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Novel *N*-(Arylalkyl)indol-3-ylglyoxylylamides Targeted as Ligands of the Benzodiazepine Receptor: Synthesis, Biological Evaluation, and Molecular Modeling Analysis of the Structure−Activity Relationships†

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A series of *N*-(arylalkyl)indol-3-ylglyoxylylamides (**4**−**8**) was synthesized as ligands of the benzodiazepine receptor (BzR) and tested for their ability to displace [^{3}H]flumazenil from bovine brain membranes. The new compounds, bearing a branched (**4**) or a geometrically constrained benzyl/phenylethyl amide side chain (**5**−**8**), represent the continuation of our research on *N*-benzylindol-3-ylglyoxylylamides **1** (Da Settimo et al., 1996), *N*′-phenylindol-3-ylglyoxylohydrazides **2** (Da Settimo et al., 1998), and *N*-(indol-3-ylglyoxylyl)alanine derivatives **3** (Primofiore et al., 1989). A few indoles belonging to the previously investigated benzylamides **1** and phenylhydrazides **2** were synthesized and tested to enrich the SARs in these two series. The affinities and the GABA ratios of selected compounds for clonal mammalian $\alpha_1\beta_2\gamma_2$, $\alpha_3\beta_2\gamma_2$, and $\alpha_5\beta_3\gamma_2$ BzR subtypes were also determined. It was hypothesized that the reduced flexibility of indoles **4**−**8** would both facilitate the mapping of the BzR binding cleft and increase the chances of conferring selectivity for the considered receptor subtypes. In the series of indoles **4**, the introduction of a methyl group on the benzylic carbon with the R configuration improved affinity of the 5-substituted (5-Cl and 5-NO_2) derivatives, whereas it was detrimental for their 5-unsubtituted (5-H) counterparts. All S enantiomers were less potent than the R ones. Replacement of the methyl with hydrophilic substituents on the benzylic carbon lowered affinity. The isoindolinylamide side chain was tolerated if the 5-position was unsubstituted (K_i of **5a** = 123 nM), otherwise affinity was abolished (**5b**, **c**). All the 2-indanylamides **6** and (S)-1-indanylamides **8** were devoid of any appreciable affinity. The 5-Cl and 5-NO_2 (R)-1-indanylamides **7b** (K_i 80 nM) and **7c** (K_i 28 nM) were the most potent among the indoles **5**−**8** geometrically constrained about the side chain. The 5-H (R)-1-indanylamide **7a** displayed a lower affinity (K_i 675 nM). The SARs developed from the new compounds, together with those collected from our previous studies, confirmed the hypothesis of different binding modes for 5-substituted and 5-unsubstituted indoles, suggesting that the shape of the lipophilic pocket L_1 (notation in accordance with Cook's BzR topological model) is asymmetric and highlighted the stereoelectronic and conformational properties of the amide side chain required for high potency. Several of the new indoles showed selectivity for the $\alpha_1\beta_2\gamma_2$ subtype compared with the $\alpha_3\beta_2\gamma_2$ and $\alpha_5\beta_3\gamma_2$ subtypes (e.g.: **4t** and **7c** bind to these three BzR isoforms with K_i values of 14 nM, 283 nM, 239 nM, and 9 nM, 1960 nM, 95 nM, respectively). The GABA ratios close to unity exhibited by all the tested compounds on each BzR subtype were predictive of an efficacy profile typical of antagonists.

Introduction

The γ-aminobutyric acid type A ($GABA_A$) receptor is the major inhibitory ligand-gated ion channel in the mammalian brain.[1,2] This membrane-bound heteropentameric receptor is made up of five subunits out of the 18 which have so far been cloned and sequenced (6α, 4β, 4γ, 1δ, 1ϵ, and 2ρ). Three subunits (α, β, and γ) are required to form a fully functional $GABA_A$ receptor. The so-called benzodiazepine receptor (BzR) is located between the α and γ subunits, and its occupation by a ligand can allosterically modulate the affinity of the GABA neurotransmitter for its specific binding site. BzR agonists and inverse agonists potentiate or decrease, respectively, the GABA-induced chloride influx, whereas antagonists have minimal or no effects on the chloride flux. These substances exhibit a wide variety of pharmacological actions spanning in a continuum from full agonists (anxiolytic, anticonvulsant, sedative-hypnotic, and myorelaxant agents) through antagonists to inverse

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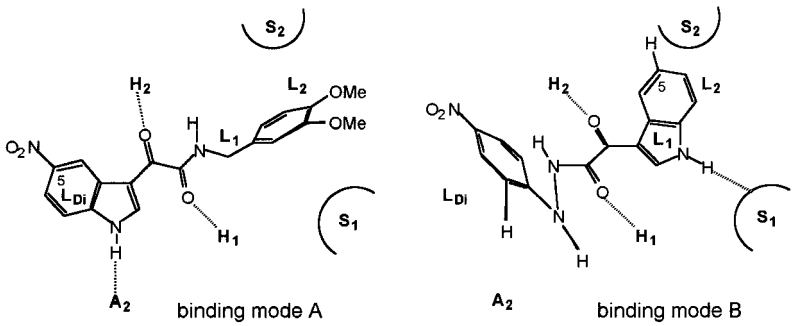


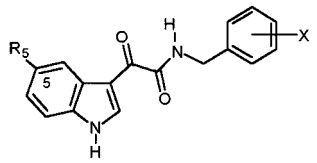
Figure 1. Binding modes A and B exemplified through the most potent ligands among benzylamides **1** and phenylhydrazides **2**, respectively. Labeling of BzR subsites are in accordance with Cook's pharmacophore model.[15]

agonists (anxiogenic and proconvulsant agents). Partial agonists exist within this efficacy spectrum and are of particular interest, as they may display antianxiety properties devoid of the undesirable side effects typical of full agonist-type ligands.[3] Partial inverse agonists have been described which can enhance general memory/learning and block or reverse the effects of barbiturate toxicity but are devoid of proconvulsant activity.[4,5] Moreover, the availability of cloned benzodiazepine receptor subtypes will probably lead soon to the discovery of subtype-selective ligands, which will open the exciting possibility to separate the many pharmacological actions of BzR ligands permitting the selective treatment of anxiety, sleep disorders, convulsions, and memory deficits with fewer side effects.[1,6]

Structure−activity relationships (SARs) of structurally diverse classes of ligands[7−14] were rationalized by Cook and co-workers[15] through a comprehensive pharmacophore/receptor model consisting of several BzR interaction (sub)sites: (i) a hydrogen bond acceptor (A_2), (ii) a hydrogen bond donor (H_1), (iii) a bifunctional hydrogen bond donor/acceptor (H_2/A_3), and (iv) four lipophilic pockets (L_1, L_2, L_3, and L_{di}). The boundaries of the receptor were defined in terms of sterically forbidden sites (S_1, S_2, and S_3). Finally, it was assumed that agonists, antagonists, and inverse agonists share the same binding cleft. Figure 1 describes the interactions of our compounds **1** and **2** at the BzR in the framework of Cook's pharmacophore model.

We have recently reported on a new class of BzR ligands designed as open chain analogues of β-carbolines, the *N*-(benzyl)indol-3-ylglyoxylylamides **1**.[16] Interestingly, in this series the effects of the R_5 and X substituents on potency are not constant but interdependent. Particularly, affinity is favored by electron-donating or electron-attracting X substituents depending on whether the 5-position of the indole nucleus is substituted (R_5 = Cl/NO_2) or not (R_5 = H). Thus, while the optimum of affinity in the 5-Cl/NO_2 series was reached with X = 3′,4′-$(OMe)_2$ (K_i 11 nM), in the 5-H series potency was optimized with X = 4′-Cl (K_i 67 nM). A few selected benzylamide derivatives were also evaluated by in vivo tests, but none of them showed any activity. Their lack of efficacy was explained in terms of poor absorption and bioavailability, partly depending on low water solubility.

The nanomolar binding constants exhibited by several benzylamides **1** prompted the design of closely related but more water-soluble and bioavailable analogues. Therefore, we prepared a series of *N*′-phenylindol-3-ylglyoxylohydrazides **2** formally derived from the previ-



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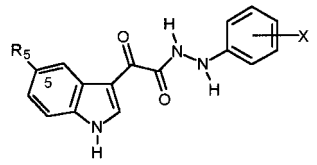
ously described benzylamides by replacing the CH_2 spacer with the isosteric NH group.[17] Surprisingly, affinity was restricted to 5-H phenylhydrazides, the 5-Cl/NO_2 counterparts being invariably inactive for either electron-donating or electron-attracting X substituents on the side phenyl ring. This discrepancy in the SARs of the isosteric series of 5-Cl/NO_2 indoles was suspected to depend on differences in the conformational properties of the NHNHAr and $NHCH_2Ar$ side chains: the former being forced in a gauche disposition about the N−N bond and the latter, more flexible, capable of assuming a staggered conformation about the N−C bond. As observed in the 5-H benzylamides, the affinity of 5-H phenylhydrazides is enhanced by electron-withdrawing X substituents such as 4′-NO_2 (K_i 11 nM) and is lowered considerably by methylation of the indole nitrogen. Selected phenylhydrazides tested in vivo revealed efficacy profiles typical of partial agonists.



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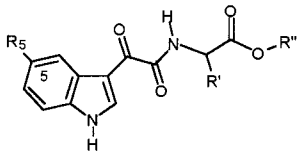
Taken together, the binding data of compounds **1** and **2** suggested that 5-Cl/NO_2 indoles interact with the receptor differently from their 5-H counterparts. Particularly, two alternative binding modes of the ligands were hypothesized, called A and B, exemplified in Figure 1 using the most potent benzylamide and phenylhydrazide derivatives, respectively.

Binding mode A requires (i) a transoid conformation of the side chain (not feasible for phenylhydrazides) and (ii) non-electron-withdrawing X substituents on the side phenyl ring. According to our hypothesis, benzylamides would engage interactions with the A_2 site (through the indole NH), the H_1 and H_2 sites (through the C=O2 and C=O1), and the L_1, L_2, and L_{Di} lipophilic regions (filled by the CH_2, the phenyl and the fused benzene ring,

respectively). An electron-withdrawing group in the 5-position of the indole, such as Cl or NO_2, strengthens the NH···A_2 hydrogen bond. The favorable electronic effects exerted by electron-donating X substituents in the series of 5-Cl/NO_2 benzylamides suggest that the side phenyl ring might be involved in a charge-transfer interaction with an electropositive function within the L_2 site.

Binding mode B takes place through the following interactions: (i) C═O2 and C═O1 are hydrogen-bound to the H_1 and H_2 sites; (ii) the lipophilic L_1 and L_2 regions are occupied by the pyrrole and benzene moieties of the indole nucleus; and (iii) a hydrogen bond is donated by the indole NH to a heteroatom belonging to the S_1 site. Electron-withdrawing substituents on the side phenyl ring, such as 4′-NO_2 in the most potent phenylhydrazide, make the terminal 2′-CH hydrogen more electropositive thus favoring its interaction with the electron-rich A_2 site. Binding mode B is accessible only to 5-H indoles because the sterically forbidden S_2 site closely faces the 5-position and is unable to host substituents larger than a hydrogen. Alternatively (or additionally), mode B might not be feasible for 5-Cl and 5-NO_2 derivatives owing to unfavorable electron-attracting effects of these substituents on the indole π-system. Collinearity between size and electron-attracting power within the limited data set (Cl and NO_2) of 5-substituents did not allow us to single out which property actually disables mode B.

Benzylamides **1** are closely related analogues of *N*-(indol-3-ylglyoxylyl)amino acid derivatives with the general formula **3**[18,19] displaying nanomolar potency when incorporating a (D)-alanine residue (R′ = Me). The much lower affinities of the corresponding glycine (R′ = H) and (L)-alanine derivatives led us to hypothesize that the L_1 pocket surrounding the α-carbon is asymmetric, so that it can be filled by the (D)-alanine methyl on one side but has no room for the (L)-alanine methyl on the opposite side.



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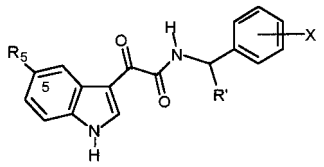
In light of the SARs in series **1** and **3**, we felt that a fruitful continuation of our research would be to prepare *N*-(α-substituted-benzyl)indol-3-ylglyoxylylamide derivatives **4** which retain the benzylamide scaffold **1** and bear an α-methyl group (R′ = Me) in the same spatial position (R configuration) as in the previously described (D)-alanine derivatives **3**. Compounds **4** were synthesized as pure enantiomers whenever the starting products were commercially available, otherwise the racemic mixture was prepared and tested.

The following indole derivatives, all featuring a geometrically constrained *N*-phenylalkyl side chain, were included in the same project: 2-(indol-3-ylglyoxylyl)-isoindolines **5**, *N*-(indan-2-yl)indol-3-ylglyoxylylamides **6**, (R) and (S) enantiomers of *N*-(indan-1-yl)indol-3-ylglyoxylylamides **7** and **8**. We reasoned that the reduced flexibility of these structures would more markedly discriminate between the two putative binding



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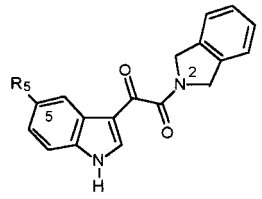
modes A and B, facilitate mapping of the BzR binding cleft, and increase the chances of improving affinity.



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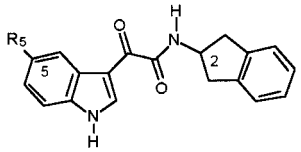

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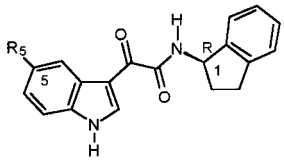

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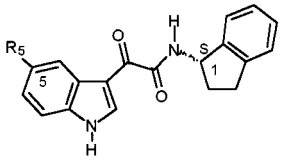

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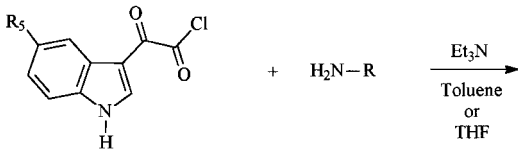
Among the newly investigated compounds there are also some benzylamides and phenylhydrazides of series **1** and **2** bearing a methoxy group in position 5 of the indole nucleus. By adding these 5-MeO derivatives to the data set of 5-Cl and 5-NO_2 indoles, we broke the collinearity between size and the electron-withdrawing character of the 5-substituent (R_5), so as to unambiguously identify the property of this substituent leading to binding mode A or B. Finally, benzylamides of type **1** were prepared featuring a nitro group in position 4′ of the side phenyl ring (X = 4′-NO_2) to compare their binding affinities with those of the 4′-nitro derivatives of type **4** (R′ = Me, X = 4′-NO_2).

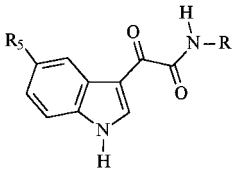
Since it is currently recognized that subtype-selective BzR ligands might represent potential selective drugs for the treatment of anxiety, sleep disorders, convulsions, and memory deficit with fewer side effects,[1,6] a few selected indole derivatives were evaluated by the radioligand technique on recombinant rat $\alpha_1\beta_2\gamma_2$, $\alpha_3\beta_2\gamma_2$, and $\alpha_5\beta_3\gamma_2$ $GABA_A$/BzR subtypes. The in vitro efficacy profile of the selected compounds for all three $GABA_A$/BzR subtypes was assessed by means of the GABA ratio.

This paper describes the synthesis, the biological evaluation, the SARs, and the molecular modeling analysis of the novel indole derivatives **1**, **2**, **4**−**8** targeted as ligands of the BzR.

Scheme 1



R_5 = H, Cl, NO_2, OCH_3
RNH_2 = arylalkylamine, phenylhydrazine, isoindoline, 1-aminoindane, 2-aminoindane



1, **2**, **4-8**

Chemistry

The general synthetic procedure used in the preparation of compounds **1**, **2**, and **4**−**8** involved the acylation of the appropriate indole with oxalyl chloride in accordance with a published procedure.[20] The indolylglyoxylyl chlorides obtained were allowed to react in mild conditions with the appropriate amine in the presence of triethylamine in toluene solution (in THF solution for **4i** and **4bb**) (Scheme 1). All products were purified by recrystallization from the appropriate solvent, and their structures were confirmed by IR, ^{1}H NMR, MS, and elemental analysis (Table 1). Spectral data of all the newly synthesized compounds **1**, **2**, and **4**−**8** are reported in the Supporting Information.

Results and Discussion

The binding affinity of each newly synthesized indole derivatives at the BzR in bovine brain membranes was determined by competition experiments against the radiolabeled antagonist [^{3}H]flumazenil[21] and expressed as the K_i value only for those compounds inhibiting radioligand binding by more than 80% at a fixed concentration of 10 μM. The in vitro efficacy of active compounds was measured by the GABA ratio which predicts the pharmacological profile of a BzR ligand.[22−24] Table 2 summarizes the biological data. The affinities of some previously reported[16,17,19] indoles (compounds **1a′**−**l′**, **2a′**−**g′**, and **3a′**−**c′**) discussed in the present paper are listed in Table 3. Molecular modeling studies, performed to aid the interpretation of SARs, were based on semiempirical quantum-mechanics and molecular mechanics calculations using the AM1 method[25] and the Tripos force field[26] available within the SYBYL suite of programs[27] (computational details are given in the Experimental Section).

Property of the R_5 Substituent Disabling Binding Mode B. As stated, binding mode B might not be feasible for 5-Cl/NO_2 indoles, due to a steric clash between a 5-substituent (R_5) larger than a hydrogen and the S_2 site (see Figure 1). Alternatively or additionally, mode B might be forbidden for 5-substituted indoles owing to the unfavorable electron-withdrawing effect exerted by a 5-Cl or a 5-NO_2 on a putative charge-transfer interaction between the indole moiety and an electron-poor function within the L_2 site. It was not possible to establish whether binding mode B is disfavored by the size and/or the electron-withdrawing ability of R_5, as these two properties remained collinear in a set of the two substituents 5-Cl and 5-NO_2.[17] The 5-OMe derivatives **1d**,**e** and **2a**,**b** were purposely prepared and tested to obtain a slightly larger data set in which the steric and electronic properties of R_5 are not correlated. Only compound **1d** (bearing a 4′-OMe on the side phenyl ring) possessed an appreciable potency, implying that a 5-OMe group affects the affinity of indole derivatives, like a 5-Cl or a 5-NO_2 substituent. Specifically, 5-Cl/NO_2 benzylamides **1** elicit nanomolar-submicromolar K_i values if X is electron-donating or a hydrogen (**1e′**−**l′**), whereas 5-Cl/NO_2 phenylhydrazides **2** are inactive for any other type of substituent X (**2d′**−**g′**). What makes 5-OMe, 5-Cl, and 5-NO_2 similar is clearly a steric rather than an electronic property. Taken together, the above-summarized SARs suggest that a 5-substituted indole cannot attain binding mode B because it would be sterically repelled by the S_2 site. Consequently, we speculate that compound **1d** binds to the receptor in accordance with mode A. The relatively low affinity of **1d** (K_i 494 nM) compared with its 5-H, 5-Cl, and 5-NO_2 counterparts **1b′**,**f′**,**j′** (163 nM, 107 nM, and 53 nM, respectively) is probably related to the electron-donating effect of the 5-OMe, weakening the hydrogen bond between the indole NH and the A_2 site.

Effects of the R′ and X Substituents on Affinity in Compounds 4. Compounds with the general formula **4** are moderately to highly potent when bearing a methyl group on the benzyl α-carbon with the R configuration; **4t** is the most potent among the newly synthesized indoles (K_i 17 nM). The remaining compounds of type **4**, where R′ is a hydrophilic (CH_2OH, CN or COOEt) or a Me group with the S configuration, displayed no affinity at the BzR, with the exception of the cyano derivative **4c′** (K_i 241 nM). A comparison of the binding constants of **4a**, **4l**, and **4t** with those of the corresponding α-desmethyl analogues **1a′**, **1e′**, and **1i′** (all the six ligands being unsubstituted on the side phenyl ring) reveals that (R)-α-Me improves the affinity of the 5-Cl/NO_2 derivatives by 4.5- and 7-fold, respectively, whereas it lowers the affinity of the 5-H derivative by 11-fold. These divergent effects of (R)-α-Me are probably related to the different binding modes of the 5-Cl/NO_2 and 5-H indoles, which direct the same (R)-α-Me to different regions of the BzR. The SARs outlined so far are consistent with our hypothesis[19] of a lipophilic L_1 pocket available to the (R)-α-Me of indoles **3** and **4** binding in accordance with mode A. The shape of this pocket is asymmetric, so that it hosts the (R)-α-Me on one side, while it has no room available for the (S)-α-Me on the opposite side. The affinity of 5-H indoles, binding in accordance with mode B, is significantly disfavored by α-methylation, probably because the receptor cleft surrounding the benzylic α-carbon is relatively narrow.

Within the set of 5-Cl/NO_2 indoles **4**, none of the substituents X on the side phenyl ring increases affinity (compare **4l** vs **4n**, **p**−**r** and **4t** vs **4v**, **x**−**z**). In contrast, 4′-OMe and 3′,4′-$(OMe)_2$ improve potency of 5-Cl/NO_2 benzylamides **1** by 16- and 10-fold (compare **1e′** vs **1g′** and **1i′** vs **1k′**). In the series of 5-H indoles **4**, affinity is favored by an electron-donating X group (compare **4a** against **4c**,**e**,**f**) or abolished if X is an electron-with-

Table 1. Physical Properties of Indolylglyoxylylamide Derivatives **1**, **2**, and **4**−**8**

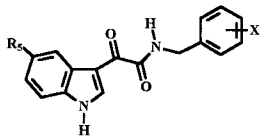
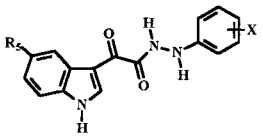
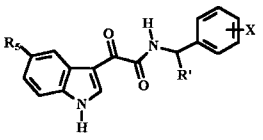
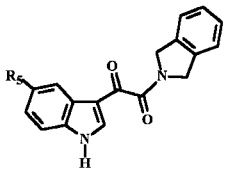
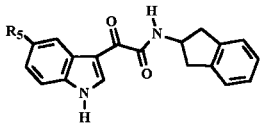
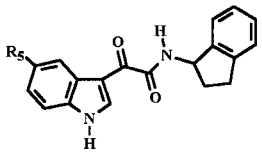
no.	R_5	X	R'	Config.	$[\alpha]_D$	Yield, (%)	Recryst. Solvent	m.p., (°C)	Formula[a]
									
1a	H	4'-NO_2				73	EtOH	194-196	$C_{17}H_{13}N_3O_4$
1b	Cl	4'-NO_2				88	EtOH	238-240	$C_{17}H_{12}ClN_3O_4$
1c	NO_2	4'-NO_2				81	DMF/H_2O	280-281	$C_{17}H_{12}N_4O_6$
1d	OCH_3	4'-OCH_3				90	EtOH	204-205	$C_{19}H_{18}N_2O_4$
1e	OCH_3	4'-Cl				89	EtOH	231-233	$C_{18}H_{15}ClN_2O_3$
									
2a	OCH_3	4'-OCH_3				65	EtOH	207-209	$C_{18}H_{17}N_3O_4$
2b	OCH_3	4'-NO_2				63	DMF/H_2O	288-290	$C_{17}H_{14}N_4O_5$
									
4a	H	H	CH_3	R	+48.61	46	EtOH	223-225	$C_{18}H_{16}N_2O_2$
4b	H	H	CH_3	S	-46.67	51	EtOH	244-245	$C_{18}H_{16}N_2O_2$
4c	H	4'-CH_3	CH_3	R	+64.48	54	MeOH	222-223	$C_{19}H_{18}N_2O_2$
4d	H	4'-CH_3	CH_3	S	-62.86	47	MeOH	215-218	$C_{19}H_{18}N_2O_2$
4e	H	4'-OCH_3	CH_3	R,S	-	47	EtOH/H_2O	175-177	$C_{19}H_{18}N_2O_3$
4f	H	3',4'-$(OCH_3)_2$	CH_3	R,S	-	46	EtOH/H_2O	138-140	$C_{20}H_{20}N_2O_4$
4g	H	4'-NO_2	CH_3	R	+53.12	82	AcOH glac.	245-247	$C_{18}H_{15}N_3O_4$
4h	H	4'-NO_2	CH_3	S	-52.37	95	AcOH glac.	245-247	$C_{18}H_{15}N_3O_4$
4i	H	H	CH_2OH	R,S	-	60	EtOH/H_2O	185-187	$C_{18}H_{16}N_2O_3$
4j	H	H	CN	R,S	-	44	Benzene	230-231	$C_{18}H_{13}N_3O_2$
4k	H	H	$CO_2C_2H_5$	R,S	-	48	Benzene	150-152	$C_{20}H_{18}N_2O_4$
4l	Cl	H	CH_3	R	-2.75	61	EtOH	182-184	$C_{18}H_{15}ClN_2O_2$
4m	Cl	H	CH_3	S	+3.60	71	EtOH	169-171	$C_{18}H_{15}ClN_2O_2$
4n	Cl	4'-CH_3	CH_3	R	+6.54	49	MeOH	186-187	$C_{19}H_{17}ClN_2O_2$
4o	Cl	4'-CH_3	CH_3	S	-4.96	52	MeOH/H_2O	181-184	$C_{19}H_{17}ClN_2O_2$
4p	Cl	4'-OCH_3	CH_3	R,S	-	51	EtOH	177-179	$C_{19}H_{17}ClN_2O_3$
4q	Cl	3',4'-$(OCH_3)_2$	CH_3	R,S	-	50	EtOH	150-151	$C_{20}H_{19}ClN_2O4$
4r	Cl	4'-NO_2	CH_3	R	-20.00	63	AcOH/H_2O	195-197	$C_{18}H_{14}ClN_3O_4$
4s	Cl	4'-NO_2	CH_3	S	+19.41	60	AcOH/H_2O	198-199	$C_{18}H_{14}ClN_3O_4$
4t	NO_2	H	CH_3	R	-45.00	81	EtOH	233-235	$C_{18}H_{15}N_3O_4$
4u	NO_2	H	CH_3	S	+45.00	77	EtOH	258-260	$C_{18}H_{15}N_3O_4$
4v	NO_2	4'-CH_3	CH_3	R	-44.57	73	EtOH	235-237	$C_{19}H_{17}N_3O_4$
4w	NO_2	4'-CH_3	CH_3	S	+46.20	69	EtOH	232-235	$C_{19}H_{17}N_3O_4$
4x	NO_2	4'-OCH_3	CH_3	R,S	-	79	EtOH/H_2O	234-235	$C_{19}H_{17}N_3O_5$
4y	NO_2	3',4'-$(OCH_3)_2$	CH_3	R,S	-	75	EtOH	209-211	$C_{20}H_{19}N_3O_6$
4z	NO_2	4'-NO_2	CH_3	R	-75.49	76	AcOH glac.	287-289	$C_{18}H_{14}N_4O_6$

Table 1. (Continued)

no.	R_5	X	R'	Config.	$[\alpha]_D$	Yield, (%)	Recryst. Solvent	m.p., (°C)	Formula[a]
4aa	NO_2	4'-NO_2	CH_3	S	+75.00	73	DMF/H_2O	278-280	$C_{18}H_{14}N_4O_6$
4bb	NO_2	H	CH_2OH	R,S	-	51	EtOH	199-201	$C_{18}H_{15}N_3O_5$
4cc	NO_2	H	CN	R,S	-	56	EtOH	243-246	$C_{18}H_{12}N_4O_4$
4dd	NO_2	H	$CO_2C_2H_5$	R,S	-	84	EtOH	200-201	$C_{20}H_{17}N_3O_6$
									
5a	H					61	EtOH	288-290	$C_{18}H_{14}N_2O_2$
5b	Cl					57	DMF	>300	$C_{18}H_{13}ClN_2O_2$
5c	NO_2					52	EtOH	>300	$C_{18}H_{13}N_3O_4$
									
6a	H					49	EtOH	222-223	$C_{19}H_{16}N_2O_2$
6b	Cl					77	EtOH	254-255	$C_{19}H_{15}ClN_2O_2$
6c	NO_2					54	EtOH	298-300	$C_{19}H_{15}N_3O_4$
									
7a	H			R	+108.43	65	EtOH	218-220	$C_{19}H_{16}N_2O_2$
7b	Cl			R	+65.94	57	EtOH	229-231	$C_{19}H_{15}ClN_2O_2$
7c	NO_2			R	+37.22	69	EtOH	282-284	$C_{19}H_{15}N_3O_4$
8a	H			S	-104.35	61	EtOH	220-222	$C_{19}H_{16}N_2O_2$
8b	Cl			S	-67.29	59	EtOH	230-231	$C_{19}H_{15}ClN_2O_2$
8c	NO_2			S	-39.41	72	EtOH	285-287	$C_{19}H_{15}N_3O_4$

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

drawing 4′-NO_2 group (compare **4a** vs **4g**). These data contrast with the effects of X in the set of 5-H indoles **1,** wherein affinity is favored by an electron-withdrawing X substituent such as a 4′-Cl (compare **1a′** vs **1d′**) or a 4′-NO_2 (featured by the newly synthesized **1a**, which is the most potent 5-H benzylamide derivative). The lack of parallelism between the effects of X in series **1** and **4** might be due to different orientations of their side phenyl rings within the BzR depending on the absence/presence of the (R)-α-Me group.

Indoles 5−8 Geometrically Constrained about the *N*-Phenylalkyl Side Chain. Also in the series of ligands **5**−**8**, 5-H derivatives exhibit divergent SARs from their 5-Cl/NO_2 counterparts. Unsubstituted indoles tolerate the isoindolinylamide side chain (**5a** being practically equipotent to **1a′**) but not the (R)-1-indanylamide moiety (**7a** is 5.6-fold less potent than **1a′**). In contrast, 5Cl/NO_2 indoles show no potency at the BzR when bearing the isoindolinylamide side chain, while the (R)-1-indanylamide residue produces an enhancement of the affinity, seeing that **7b** and **7c** are 6-fold and 4-fold more potent than the corresponding benzylamides **1e′** and **1i′**. None of the compounds **6a**−**c** and **8a**−**c** possesses any significant potency. These data further support our hypothesis of different interaction modes available for 5-H and 5-Cl/NO_2 indoles at the BzR. The binding data of the 1-indanylamides **7a**,**b**,**c** and **8a**,**b**,**c** parallel those of their open chain analogues **4a**,**l**,**t** and **4b**,**m**,**u**, thus suggesting that the methylene in position 2 of the 1-indane ring (2-CH_2) fits into the same lipophilic pocket of the L_1 site hosting the (R)-α-Me group.

Isoindolinylamides **5** are the most rigid structures among those discussed in the present paper: the torsion angles (O=C)−N−C−C1′ and N−C−C1′−C2′ are both frozen in a staggered conformation (their values are 180°). Based on our model of binding mode A,[17] the transoid disposition of the former torsion angle should favor affinity of the 5-Cl and 5-NO_2 derivatives **5b** and **5c**. Actually, the inactivity of these two compounds

Table 2. Inhibition of [^{3}H]flumazenil Specific Binding to Bovine Brain Membranes and GABA Ratios of Indolylglyoxylylamide Derivatives **1**, **2**, and **4**−**8**

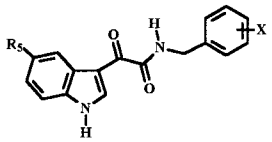
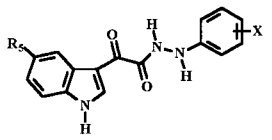
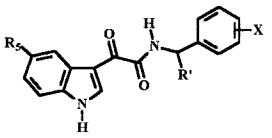
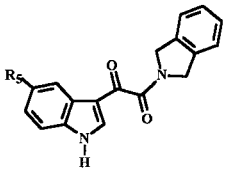
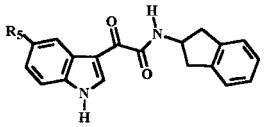
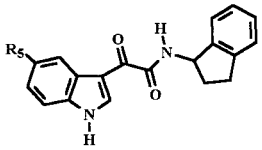
no.	R_5	X	R'	Config.	K_i [a](nM)	GABA ratio[b]
						
1a	H	4'-NO_2			21 ± 2	0.75
1b	Cl	4'-NO_2			1370 ± 128	0.73
1c	NO_2	4'-NO_2			620 ± 49	1.20
1d	OMe	4'-OMe			494 ± 39	1.15
1e	OMe	4'-Cl			ND[c]	
						
2a	OMe	4'-OMe			ND	
2b	OMe	4'-NO_2			ND	
						
4a	H	H	Me	R	1307 ± 124	1.00
4b	H	H	Me	S	ND	
4c	H	4'-Me	Me	R	1150 ± 106	0.97
4d	H	4'-Me	Me	S	ND	
4e	H	4'-OMe	Me	R,S	1168 ± 111	0.76
4f	H	3',4'-$(OMe)_2$	Me	R,S	850 ± 81	1.20
4g	H	4'-NO_2	Me	R	ND	
4h	H	4'-NO_2	Me	S	ND	
4i	H	H	CH_2OH	R,S	ND	
4j	H	H	CN	R,S	ND	
4k	H	H	COOEt	R,S	ND	
4l	Cl	H	Me	R	100 ± 8	1.20
4m	Cl	H	Me	S	ND	
4n	Cl	4'-Me	Me	R	105 ± 9	1.00
4o	Cl	4'-Me	Me	S	ND	
4p	Cl	4'-OMe	Me	R,S	103 ± 11	0.72
4q	Cl	3',4'-$(OMe)_2$	Me	R,S	153 ± 13	0.97
4r	Cl	4'-NO_2	Me	R	695 ± 66	0.82
4s	Cl	4'-NO_2	Me	S	ND	
4t	NO_2	H	Me	R	17 ± 1	1.10
4u	NO_2	H	Me	S	ND	
4v	NO_2	4'-Me	Me	R	34 ± 3	0.95
4w	NO_2	4'-Me	Me	S	ND	
4x	NO_2	4'-OMe	Me	R,S	113 ± 9	0.80
4y	NO_2	3',4'-$(OMe)_2$	Me	R,S	55 ± 3	1.10
4z	NO_2	4'-NO_2	Me	R	78 ± 5	0.88
4aa	NO_2	4'-NO_2	Me	S	ND	
4bb	NO_2	H	CH_2OH	R,S	ND	
4cc	NO_2	H	CN	R,S	241 ± 19	1.20
4dd	NO_2	H	COOEt	R,S	ND	

Table 2. (Continued)

no.	R_5	X	R'	Config.	K_i [a] (nM)	GABA ratio [b]
						
5a	H				123 ± 9	1.00
5b	Cl				ND	
5c	NO_2				ND	
						
6a	H				ND	
6b	Cl				ND	
6c	NO_2				ND	
						
7a	H			R	675 ± 63	1.10
7b	Cl			R	80 ± 6	1.20
7c	NO_2			R	28 ± 2	1.13
8a	H			S	ND	
8b	Cl			S	ND	
8c	NO_2			S	ND	
flumazenil					0.90 ± 0.05	0.90
clonazepam					0.85 ± 0.02	1.97

[a] K_i values are means ± SEM of three determinations. [b] GABA ratio = (K_i without GABA)/(K_i with GABA). [c] Not determined for the compounds (10 μM) showing percentages of inhibition of specific [^{3}H]flumazenil binding ≤80%.

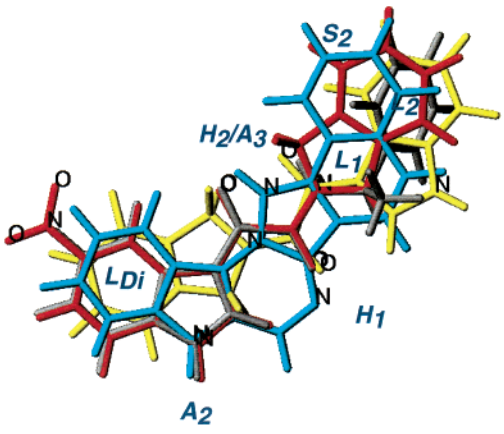
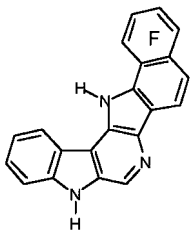


Figure 2. The isoindolinylamides **5c** (inactive, in red) and **5a** (active, in yellow) oriented according to mode A and, respectively, mode B are aligned on the (R)-α-Me-benzylamide **4t** (active, in gray) and the benzopyridodiindole **9** (inactive, in cyan). The fused benzene ring F of **9** maps the sterically forbidden S_2 site.

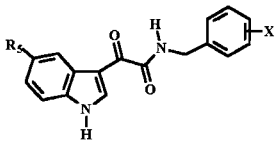
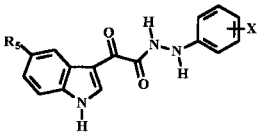
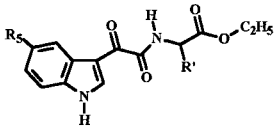
depends on poor shape complementarity with the receptor rather than on lack of conformational requirements. In fact, Figure 2 shows that when **5c** is oriented in accordance with mode A, the isoindoline benzene moiety projects into the sterically hindered S_2 site mapped by ring F of the inactive benzopyridodiindole **9**.[28] The active (R)-α-Me benzylamide **4t**, also aligned in accordance with mode A, avoids the steric clash with the S_2 site by virtue of its side phenyl ring twisted out of the main plane of the molecule. More specifically, the torsion angle N−C−C1′−C2′ is 60° in **4t**, whereas, as already mentioned, it is fixed to 180° in the isoindolinylamides **5**. On the other hand, it is worth noting that when the active 5-H isoindoline **5a** adopts binding mode B, the indole moiety fills the L_2 site without contacting the S_2 site.



9

The lack of affinity exhibited by the 2-indanylamides **6a**−**c** and the (S)-1-indanylamides **8a**−**c** implies that neither mode A nor mode B of interaction are allowed for these compounds. The 5-Cl/NO_2 (R)-1-indanylamides **7b** and **7c** are the only ones, among the newly investi-

Table 3. Inhibition of [^{3}H]flumazenil Specific Binding to Bovine Brain Membranes and GABA Ratios of Indolylglyoxylylamide Derivatives **1′**−**3′**[16,17,19]

no.	R_5	X	R'	Config.	Ki (nM)[a]	GABA ratio[b]
						
1a'	H	H			120	-
1b'	H	4'-OMe			163	-
1c'	H	3',4'-(OMe)2			94	-
1d'	H	4'-Cl			67	ND[c]
1e'	Cl	H			490	-
1f'	Cl	4'-OMe			107	-
1g'	Cl	3',4'-(OMe)2			30	-
1h'	Cl	4'-Cl			ND	ND
1i'	NO_2	H			117	-
1j'	NO_2	4'-OMe			53	-
1k'	NO_2	3',4'-(OMe)2			11	-
1l'	NO_2	4'-Cl			ND	ND
						
2a'	H	H			203	0.88
2b'	H	4'-OMe			430	0.84
2c'	H	4'-NO_2			11	0.90
2d'	Cl	4'-OMe			ND	ND
2e'	Cl	4'-NO_2			ND	ND
2f'	NO_2	4'-OMe			ND	ND
2g'	NO_2	4'-NO_2			ND	ND
						
3a'	NO_2		Me	R	45	0.67
3b'	NO_2		Me	S	1950	0.79
3c'	NO_2		H		2700	0.70

[a−c] See Table 2 footnotes.

gated closed-chain analogues, capable of ensuring a tight binding (probably in accordance with mode A).

Figure 3 shows an overlay of the 5-NO_2 derivatives **7c** (active), **6c** (inactive), and **8c** (inactive) illustrating how we interpret the binding data of indoles **6**−**8** at the molecular level. The side chains of the three ligands attain a transoid conformation (required for binding mode A) which ensures the match of the corresponding terminal fused-benzene rings expected to fill the L_2 pocket. An arrow highlights a region of the receptor whose occupancy by a ligand, such as **6c** or **8c**, compromises binding for steric reasons. The indane 2-CH_2 fragment of the (R)-1-indanylamide **7c**, supposed to enhance affinity through a hydrophobic interaction, is surrounded by a cartoon of the lipophilic pocket L_1. We believe that this BzR site hosts similarly also the (R)-α-Me group of indole derivatives **4**.

The 5-H derivatives **6a**, **7a,** and **8a** are poorly active or not active at all, probably because branching at the

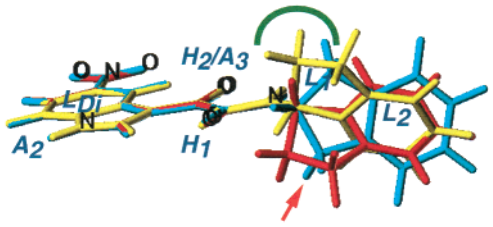


Figure 3. Superposition of the (R)-1-indanylamide **7c** (active, in yellow) on the (S)-1-indanylamide **8c** (inactive, in red) and the 2-indanylamide **6c** (inactive, in cyan), all oriented in accordance with mode A. Sterically unfavorable fragments of the inactive compounds **8c** and **6c** are marked by an arrow. A cartoon of the L_1 lipophilic pocket surrounds the favorable 2-CH_2 fragment of the potent ligand **7c**.

carbon bound to the amidic nitrogen, a structural motif common to these compounds as well as to the 5-H α-methylbenzylamides **4**, interferes sterically with bind-

Table 4. Inhibition of [^{3}H]Flumazenil Specific Binding and GABA Ratios of Selected Compounds at Rat $\alpha_1\beta_2\gamma_2$, $\alpha_3\beta_2\gamma_2$, and $\alpha_5\beta_3\gamma_2$ Gaba$_A$/Bz Receptor Subtypes[a]

	K_i (nM)[b] or % inhibition (10 μM)[c]					
no.	$\alpha_1\beta_2\gamma_2$	GABA ratio[d]	$\alpha_3\beta_2\gamma_2$	GABA ratio[d]	$\alpha_5\beta_3\gamma_2$	GABA ratio[d]
1a	16 ± 2	0.90	1600 ± 110	0.91	58 ± 6	0.73
4a	1150 ± 86	0.92	5% ± 0.4		5500 ± 360	1.05
4t	14 ± 2	1.17	283 ± 19	1.30	239 ± 21	1.30
5a	224 ± 20	1.06	3200 ± 150	1.10	43% ± 4	
7a	225 ± 13	1.13	28% ± 3		2160 ± 160	1.10
7c	9 ± 0.6	1.13	1960 ± 150	1.12	95 ± 8	0.98
1j′	42 ± 3	1.11	137 ± 11	0.96	126 ± 11	0.90
2c′	25 ± 3	0.96	40 ± 4	0.98	43 ± 5	0.80
zolpidem	50 ± 3		765 ± 63		35% ± 3	

[a] The ability of the compounds to displace [^{3}H]flumazenil was measured in membranes from HEK293 cells expressing the $\alpha_1\beta_2\gamma_2$, $\alpha_3\beta_2\gamma_2$, and $\alpha_5\beta_3\gamma_2$ subtypes, as described in the Experimental Section. [b] K_i values are means ± SEM of three determinations. [c] Percentage inhibition values of specific [^{3}H]flumazenil binding at 10 μM concentration are means ± SEM of three determinations. [d] GABA ratio = (K_i without GABA)/(K_i with GABA).

ing mode B. This hypothesis is consistent with the significantly higher potency elicited by the isoindolinylamide **5a** which is characterized by two nonbranched benzylic CH_2 fragments.

Using an exhaustively washed membrane preparation, the GABA ratio values of the most active compounds of each series **1**, **4**, **5**, and **7** were evaluated. All the products tested showed values close to unity, predicting antagonist properties. A correspondence between the GABA ratio and the pharmacological profile has already been reported for the structurally analogous *N*-(phenylethyl)indol-3-ylglyoxylylamides.[29]

Binding of Selected Compounds at $\alpha_1\beta_2\gamma_2$, $\alpha_3\beta_2\gamma_2$, and $\alpha_5\beta_3\gamma_2$ GABA$_A$/Bz Receptor Subtypes. A number of compounds (**1a**, **4a**, **4t**, **5a**, **7a**, **7c**, **1j′**, and **2c′**) were tested for their ability to displace [^{3}H]flumazenil from recombinant rat $\alpha_1\beta_2\gamma_2$, $\alpha_3\beta_2\gamma_2$, and $\alpha_5\beta_3\gamma_2$ GABA$_A$/Bz receptor subtypes (Table 4). A good correlation exists between the affinities at wild-type and $\alpha_1\beta_2\gamma_2$ subtype receptors. Most of the ligands showed enhanced affinities for the $\alpha_1\beta_2\gamma_2$ isoform, with compounds **4t** and **7a** exhibiting the highest selectivity over both $\alpha_3\beta_2\gamma_2$ and $\alpha_5\beta_3\gamma_2$ subtypes.

The GABA ratios close to unity exhibited by these compounds at the $\alpha_1\beta_2\gamma_2$ receptor subtype were predictive of antagonist properties. Interestingly, it has recently been proposed that α_1-selective ligands, such as the antagonist *tert*-butyl β-carboline-3-carboxylate (BCCT), may be useful for the treatment of alcohol abuse.[1]

Conclusions

The SARs developed from the new series of *N*-(arylalkyl)indol-3-ylglyoxylylamide derivatives **1** and **4**−**8** further support our hypothesis of two different binding modes selected by these ligands depending on the size of the substituent in the 5-position of the indole nucleus. Specifically, 5-substituted and 5-unsubstituted indoles bind preferentially in accordance with mode A and B, respectively. Using molecular modeling methods, we inferred the conformational and stereoelectronic properties of the amide side chains leading to a high affinity. The binding data of optically active indole derivatives suggested that the shape of the lipophilic pocket L_1 is asymmetric. A subset of the compounds tested on recombinant GABA$_A$/BzR $\alpha_1\beta_2\gamma_2$, $\alpha_3\beta_2\gamma_2$, and $\alpha_5\beta_3\gamma_2$ subtypes showed an enhanced affinity at the α_1 containing GABA$_A$ isoform.

Experimental Section

Chemistry. Melting points were determined using a Reichert Köfler hot-stage apparatus and are uncorrected. The $[\alpha]_D$ values were measured with a Perkin-Elmer Model 241 polarimeter in freshly distilled DMF solution. Infrared spectra were obtained on a PYE/UNICAM mod. PU 9561 spectrophotometer in Nujol mulls. Nuclear magnetic resonance spectra were recorded in DMSO-d_6 on a Varian CFT-20 spectrometer operating at 80 MHz using tetramethylsilane (TMS) as the internal standard. Mass spectra were obtained on a Hewlett-Packard 5988 A spectrometer using a direct injection probe and an electron beam energy of 70 eV. Magnesium sulfate was always used as the drying agent. Evaporations were made in vacuo (rotary evaporator). Analytical TLC was carried out on Merck 0.2 mm precoated silica gel aluminum sheets (60 F-254). Elemental analyses were performed by our Analytical Laboratory and agreed with theoretical values to within ±0.4%.

Besides the commercially available starting materials, the following products were prepared in accordance with reported methods: 1-(4-methoxyphenyl)ethylamine,[30] 1-(3,4-dimethoxyphenyl)ethylamine,[30] and 1,3-dihydroisondole.[31]

General Procedure for the Synthesis of *N*-[(5-Substituted indol-3-yl)glyoxylyl]amide Derivatives 1, 2, and 4−8. Triethylamine (3.0 mmol) was added dropwise to a stirred suspension, cooled at 0 °C, of indolylglyoxylyl chloride (2.5 mmol) and the appropriate amine (2.75 mmol) in 50 mL of dry toluene (THF for compounds **4i** and **4bb**). The reaction mixture was left to warm to room temperature, stirred for 24−36 h (TLC analysis), 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 toluene (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 amide derivatives obtained from the initial insoluble precipitate or from the toluene (or THF) solution were variable, depending upon the solubility of the various compounds. All products **1**, **2**, and **4**−**8** were purified by recrystallization from the appropriate solvent. Yields, recrystallization solvents, and melting points are listed in Table 1. IR, ^{1}H NMR, and MS spectral data are reported in the Supporting Information.

Binding Studies. [^{3}H]Flumazenil (specific activity 70.8 Ci/mmol) was obtained from NEN Life Sciences Products. All other chemicals were of reagent grade and were obtained from commercial suppliers.

Bovine cerebral cortex membranes were prepared in accordance with ref 32. 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.[33]

[^{3}H]Flumazenil binding studies were performed as previously reported.[17]

Clonal mammalian cell lines expressing relatively high levels of rat GABA$_A$ receptor subtypes ($\alpha_1\beta_2\gamma_2$, $\alpha_3\beta_2\gamma_2$, $\alpha_5\beta_3\gamma_2$) were maintained, as previously described[34] in Minimum Essential Medium Eagle with EBSS, supplemented with 10% fetal calf serum, L-glutamine (2 mM), penicillin (100 units/mL), and streptomycin (100 μg/mL) in a humidified atmosphere of 5% CO_2/95% air at 37 °C. Cells were harvested and then centrifuged at 500 × g. The crude membranes were prepared after homogenization in 10 mM potassium phosphate, pH 7.4, and differential centrifugation at 48 000 × g for 30 min at 4 °C. The pellets were washed twice in this manner before final resuspension in 10 mM potassium phosphate, pH 7.4, containing 100 mM potassium chloride.[34]

[^{3}H]Flumazenil binding assays to transfected cells membranes were carried out as previously described.[34] In brief, the cell lines membranes were incubated in a volume of 500 μL which contained [^{3}H]flumazenil at a concentration of 1−2 nM and the test compound in the range 10^{-9}−10^{-5} M. Nonspecific binding was defined by 10^{-5} M diazepam. Assays were incubated to equilibrium for 1 h at 4 °C.

The potencies of the new synthesized compounds to inhibit [^{3}H]flumazenil binding in the presence and absence of GABA were compared. The differences obtained were expressed as the GABA ratio, namely the ratios of the K_i values obtained in the absence of GABA over the K_i values obtained in the presence of GABA.

Computational Chemistry. All molecular modeling was performed using the software package SYBYL[27] running on a Silicon Graphics R10000 workstation. Most of the models were built starting from benzylamide structures of type **1** (available from our previous works)[16,17] in accordance with SYBYL standard bond lengths and valence angles. Atom centered charges were calculated by the Gasteiger-Hückel method.[35,36] Preliminary geometry optimizations were carried out using the SYBYL/MAXIMIN2 minimizer based on the molecular mechanics Tripos force field[26] and the BFGS (Broyden, Fletcher, Goldfarb, and Shanno) algorithm.[37] A root-mean-square gradient of the forces acting on each atom of 0.05 kcal/mol Å was set as the convergence criterion.

Global minimum and pharmacophore-consistent conformations were identified using the SYBYL/SEARCH routine. With the exception of compounds **5a** and **5c**, featuring a totally rigid isoindolinylamide moiety, rotatable bonds of the arylalkyl side chain were generally scanned through 10° increments within the 0−350° interval. A 0.75 van der Waals scaling factor was applied to "soften" steric contacts in the rigid rotamers. All the conformations subjected to further modeling had a strain energy (difference with respect to the global minimum conformation) not greater than 3 kcal/mol.[38] For each of the compounds **4t**, **6c**, **7c**, and **8c** we selected a pharmacophore-consistent conformation as the one featuring the largest distance between the amidic nitrogen and the phenyl ring centroid. This criterion allowed us to identify geometries characterized by a side chain aryl moiety positioned within the plane of the indole-COCONH system and therefore compatible with binding mode A.

The selected global minimum and pharmacophore-consistent conformations were subjected to full geometry optimizations performed with the semiempirical quantum-mechanics method AM1[25] available in the MOPAC program.[39] MOPAC was run using the keywords "XYZ" and "MMOK". The resulting pharmacophore-consistent conformers, defined by the following torsion angles, were all coincident with global minima: **4t**: (O=)C−N−C−C1′$_{Ar}$ = 159°, N−C−C1′$_{Ar}$−C2′$_{Ar}$ = 60°; **5a** and **5c**: (O=)C−N−C−C$_{Ar}$ = 180°; **6c**: (O=)C−N−C2−C1 =108°; **7c**: (O=)C−N−C−C$_{Ar}$ = 155°; **8c**: (O=)C−N−C−C$_{Ar}$ = 162°.

The model of the benzopyridodiindole **9** shown in Figure 2 was available from a previous work.[17] Molecular superpositions were accomplished following the procedures described by Cook et al.[15]

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Supporting Information Available: Table containing the IR, ^{1}H NMR, and MS spectral data of compounds **1**, **2**, **4**−**7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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