modulate the channel function, such as the benzodiazepine receptor (BzR), the picrotoxin site, and the barbiturate site.[3] Among these, the BzR is one of the most widely studied, as demonstrated by the great number of scientific papers published every year on this subject.[4−8] The BzR ligands (Bz) include not only substances with a benzodiazepine structure but also others chemically quite different,[9−15] and they mediate a wide variety of pharmacological actions. They have a continuum of intrinsic activity ranging from full agonists (anxiolytic, anticonvulsant, sedative−hypnotic, and myorelaxant agents) through antagonists to inverse agonists (anxiogenic, somnolytic, proconvulsant, or even convulsant agents, i.e., compounds that produce pharmacological effects which are exactly the opposite of those of the benzodiazepines).[16,17]

In the past, benzodiazepines have been widely employed in clinical practice as anxiolytics, sedative−hypnotics, and anticonvulsants.[18] In recent years, their use has declined, due to the increasing unacceptability of their side effects, such as sedation, dizziness, interaction with alcohol, and the risk of dependence with long-term use.[3] For these reasons, current research is directed toward the search for novel non-benzodiazepine tranquilizers devoid of the unwanted side effects associated with the classic benzodiazepines. For therapeutic use, it would be useful to have partial agonists with anxiolytic and anticonvulsant properties in the absence of myorelaxant or sedative−hypnotic activity[19] or to have partial inverse agonists which can enhance general memory-learning and block or reverse the effects of barbiturate toxicity but are devoid of proconvulsant or convulsant activity.[8,20] New perspectives in achieving this purpose derive from the discovery of different BzR subtypes in different brain areas,[21] performing different physiological functions. Initially, two receptor subtypes were discovered, the BzR_1 subtype, predominant in the cerebellum, and the BzR_2 subtype, found principally in the cortex, hippocampus, and spinal cord.[22−25] So far, a total of at least six receptor subtypes, BzR_1−BzR_6, have been cloned and sequenced from mammalian brain, which derive from the combination of different subunits α, β, γ, δ, and ρ. One of the more attractive consequences of these findings is the possibility of developing molecules selective for these different receptor subtypes, showing only part of the benzodiazepine spectrum of behavioral effects.

We have recently described several [[(arylethyl)-amino]glyoxylyl]indole derivatives.[26] They represent a new structural class of ligands at the BzR, in which the indoleglyoxylyl group mimics the β-carboline system.[27] Though these compounds generally exhibit lower affinity than the β-carboline derivatives, they show an interesting pharmacological profile, ranging in a continuum from inverse agonists to antagonists and partial

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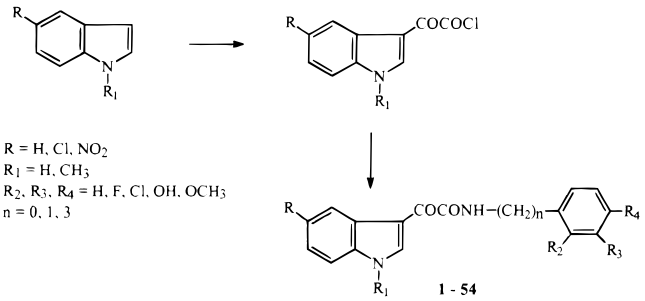
Scheme 1



agonists, depending on the nature of the substituents on the aryl group.

With the aim of finding new compounds with a higher affinity at the BzR and with this interesting pharmacological profile, in this paper we report the synthesis, the affinity data, and the pharmacological profile, as determined *in vitro* by the GABA ratio[28−30] and confirmed by the [^{35}S]-*tert*-butylbicyclophosphorothionate ([^{35}S]TBPS) binding shift,[31−33] of several derivatives with the [(phenylpropyl)amino]-, (benzylamino)-, and (anilinoglyoxylyl)indole structure. A further aim of this work was that of collecting new structure−activity relationship (SAR) data for this class of compounds which, together with molecular modeling studies, could shed light on the structural features of these ligands responsible for both the binding at the receptor site (affinity) and the intrinsic activity (efficacy) and could also allow a better definition of the topography of the receptor site.

Chemistry

The general synthetic procedure used in the preparation of compounds **1**−**54** involved the acylation of the appropriate indole with oxalyl chloride in accordance with a published procedure.[34] The indolylglyoxylyl chlorides obtained were allowed to react in mild conditions with the appropriate amine in the presence of triethylamine in benzene solution for compounds **1**−**32** and **39**−**54** and in THF solution for **33**−**38** (Scheme 1). All products were purified by recrystallization from appropriate solvent, and their structure was confirmed by IR, ^{1}H NMR, and elemental analyses (Table 1).

Results

Compounds **1**−**54** were tested for their ability to displace the specific binding of [^{3}H]flunitrazepam from bovine cortical membranes. The percentage of inhibition was determined at 10 μM compound concentration, and then the IC_{50} values for the most active compounds were calculated by log-probit plots, from which the K_i values were also derived to define the BzR affinity. Moreover, the *in vivo* efficacy of these ligands was predicted *in vitro* by the determination of the GABA ratio, which, according to different authors, generally predicts the expected pharmacological properties of a BzR ligand.[28−30] As the usefulness of the GABA ratio as a predictor of efficacy has sometimes been questioned,[35,36] the [^{35}S]TBPS binding shift was also determined.[31−33]

As shown in Table 2, the phenylpropylamine derivatives **1**−**3** and the aniline derivatives **46**−**54** showed poor affinity at the BzR. Practically, the affinity was restricted to the benzylamine compounds **4**−**45**, which exhibited moderate to high potency, with K_i values ranging from 2700 to 11 nM. In all the benzylamine series, the most potent derivatives were those bearing a nitro group in the 5-position of the indole nucleus (**6**, K_i = 117 nM; **9**, K_i = 53 nM; **12**, K_i = 38 nM; **15**, K_i = 11 nM; **35**, K_i = 13 nM; **38**, K_i = 12 nM; **41**, K_i = 65 nM; **45**, K_i = 48 nM, compared with the 5-unsubstituted derivatives **4**, **7**, **10**, **13**, **33**, **36**, **39**, and **43** showing K_i values of 120, 163, 290, 94, 140, 430, 820, and 349 nM, respectively). No such effect was observed in the series of compounds **17**−**32**, which bear a chloro or a fluoro substituent on the side phenyl ring; in this series all the 5-substituents examined lowered the affinity compared with the unsubstituted derivatives **17**, **21**, **24**, **27**, and **30** (with K_i values of 67, 160, 2600, 52, and 660 nM, respectively). Regarding the effects on the affinity of the position of the substituent(s) on the side phenyl ring, ortho substitution always led to a lowering of the affinity (compounds **24**−**26** and **30**−**32** compared with the corresponding parents **4**−**6**), while the para and/or meta substitution(s) gave variable results. Thus, in the 5-unsubstituted series a retention of affinity was observed for compounds **7**, **13**, **21**, and **33** compared with the parent compound **4** and an enhancement of affinity for compounds **17** and **27**, while a decrease was seen for the remaining **10**, **36**, **39**, and **43**.

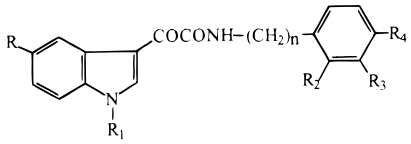
The 5-chloro- and 5-nitro-substituted series displayed a similar affinity trend, with the exclusion of the *m*-hydroxy-substituted compound **40**, which showed an affinity almost equal, or at least within the range of experimental error, to that of the parent compound **5**: Affinity improved for the methoxy- and/or hydroxy-substituted **8**, **11**, **14**, **34**, **37**, **44** and **9**, **12**, **15**, **35**, **38**, **41**, **45** compared with the parent compounds **5** and **6**, respectively, and decreased with the chloro- and fluoro-substituted compounds **18**, **22**, **28**, and **19**, **23**, **29**, respectively. In each of the 5-substituted indole series, the products monosubstituted in the para or meta position, or disubstituted in these two positions of the side phenyl ring, did not show, however, any significant affinity differences among them. In any case, the methylation of the indole NH led to a drastically lower affinity (see products **16**, **20**, and **42** compared with **13**, **17**, and **39**, respectively).

Furthermore, the products with a hydrogen, a methoxy, a chloro, a fluoro, or a *p*-hydroxy group on the side phenyl ring (compounds **4**−**38**, with the exception of **36**) showed GABA ratio values which were lower than, or close to, unity, predicting partial inverse agonist or antagonist properties. For compounds **39**−**45**, characterized by the presence of a *m*-hydroxy group, the GABA ratio values higher than unity predicted a partial agonist profile.

The most active compounds (**4**, **9**, **12**, **15**, **17**, **27**, **35**, **38**, **41**, and **45**) were also assayed with the [^{35}S]TBPS shift test. The effect of a 0.5 μM concentration of these compounds on [^{35}S]TBPS binding in bovine membranes, in the presence of 1 μM exogenous GABA, was measured.[37,38]

The data obtained, reported in Table 2 as [^{35}S]TBPS shift, confirmed the efficacy profile predicted by the GABA ratio values, seeing that compounds **4, 9, 12, 15, 17, 27, 35,** and **38** showed [^{35}S]TBPS shift values ranging between 2 and 31, similar to the value of 15 obtained for the antagonist Ro 15-1788, and compounds

Table 1. Physical Properties of *N*-[(Substituted indol-3-yl)glyoxylyl]amine Derivatives



no.	R	R_1	R_2	R_3	R_4	n	yield (%)	cryst solvent	mp (°C)	formula[a]
1	H	H	H	H	H	3	50	benzene	141−143	$C_{19}H_{18}N_2O_2$
2	Cl	H	H	H	H	3	59	methanol	160−162	$C_{19}H_{17}ClN_2O_2$
3	NO_2	H	H	H	H	3	47	methanol	245−247	$C_{19}H_{17}N_3O_4$
4	H	H	H	H	H	1	53	benzene	172−173	$C_{17}H_{14}N_2O_2$
5	Cl	H	H	H	H	1	61	methanol	251−253	$C_{17}H_{13}ClN_2O_2$
6	NO_2	H	H	H	H	1	47	ethanol	290−292	$C_{17}H_{13}N_3O_4$
7	H	H	H	H	OCH_3	1	66	benzene	157−158	$C_{18}H_{16}N_2O_3$
8	Cl	H	H	H	OCH_3	1	60	methanol	209−211	$C_{18}H_{15}ClN_2O_3$
9	NO_2	H	H	H	OCH_3	1	43	ethanol	274−275	$C_{18}H_{15}N_3O_5$
10	H	H	H	OCH_3	H	1	46	benzene	132−134	$C_{18}H_{16}N_2O_3$
11	Cl	H	H	OCH_3	H	1	69	benzene	161−162	$C_{18}H_{15}ClN_2O_3$
12	NO_2	H	H	OCH_3	H	1	50	methanol	232−234	$C_{18}H_{15}N_3O_5$
13	H	H	H	OCH_3	OCH_3	1	72	benzene	160−162	$C_{19}H_{18}N_2O_4$
14	Cl	H	H	OCH_3	OCH_3	1	64	methanol	197−198	$C_{19}H_{17}ClN_2O_4$
15	NO_2	H	H	OCH_3	OCH_3	1	63	methanol	207−209	$C_{19}H_{17}N_3O_6$
16	H	CH_3	H	OCH_3	OCH_3	1	74	benzene	153−154	$C_{20}H_{20}N_2O_4$
17	H	H	H	H	Cl	1	48	benzene	205−206	$C_{17}H_{13}ClN_2O_2$
18	Cl	H	H	H	Cl	1	71	methanol	248−249	$C_{17}H_{12}Cl_2N_2O_2$
19	NO_2	H	H	H	Cl	1	59	DMF/H_2O	>300	$C_{17}H_{12}ClN_3O_4$
20	H	CH_3	H	H	Cl	1	75	benzene	156−157	$C_{18}H_{15}ClN_2O_2$
21	H	H	H	Cl	H	1	51	benzene	186−188	$C_{17}H_{13}ClN_2O_2$
22	Cl	H	H	Cl	H	1	57	methanol	229−230	$C_{17}H_{12}Cl_2N_2O_2$
23	NO_2	H	H	Cl	H	1	60	ethanol	282 dec	$C_{17}H_{12}ClN_3O_4$
24	H	H	Cl	H	H	1	67	benzene	191−193	$C_{17}H_{13}ClN_2O_2$
25	Cl	H	Cl	H	H	1	57	methanol	222−224	$C_{17}H_{12}Cl_2N_2O_2$
26	NO_2	H	Cl	H	H	1	40	ethanol	272−274	$C_{17}H_{12}ClN_3O_4$
27	H	H	H	H	F	1	72	benzene	202−204	$C_{17}H_{13}FN_2O_2$
28	Cl	H	H	H	F	1	73	ethanol	273−275	$C_{17}H_{12}ClFN_2O_2$
29	NO_2	H	H	H	F	1	61	DMF/H_2O	>300	$C_{17}H_{12}FN_3O_4$
30	H	H	F	H	H	1	47	benzene	177−178	$C_{17}H_{13}FN_2O_2$
31	Cl	H	F	H	H	1	63	ethanol	256−258	$C_{17}H_{12}ClFN_2O_2$
32	NO_2	H	F	H	H	1	44	ethanol	298 dec	$C_{17}H_{12}FN_3O_4$
33	H	H	H	OCH_3	OH	1	50	benzene	212−214	$C_{18}H_{16}N_2O_4$
34	Cl	H	H	OCH_3	OH	1	61	benzene	200−202	$C_{18}H_{15}ClN_2O_4$
35	NO_2	H	H	OCH_3	OH	1	40	ethanol	252−254	$C_{18}H_{15}N_3O_6$
36	H	H	H	H	OH	1	60	methanol	221−222	$C_{17}H_{14}N_2O_3$
37	Cl	H	H	H	OH	1	43	methanol	234−235	$C_{17}H_{13}ClN_2O_3$
38	NO_2	H	H	H	OH	1	50	methanol	239−241	$C_{17}H_{13}N_3O_5$
39	H	H	H	OH	H	1	50	methanol	224−226	$C_{17}H_{14}N_2O_3$
40	Cl	H	H	OH	H	1	47	methanol	220−222	$C_{17}H_{13}ClN_2O_3$
41	NO_2	H	H	OH	H	1	54	ethanol	259−261	$C_{17}H_{13}N_3O_5$
42	H	CH_3	H	OH	H	1	51	ethanol	201−202	$C_{18}H_{16}N_2O_3$
43	H	H	H	OH	OH	1	42	methanol	212−213	$C_{17}H_{14}N_2O_4$
44	Cl	H	H	OH	OH	1	36	methanol	215−217	$C_{17}H_{13}ClN_2O_4$
45	NO_2	H	H	OH	OH	1	60	methanol	286−288	$C_{17}H_{13}N_3O_6$
46	H	H	H	H	H	0	73	ethanol	244−246	$C_{16}H_{12}N_2O_2$
47	Cl	H	H	H	H	0	60	ethanol	269−271	$C_{16}H_{11}ClN_2O_2$
48	NO_2	H	H	H	H	0	69	DMF/H_2O	>300	$C_{16}H_{11}N_3O_4$
49	H	H	H	H	OCH_3	0	47	ethanol	234−236	$C_{17}H_{14}N_2O_3$
50	Cl	H	H	H	OCH_3	0	58	ethanol	263−264	$C_{17}H_{13}ClN_2O_3$
51	NO_2	H	H	H	OCH_3	0	22	DMF/H_2O	>300	$C_{17}H_{13}N_3O_5$
52	H	H	H	H	OH	0	60	methanol	>300	$C_{16}H_{12}N_2O_3$
53	Cl	H	H	H	OH	0	69	methanol	>300	$C_{16}H_{11}ClN_2O_3$
54	NO_2	H	H	H	OH	0	28	ethanol	>300	$C_{16}H_{11}N_3O_5$

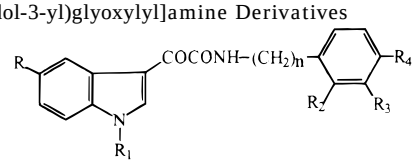
[a] Elemental analyses for C, H, N were within ±0.4% of the calculated values.

41 and **45** showed values of 50 and 52, respectively, intermediate between those of clonazepam (fixed here at a value of 100) and Ro 15-1788.

The efficacy profile of the most potent compounds (**6**, **9**, **17**, **27**, **38**, and **45**) was also checked by *in vivo* tests for anticonvulsant, proconvulsant, and diazepam antagonism action, essentially carried out as previously described.[26] None of the tested compounds were proconvulsant, even at the highest dose of 250 mg/kg, nor did they antagonize the convulsant effect of pentylenetetrazole (PTZ) or the anticonvulsant effect of diazepam at the same dose, thus showing no *in vivo* efficacy.

As the lack of *in vivo* activity of these products could have been ascribed to a nonoptimal hydrophilic−lipophilic balance, the partition coefficients (log *P*) of **9**, **27**, and **38** were determined, since this parameter has often been successfully employed to measure the ability of a compound to cross the blood−brain barrier.[39] The data obtained, reported in Table 3, showed that these compounds have log *P* values analogous to those of well-

Table 2. *In Vitro* Data of *N*-[(Substituted indol-3-yl)glyoxylyl]amine Derivatives



no.	R	R_1	R_2	R_3	R_4	n	inhibn[a] (%) (10 μM)	K_i[b] (nM)	GABA ratio[c]	[^{35}S]TBPS binding with GABA[d] (% clonazepam)
1	H	H	H	H	H	3	78 ± 6	1260 ± 130	1.00	
2	Cl	H	H	H	H	3	32 ± 2			
3	NO_2	H	H	H	H	3	52 ± 4			
4	H	H	H	H	H	1	98 ± 2	120 ± 11	0.87	29 ± 6
5	Cl	H	H	H	H	1	34 ± 4[e]	490 ± 39	0.95	
6	NO_2	H	H	H	H	1	55 ± 5[e]	117 ± 12	1.00	
7	H	H	H	H	OCH_3	1	97 ± 3	163 ± 12	1.00	
8	Cl	H	H	H	OCH_3	1	81 ± 7	107 ± 10	1.00	
9	NO_2	H	H	H	OCH_3	1	71 ± 6	53 ± 5.3	1.06	2 ± 4
10	H	H	H	OCH_3	H	1	97 ± 3	290 ± 23	0.74	
11	Cl	H	H	OCH_3	H	1	89 ± 4	162 ± 8.5	0.79	
12	NO_2	H	H	OCH_3	H	1	84 ± 6	38 ± 5	0.91	10 ± 3
13	H	H	H	OCH_3	OCH_3	1	99 ± 3	94 ± 10	0.80	
14	Cl	H	H	OCH_3	OCH_3	1	94 ± 6	30 ± 5	0.82	
15	NO_2	H	H	OCH_3	OCH_3	1	97 ± 7	11 ± 3	1.00	31 ± 7
16	H	CH_3	H	OCH_3	OCH_3	1	70 ± 5	2700 ± 300	0.88	
17	H	H	H	H	Cl	1	99 ± 4	67 ± 5	1.03	27 ± 6
18	Cl	H	H	H	Cl	1	38 ± 3			
19	NO_2	H	H	H	Cl	1	2 ± 3			
20	H	CH_3	H	H	Cl	1	86 ± 8	1000 ± 300	1.00	
21	H	H	H	Cl	H	1	96 ± 5	160 ± 30	0.95	
22	Cl	H	H	Cl	H	1	14 ± 4			
23	NO_2	H	H	Cl	H	1	0			
24	H	H	Cl	H	H	1	77 ± 3	2600 ± 400	0.93	
25	Cl	H	Cl	H	H	1	40 ± 4			
26	NO_2	H	Cl	H	H	1	48 ± 3			
27	H	H	H	H	F	1	99 ± 4	52 ± 6	1.16	21 ± 6
28	Cl	H	H	H	F	1	21 ± 3			
29	NO_2	H	H	H	F	1	24 ± 3			
30	H	H	F	H	H	1	94 ± 4	660 ± 5	0.86	
31	Cl	H	F	H	H	1	39 ± 3			
32	NO_2	H	F	H	H	1	42 ± 5			
33	H	H	H	OCH_3	OH	1	96 ± 5	140 ± 3	1.00	
34	Cl	H	H	OCH_3	OH	1	99 ± 3	27 ± 5	1.03	
35	NO_2	H	H	OCH_3	OH	1	100 ± 2	13 ± 4	1.07	27 ± 8
36	H	H	H	H	OH	1	96 ± 1	430 ± 20	1.35	
37	Cl	H	H	H	OH	1	98 ± 2	150 ± 20	1.10	
38	NO_2	H	H	H	OH	1	100 ± 3	12 ± 2	1.05	10 ± 4
39	H	H	H	OH	H	1	91 ± 6	820 ± 60	1.25	
40	Cl	H	H	OH	H	1	95 ± 4	570 ± 70	1.45	
41	NO_2	H	H	OH	H	1	95 ± 7	65 ± 4	1.32	50 ± 9
42	H	CH_3	H	OH	H	1	17 ± 5			
43	H	H	H	OH	OH	1	95 ± 3	349 ± 49	1.56	
44	Cl	H	H	OH	OH	1	96 ± 2	180 ± 25	1.33	
45	NO_2	H	H	OH	OH	1	98 ± 3	48 ± 2	1.35	52 ± 11
46	H	H	H	H	H	0	10 ± 4			
47	Cl	H	H	H	H	0	0			
48	NO_2	H	H	H	H	0	0			
49	H	H	H	H	OCH_3	0	0			
50	Cl	H	H	H	OCH_3	0	10 ± 4			
51	NO_2	H	H	H	OCH_3	0	12 ± 2			
52	H	H	H	H	OH	0	65 ± 5			
53	Cl	H	H	H	OH	0	23 ± 3			
54	NO_2	H	H	H	OH	0	76 ± 6	1800 ± 400	1.0	
clonazepam									1.97	100 ± 10
Ro 15-1788									0.90	15 ± 5

[a] Percents of inhibition of specific [^{3}H]flunitrazepam binding at 10 μM compound concentration are means ± SEM of five determinations. [b] K_i values are means ± SEM of three determinations. [c] GABA ratio = K_i without GABA/K_i with GABA. [d] The effects of the compounds at 0.5 μM on TBPS binding were normalized with respect to the corresponding action of clonazepam. The data represent the mean ± standard error of three separate experiments. [e] Determined at 200 nM concentration of the compound due to its insolubility at 10 μM.

known therapeutically used benzodiazepines (diazepam, log P = 2.80; lorazepam, log P = 2.39; flunitrazepam, log P = 2.06).[40]

These unexpected results led us to verify whether our compounds, after ip administration, could be present in brain tissue in a sufficient concentration to induce a pharmacological response in mice. We directly determined the presence of compounds **9**, **27**, and **38** in the CNS, essentially following the method described by Chang and Snyder[41] (see the Experimental Section). They did not displace [^{3}H]flunitrazepam binding *in vivo*, while the binding was reduced by 90% in diazepam-injected animals compared with vehicle-injected animals (control). These experiments showed that the com-

Table 3. Partition Coefficients of Compounds **9**, **27**, and **38**

no.	log P^a
9	2.17
27	2.88
38	2.67

[a] Experimentally determined in accordance with ref 39.

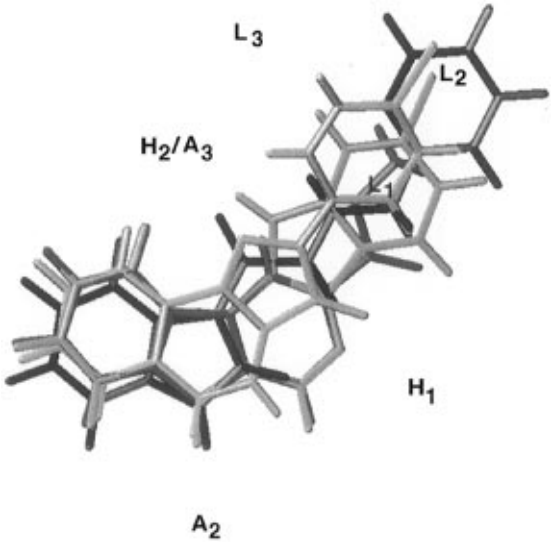


Figure 1. Superimposition of the benzylamine derivative **4** (blue), the pyridodiindole **I** (yellow), the β-carboline **II** (red), and the pyrazoloquinolinone **III** (gray). Ligand−BzR interaction sites are labeled according to Cook's pharmacophore model.[8] The L_3 site is not occupied by any of the ligands considered.

pounds did not reach the mouse CNS, and their *in vivo* inactivity might have been due to unfavorable pharmacokinetics.

Discussion

The remarkable differences in affinity between the benzylamine derivatives and their phenylpropylamine and aniline analogs can be rationalized in the framework of the pharmacophore model devised by Cook et al.[8,42−44] This model, which assumes that the three pharmacological classes of BzR ligands (agonists, antagonists, and inverse agonists) share the same binding domain, consists of the following interaction sites: (i) a hydrogen bond acceptor site (A_2), (ii) a hydrogen bond donor site (H_1), (iii) a "bifunctional" hydrogen bond donor/acceptor site (H_2/A_3), and (iv) three lipophilic pockets (L_1, L_2, and L_3).

Figure 1 shows a superimposition of the benzylamine **4** on three BzR ligands exhibiting nanomolar potency: the pyridodiindole **I** (antagonist),[44] the β-carboline **II** (full inverse agonist),[42] and the pyrazoloquinolinone **III** (partial agonist).[12] The formulas of **I**−**III** are given in Chart 1. The fairly rigid structures of the templates **I**−**III** are available from crystallographic studies,[45−47] while a molecular model of **4** was generated using the semiempirical quantum-mechanics AM1 method[48] (computational details are summarized in the Experimental Section). Looking at Figure 1, it may be noted that **4** attains a coplanar conformation mimicking the shape of the potent templates **I**−**III**. Overlaps between the pharmacophoric features of **4** and **I**−**III** occur about the sites A_2 (indole NH), H_1 (C═O2), H_2 (C═O1), L_1 (CH_2), and L_2 (phenyl ring). None of the ligands considered

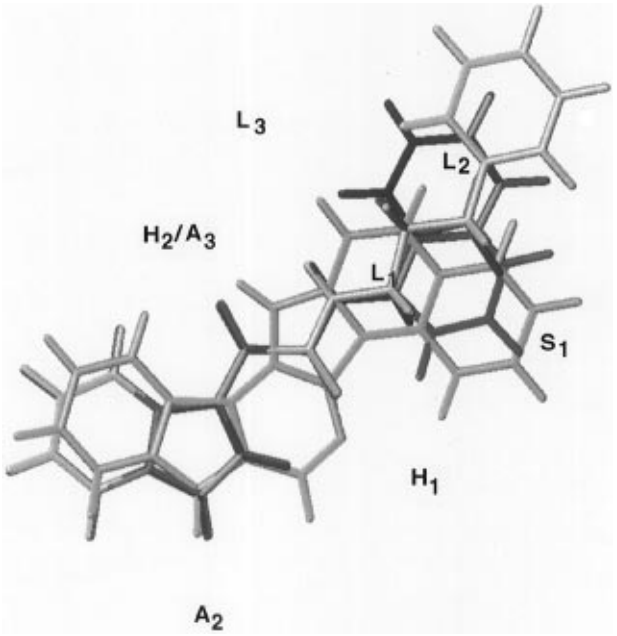
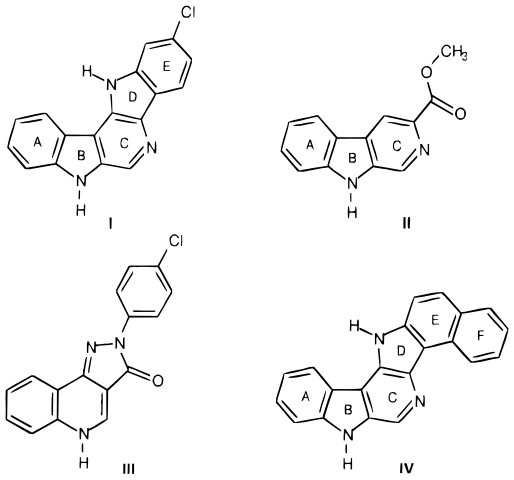


Figure 2. Superimposition of the aniline derivative **46** (red), the phenylpropylamine derivative **1** (gray), the benzylamine derivative **4** (blue), and the benzopyridodiindole **IV** (yellow). The S_1 site is a sterically repulsive region of the BzR.[8]

Chart 1



occupy the L_3 region. The alignment of **4** is in agreement with the modest affinities of the N1-methylated analogs **16**, **20**, and **42** which cannot receive a hydrogen bond from the A_2 protic function of the receptor. Additionally, we have recently demonstrated that the presence of the C═O2 group of the oxalyl bridge engaging a hydrogen bond with the H_1 site is crucial for the expression of an appreciable affinity.[49]

Figure 2 shows the AM1-derived geometries of the aniline **46** and phenylpropylamine **1** superimposed on the benzylamine **4** and the benzopyridodiindole **IV**[50] (Chart 1). Cook et al. have explained the relatively low potency of **IV** with respect to **I** by postulating that the fused benzene ring F of **IV** is sterically repelled by one of the receptor walls defined as the S_1 region.[8,50] As the phenyl ring of the aniline **46** overlaps part of the F ring of **IV**, it is reasonable to assume that the poor affinity of **46** and its congeners arises from interaction with the sterically forbidden S_1 region.

The molecular alignment illustrated in Figure 2 also rationalizes the drop in affinity observed in the benzyl-

amine series when a chlorine or fluorine atom is inserted at the ortho position of the phenyl ring (see compounds **24**−**26** and **30**−**32**). AM1 calculations on **24** and **30** indicate that the ortho halogen is oriented toward the S_1 area, with the ligand assuming a stable conformation identical with that of benzylamine **4**. Considering that fluorine is larger than hydrogen and smaller than chlorine,[51] an unfavorable steric interaction at the S_1 site involving the ortho halogen would account for the increasing K_i values exhibited by **4**, **30,** and **24**.

Finally, inspection of Figure 2 reveals that the phenyl ring of **1** extends far beyond the L_2 site, where it probably makes an unfavorable contact with the boundaries of this lipophilic cleft. Structure−affinity relationships developed by Cook et al.,[8] showing that the L_2 region is relatively small, strongly suggest that the poor affinity of **1** depends on the excessive length of the phenylpropylamine chain.

A careful examination of the binding data for the benzylamine derivatives **4**−**45** reveals that the effects of the R, R_3, and R_4 substituents on affinity are not constant but interdependent. In other words, parallel structural modifications do not always produce parallel effects on affinity. The following are examples of "irregularities" in the structure−affinity relationships. Within the series of analogs of **4** (R = H), the introduction of methoxy and/or hydroxy groups on the phenyl ring retains (**7**, **13**, and **33**) or lowers (**10**, **36**, **39**, and **43**) affinity. In contrast, within the series of analogs of **6** ($R = NO_2$), potency is invariably improved by the same substituents on the phenyl ring (**9**, **12**, **15**, **35**, **38**, **41**, and **45**). The insertion of methoxy and/or hydroxy groups on the phenyl ring of **5** (R = Cl) generally enhances potency (**8**, **11**, **14**, **34**, **37**, and **44**). However, the *m*-hydroxy derivative **40** is an exception, since it is slightly less potent than **5**.

What is most puzzling is the effect of halogens in the meta and para positions of the phenyl ring. While analogs unsubstituted in the 5-position of the indole moiety are highly potent when R_3 or R_4 = Cl or F (**17**, **21**, and **27**), the 5-chloro and 5-nitro analogs halogenated in the same position of the phenyl ring are surprisingly devoid of affinity (**18**, **19**, **22**, **23**, **28,** and **29**).

The above observations suggest that the interactions of our benzylamine derivatives with the BzR cannot entirely be fitted into a simple "key-and-lock" model. One hypothesis is that recognition of the arylmethyl moiety by a receptor subsite, particularly demanding in terms of shape complementarity, "drives" the anchoring of the rest of the ligand structure. The ligands might therefore assume slightly different orientations within the receptor cavity, somehow shifted one with respect to another. Thus, since the positioning of a given substructure common to several analogs is not identical, that substructure will not produce a constant effect on affinity. According to this dynamic model, the alignments shown in Figures 1 and 2 must be regarded as an "averaged" representation of similar, but not identical, binding models. A higher resolution topography of the receptor cannot be drawn owing to the limited amount of information compared with the complexity of the biological system.

Unfortunately, these derivatives were completely lacking in *in vivo* efficacy, and this was unexpected since some of them exhibited a high potency *in vitro*. The log *P* values, determined for compounds **9**, **27**, and **38**, similar to those of well-known therapeutically used benzodiazepines, could have led us to hypothesize an easy crossing of the blood−brain barrier.[41] Their lack of bioavailability could be due to transport and/or metabolism problems. Although this class of ligands was inactive *in vivo*, the derived SARs may be useful for the design of new molecules with a high-affinity and selective efficacy profile at the BzR.

In the benzylamine series, the nature of the R_3 and R_4 substituents of the side phenyl ring modulates not only the affinity, as reported above, but also the way the ligand interacts with the membrane receptor system as assessed by the GABA ratio values.[28−30] As the GABA ratios can sometimes give questionable results,[35,36] especially with the β-carboline series, the [^{35}S]-TBPS binding shifts were determined for the most active compounds. Thus, the binding of a ligand at the BzR allosterically affects the binding of TBPS, which binds with high affinity to a site located near the chloride channel of the $GABA_A$ receptor complex, and the measurement of the shift in TBPS binding in the presence and absence of a test compound makes it possible to estimate its intrinsic efficacy. Benzodiazepine receptor agonists enhance the [^{35}S]TBPS binding shift, while antagonists have no effect ([^{35}S]TBPS shift = 0). Partial agonists have an intermediate effect, and inverse agonists reduce the [^{35}S]TBPS shift.[31−33]

Compounds **36**, **39**−**41**, and **43**−**45**, featuring at least one hydroxy group on the phenyl ring, showed GABA ratio values higher than unity and [^{35}S]TBPS binding shifts in the range 50−52 (determined for compounds **41** and **45**). The remaining benzylamine derivatives had GABA ratio values close to unity and [^{35}S]TBPS binding shifts of 2−31 (Table 2), showing a different efficacy profile. It seems unlikely that the two groups of benzylamines assume bound-conformations differing significantly from those illustrated in Figures 1 and 2. As already mentioned, it has been proposed that all pharmacological classes of BzR ligands occupy the same binding domain and that different pharmacological activities may result from distinct conformational states of the receptor.[8] It is thus possible that the R_3 and R_4 substituents of (indolylglyoxylyl)benzylamines modulate the efficacy by interaction with a receptor site working as a "sensor". The triggering of this site would in turn stabilize to different extents the various conformational/functional states of the receptor complex. Further investigations are required to identify a relationship between the physicochemical properties of R_3 and R_4 and their efficacy profile.

Experimental Section

Chemistry. Melting points were determined on a Köfler hot-stage apparatus and are uncorrected. IR spectra were recorded with a Pye Unicam Infracord Model PU 9516 spectrometer in Nujol mulls. Routine ^{1}H NMR spectra were determined on a Varian CFT 20 spectrometer operating at 80 MHz, using tetramethylsilane (TMS) as the internal standard. Magnesium sulfate was always used as the drying agent. Evaporations were made *in vacuo* (rotating evaporator). Analytical TLC was carried out on Merck 0.2 mm precoated silica gel aluminum sheets (60 F-254). Silica gel 60 (230−400 mesh) was used for column chromatography. Elemental analyses were performed by our Analytical Laboratory and agreed with theoretical values to within ±0.4%.

General Procedure for the Synthesis of *N*-[(5-Substituted indol-3-yl)glyoxylyl]amine Derivatives 1−54. Triethylamine (3.0 mmol) was added dropwise to a stirred suspension of indolylglyoxylyl chloride (2.5 mmol) and the appropriate amine (2.75 mmol) in 50 mL of dry benzene (THF for compounds **33**−**38**), cooled at 0 °C. The reaction mixture was left to warm at room temperature, stirred for 24 h, refluxed for 2 h, and then filtered. The precipitate collected 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 amine derivatives obtained from the initial insoluble precipitate or from the benzene (or THF) solution were variable, depending upon the solubility of the various compounds. All products **1**−**54** were purified by recrystallization from the appropriate solvent. Yields, recrystallization solvents, and melting points are listed in Table 1.

Molecular Modeling. All molecular modeling was conducted using the software package SYBYL[52] running on a Silicon Graphics Indigo XS24 workstation. Geometry optimizations were performed with the semiempirical quantum-mechanics methods AM1,[48] available in the MOPAC program.[53] MOPAC was run using default settings and the keyword "MMOK" for compounds containing an amide bond.

Crystal structures of compounds **I**−**III** were retrieved from the October 1995 release (3D graphics 5.10 version for UNIX platforms) of the Cambridge Structural Database (CSD).[54] The CSD refcodes of **I**−**III** are JOJHIZ, CITRIG01, and COVLOO, respectively.

A molecular model of **IV** was built by modifying the crystal structure of **I** using the SYBYL fragment library. The structure obtained was partially geometry optimized with the AM1 method about the ring F.

Input coordinates of compounds **1**, **4**, and **46** were generated using SYBYL standard bond lengths and bond angles. Torsional angles were adjusted so as to produce trans-like coplanar conformations. The trial geometries were fully energy-minimized with the AM1 Hamiltonian, yielding, for **1** and **4**, nearly unmodified conformations. In contrast, the output geometry of **46** displayed the indole−CO and CONHPh moieties lying in planes twisted by about 30°.

The AM1 results were compared with the crystal structure of *p*-tolylglyoxylic acid *p*-chloroanilide (CSD refcode MPGCAN),[55] which is almost entirely coplanar. The conformation of MPGCAN resembles the AM1-derived geometries of **1** and **4** but not the "twisted" one of the aniline derivative **46**. Thus, AM1 single-point calculations were performed on a geometry of **46** whose torsional angles about the C−COCONH−C fragment were given the same values measured on MPGCAN. Interestingly, this planar arrangement turned out to be only 0.6 kcal/mol less stable than the "twisted" one. Considering that the structures of the highly potent ligands **I**−**III** are essentially flat, the planar conformation of **46** was selected for the molecular superposition shown in Figure 2.

According to the AM1 calculations, the coplanar disposition of the X−C−Ph system in **1** (X = C) and **4** (X = N) is 1 kcal/mol less stable than those in which the torsional angle about the C−Ph bond is 94°. This small energy difference probably does not impede **1** and **4** from binding to the receptor in coplanar conformations. Hence, we have modeled the hypothetical receptor-recognized conformations of **1** and **4** in their coplanar arrangements (see Figures 1 and 2). It is worth noting that the insertion of a chlorine or a fluorine in the ortho position of the phenyl moiety (as in compounds **24** and **30**) does not alter the energy gap between the two above-mentioned rotameric states.

Molecules were superimposed by minimization of the root mean square distance between selected atom pairs using the SYBYL/FIT command. The pseudoatoms H_1 and H_2 (not displayed in Figures 1 and 2) were added to the structures being compared to simulate hypothetical positions of receptor hydrogen bond donor functions. Positioning of these pseudoatoms and overlap of compounds **I**−**IV** were accomplished following the procedures described by Cook et al.[8]

The benzylamine derivative **4** was fitted on the pyridodiindole **I** about the following points: (i) the indole hydrogen complementary to the A_2 site, (ii) the H_1 pseudoatom, and (iii) the L_2 pharmacophoric element (this latter corresponds to the benzylic carbon atom in **4** and the centroid of the E ring in **I**). The phenylpropylamine **1** and the aniline **46** were fitted on **4** by matching the common indolylglyoxylylamide fragment.

log *P* Measurements. The log *P* values of compounds **9**, **27**, and **38** were determined according to the classic shake-flask procedure[39] at room temperature, using octanol as the lipophilic phase and phosphate buffer, pH 7.4, as the hydrophilic phase. The concentration of the partitioned solute was measured in both phases using a Beckman DU-40 spectrophotometer. The two phases were adjusted in volume so that satisfactory amounts of the compound were present in each phase. Partitioning was carried out at four different concentrations to ensure that special interactions were not occurring and to check against other errors. Variation among four measurements was less than 2.5%.

Binding Studies. [^{3}H]Flunitrazepam (specific activity 82.5 Ci/mol, radiochemical purity > 99%) and [^{35}S]TBPS (specific activity 80 Ci/mol) were obtained from Du Pont de Nemours, New England Nuclear Division (Dreieichenhaim, Germany). All other chemicals were of reagent grade and obtained from commercial suppliers.

Bovine cerebral cortex membranes were prepared in accordance with ref 56. The membrane preparations were subjected to a freeze−thaw cycle, washed by suspension and centrifugation in 50 mM Tris-citrate buffer, pH 7.4 (T1), and then used in the binding assay. Protein concentration was assayed by the method of Lowry et al.[57]

[^{3}H]Flunitrazepam Binding Studies. These studies were performed by using a filtration technique essentially as previously reported.[27]

[^{35}S]TBPS Binding Studies. The membrane suspension was incubated together with 5 nM [^{35}S]TBPS for 90 min at 25°C in 500 μL (final volume) of T1 buffer containing 200 mM KBr and 0.1 mM EDTA. The binding assay was performed by using a filtration technique. After incubation, the samples were diluted with 5 mL of assay buffer, immediately filtered under reduced pressure through glass filter disks (Whatman GF/C), and then washed with 5 mL of the same buffer. The filter disks were then placed in polypropylene scintillation vials together with 8 mL of Ready Safe Beckman scintillation cocktail; the radioactivity of the filters was determinated by a Beckman LS 1800 scintillometer. Drugs were added as concentrated ethanolic solutions (0.5 μM). The level of ethanol did not exceed 0.2% and was maintained constant in all tubes. Nonspecific [^{35}S]TBPS binding was estimated in the presence of 600 μM picrotoxinin and was subtracted to compute specific binding. The characterization of the actions of various drugs on [^{35}S]TBPS binding was performed as described elsewhere.[33]

***In Vivo* Studies: Procovulsant, Anticonvulsant, and Diazepam Antagonism Action.** Groups of 10 mice were injected intraperitoneally (0.1 mL) with graded doses of the compounds (up to the highest dose of 250 mg/kg), suspended in 20% dilute Emulphor−80% saline solution (vehicle) (dilute Emulphor is Emulphor diluted 1:1, w/w, with ethanol), or an equal volume of the 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.[58]

Antagonism of the anticonvulsant effects of diazepam was carried out as described by Cain et al.[59] Groups of 10 mice were injected with diazepam (2.5 mg/kg ip) followed 10 min later by administration of graded doses of test compound or vehicle. Fifteen minutes after injection of the compound, animals were injected with PTZ (80 mg/kg).

***In Vivo* Determination of Benzodiazepine Receptor Occupancy.** *In vivo* determination of receptor occupancy by (indolylglyoxylyl)benzylamines was determined essentially as described by Chang and Snyder.[41]

Groups of 15 mice were divided into three groups: The first group was intraperitoneally injected with 100 mg/kg test compound, suspended in 20% dilute Emulphor−80% saline,

the second with the vehicle, and the third with 2.5 mg/kg diazepam 30 min before the injection of [^{3}H]flunitrazepam diluted with a 0.9% NaCl solution to 167 μCi/kg of body weight into the tail vein. After 20 min, mice were decapitated, and their brains were rapidly removed, weighed, and homogenized in 40 vol of ice cold Tris-HCl buffer (50 mM, pH 7.7) for 30 s. Triplicate 1 mL aliquots were immediately filtered through Whatman glass fiber filters, and the filters were rinsed with two 5 mL portions of ice cold Tris-HCl buffer. The bound [^{3}H]-flunitrazepam was counted by liquid scintillation spectroscopy. The total [^{3}H]flunitrazepam in the brain was determined by counting 1 mL aliquots of the homogenates prior to filtration.

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