

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/231561188>

Novel N-(Arylalkyl)indol-3-ylglyoxylylamides Targeted as Ligands of the Benzodiazepine Receptor: Synthesis, Biological Evaluation, and Molecular Modeling Analysis of the Structure—...

ARTICLE in JOURNAL OF MEDICINAL CHEMISTRY · JUNE 2001

Impact Factor: 5.45 · DOI: 10.1021/jm010827j · Source: PubMed

CITATIONS

25

READS

59

11 AUTHORS, INCLUDING:



Sabrina Taliani

Università di Pisa

85 PUBLICATIONS 1,064 CITATIONS

SEE PROFILE



Anna Maria Marini

Università di Pisa

93 PUBLICATIONS 1,050 CITATIONS

SEE PROFILE



Ettore Novellino

University of Naples Federico II

618 PUBLICATIONS 9,097 CITATIONS

SEE PROFILE



Antonio Lavecchia

University of Naples Federico II

119 PUBLICATIONS 2,478 CITATIONS

SEE PROFILE

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†

Giampaolo Primofiore,*[§] Federico Da Settimo,[§] Sabrina Taliani,[§] Anna Maria Marini,[§] Ettore Novellino,[‡] Giovanni Greco,[‡] Antonio Lavecchia,[‡] François Besnard,[‡] Letizia Trincavelli,[#] Barbara Costa,[#] and Claudia Martini[#]

Dipartimento di Scienze Farmaceutiche and Dipartimento di Psichiatria, Neurobiologia, Farmacologia e Biotecnologie, Università di Pisa, Via Bonanno 6, 56126 Pisa, Italy, Dipartimento di Chimica Farmaceutica e Tossicologica, Università di Napoli "Federico II", Via D. Montesano, 49, 80131 Napoli, Italy, and Department of Molecular and Functional Genomics, Synthelabo, 10 rue des Carrieres, 92500 Rueil-Malmaison, France

Received January 23, 2001

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 [³H]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-NO₂) derivatives, whereas it was detrimental for their 5-unsubstituted (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-NO₂ (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 L₁ (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 (6 α , 4 β , 4 γ , 1 δ , 1 ϵ , and 2 ρ). 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

* To whom correspondence should be addressed. Tel: 39 50 500209. Fax: 39 50 40517. E-mail: primofiore@farm.unipi.it.

† Presented at the Italian–Hungarian–Polish Joint Meeting on Medicinal Chemistry, Giardini Naxos-Taormina, September 28–October 1, 1999 and at the IX Meeting on Heterocyclic Structures in Medicinal Chemistry Research, Palermo, May 14–17, 2000.

[§] Dipartimento di Scienze Farmaceutiche, Università di Pisa.

[‡] Università "Federico II" di Napoli.

[‡] Department of Molecular and Functional Genomics, Synthelabo.

[#] Dipartimento di Psichiatria, Neurobiologia, Farmacologia e Biotecnologie, Università di Pisa.

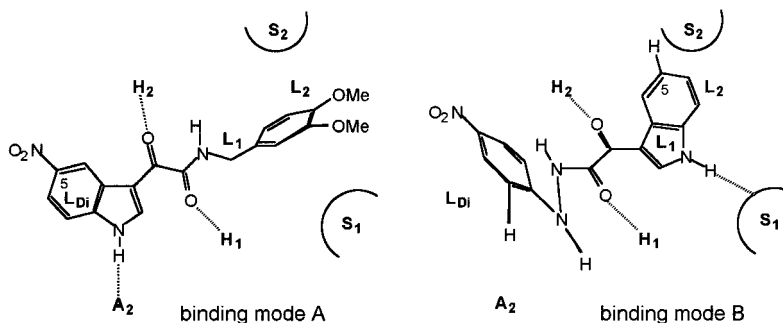


Figure 1. Binding modes A and B exemplified through the most potent ligands among benzylamides **1** and phenylhydrazides **2**, respectively. Labeling of BzR subsites are in accordance with Cook's pharmacophore model.¹⁵

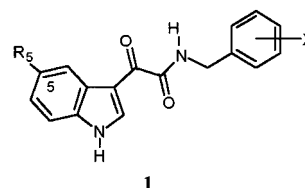
agonists (anxiogenic and proconvulsant agents). Partial agonists exist within this efficacy spectrum and are of particular interest, as they may display antianxiety properties devoid of the undesirable side effects typical of full agonist-type ligands.³ Partial inverse agonists have been described which 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-workers¹⁵ through a comprehensive pharmacophore/receptor model consisting of several BzR interaction (sub)sites: (i) a hydrogen bond acceptor (A₂), (ii) a hydrogen bond donor (H₁), (iii) a bifunctional hydrogen bond donor/acceptor (H₂/A₃), and (iv) four lipophilic pockets (L₁, L₂, L₃, and L_{Di}). The boundaries of the receptor were defined in terms of sterically forbidden sites (S₁, S₂, and S₃). 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 R₅ 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 (R₅ = Cl/NO₂) or not (R₅ = H). Thus, while the optimum of affinity in the 5-Cl/NO₂ 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.

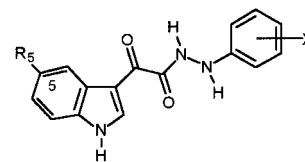
The nanomolar binding constants exhibited by several benzylamides **1** prompted the design of closely related but more water-soluble and bioavailable analogues. Therefore, we prepared a series of *N*-phenylindol-3-

ylglyoxylyhydrazides **2** formally derived from the previ-



1

ously described benzylamides by replacing the CH₂ spacer with the isosteric NH group.¹⁷ Surprisingly, affinity was restricted to 5-H phenylhydrazides, the 5-Cl/NO₂ 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/NO₂ indoles was suspected to depend on differences in the conformational properties of the NHNHAr and NHCH₂Ar 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'-NO₂ (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.



2

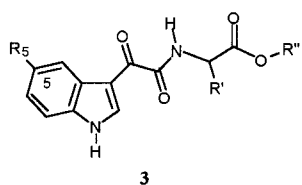
Taken together, the binding data of compounds **1** and **2** suggested that 5-Cl/NO₂ indoles interact with the receptor differently from their 5-H counterparts. Particularly, two alternative binding modes of the ligands were hypothesized, called A and B, exemplified in Figure 1 using the most potent 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 A₂ site (through the indole NH), the H₁ and H₂ sites (through the C=O and C=O1), and the L₁, L₂, and L_{Di} lipophilic regions (filled by the CH₂, the phenyl and the fused benzene ring,

respectively). An electron-withdrawing group in the 5-position of the indole, such as Cl or NO₂, strengthens the NH...A₂ hydrogen bond. The favorable electronic effects exerted by electron-donating X substituents in the series of 5-Cl/NO₂ benzylamides suggest that the side phenyl ring might be involved in a charge-transfer interaction with an electropositive function within the L₂ site.

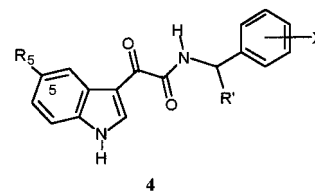
Binding mode B takes place through the following interactions: (i) C=O₂ and C=O₁ are hydrogen-bonded to the H₁ and H₂ sites; (ii) the lipophilic L₁ and L₂ regions are occupied by the pyrrole and benzene moieties of the indole nucleus; and (iii) a hydrogen bond is donated by the indole NH to a heteroatom belonging to the S₁ site. Electron-withdrawing substituents on the side phenyl ring, such as 4'-NO₂ in the most potent phenylhydrazide, make the terminal 2'-CH hydrogen more electropositive thus favoring its interaction with the electron-rich A₂ site. Binding mode B is accessible only to 5-H indoles because the sterically forbidden S₂ 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-NO₂ 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 NO₂) 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 L₁ 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.

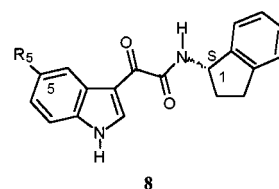
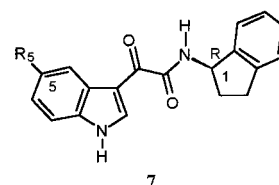
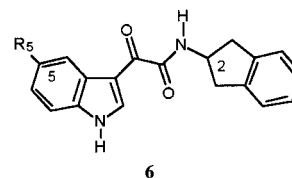
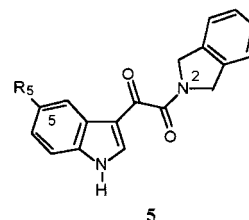


In light of the SARs in series **1** and **3**, we felt that a fruitful continuation of our research would be to prepare *N*-(α -substituted-benzyl)indol-3-ylglyoxylylamide derivatives **4** which retain the benzylamide scaffold **1** and bear an α -methyl group (R' = Me) in the same spatial position (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



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

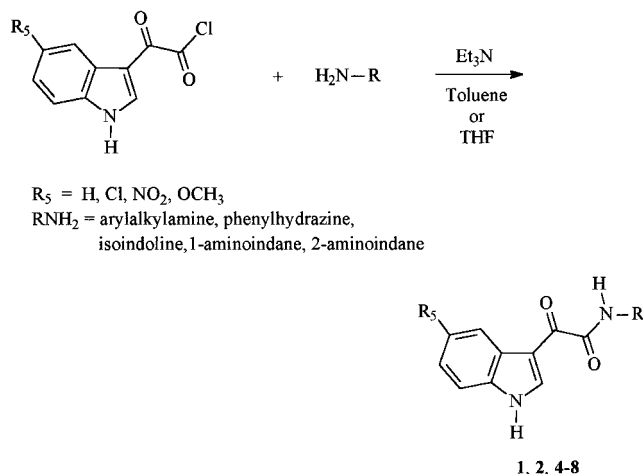


Among the newly investigated compounds there are also some benzylamides and phenylhydrazides of series **1** and **2** bearing a methoxy group in position 5 of the indole nucleus. By adding these 5-MeO derivatives to the data set of 5-Cl and 5-NO₂ indoles, we broke the collinearity between size and the electron-withdrawing character of the 5-substituent (R₅), 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'-NO₂) to compare their binding affinities with those of the 4'-nitro derivatives of type **4** (R' = Me, X = 4'-NO₂).

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



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, ¹H 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 [³H]flumazenil²¹ 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'–I'**, **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 method²⁵ and the Tripos force field²⁶ available within the SYBYL suite of programs²⁷ (computational details are given in the Experimental Section).

Property of the R₅ Substituent Disabling Binding Mode B. As stated, binding mode B might not be feasible for 5-Cl/NO₂ indoles, due to a steric clash between a 5-substituent (R₅) larger than a hydrogen and the S₂ 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-NO₂ on a putative charge-transfer interaction between the indole moiety and an electron-poor function within the L₂ site. It was not possible to establish whether binding mode B is dis-

favored by the size and/or the electron-withdrawing ability of R₅, as these two properties remained collinear in a set of the two substituents 5-Cl and 5-NO₂.¹⁷ 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 R₅ 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-NO₂ substituent. Specifically, 5-Cl/NO₂ benzylamides **1** elicit nanomolar-submicromolar *K_i* values if X is electron-donating or a hydrogen (**1e'–I'**), whereas 5-Cl/NO₂ phenylhydrazides **2** are inactive for any other type of substituent X (**2d'–g'**). What makes 5-OMe, 5-Cl, and 5-NO₂ 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 S₂ 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-NO₂ 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 A₂ 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 (CH₂OH, 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/NO₂ 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/NO₂ 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 hypothesis¹⁹ of a lipophilic L₁ 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/NO₂ 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/NO₂ 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**

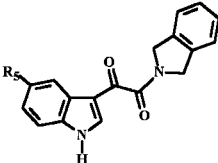
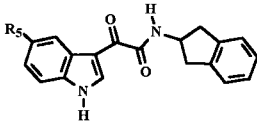
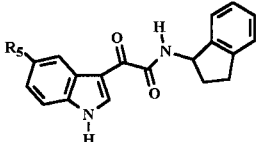
no.	R ₅	X	R'	Config.	[α] _D	Yield, (%)	Recryst. Solvent	m.p., (°C)	Formula ^a
-----	----------------	---	----	---------	------------------	---------------	---------------------	---------------	----------------------

1a	H	4'-NO ₂				73	EtOH	194-196	C ₁₇ H ₁₃ N ₃ O ₄
1b	Cl	4'-NO ₂				88	EtOH	238-240	C ₁₇ H ₁₂ ClN ₃ O ₄
1c	NO ₂	4'-NO ₂				81	DMF/H ₂ O	280-281	C ₁₇ H ₁₂ N ₄ O ₆
1d	OCH ₃	4'-OCH ₃				90	EtOH	204-205	C ₁₉ H ₁₈ N ₂ O ₄
1e	OCH ₃	4'-Cl				89	EtOH	231-233	C ₁₈ H ₁₅ ClN ₂ O ₃

2a	OCH ₃	4'-OCH ₃				65	EtOH	207-209	C ₁₈ H ₁₇ N ₃ O ₄
2b	OCH ₃	4'-NO ₂				63	DMF/H ₂ O	288-290	C ₁₇ H ₁₄ N ₄ O ₅

4a	H	H	CH ₃	R	+48.61	46	EtOH	223-225	C ₁₈ H ₁₆ N ₂ O ₂
4b	H	H	CH ₃	S	-46.67	51	EtOH	244-245	C ₁₈ H ₁₆ N ₂ O ₂
4c	H	4'-CH ₃	CH ₃	R	+64.48	54	MeOH	222-223	C ₁₉ H ₁₈ N ₂ O ₂
4d	H	4'-CH ₃	CH ₃	S	-62.86	47	MeOH	215-218	C ₁₉ H ₁₈ N ₂ O ₂
4e	H	4'-OCH ₃	CH ₃	R,S	-	47	EtOH/H ₂ O	175-177	C ₁₉ H ₁₈ N ₂ O ₃
4f	H	3',4'-(OCH ₃) ₂	CH ₃	R,S	-	46	EtOH/H ₂ O	138-140	C ₂₀ H ₂₀ N ₂ O ₄
4g	H	4'-NO ₂	CH ₃	R	+53.12	82	AcOH glac.	245-247	C ₁₈ H ₁₅ N ₃ O ₄
4h	H	4'-NO ₂	CH ₃	S	-52.37	95	AcOH glac.	245-247	C ₁₈ H ₁₅ N ₃ O ₄
4i	H	H	CH ₂ OH	R,S	-	60	EtOH/H ₂ O	185-187	C ₁₈ H ₁₆ N ₂ O ₃
4j	H	H	CN	R,S	-	44	Benzene	230-231	C ₁₈ H ₁₃ N ₃ O ₂
4k	H	H	CO ₂ C ₂ H ₅	R,S	-	48	Benzene	150-152	C ₂₀ H ₁₈ N ₂ O ₄
4l	Cl	H	CH ₃	R	-2.75	61	EtOH	182-184	C ₁₈ H ₁₅ ClN ₂ O ₂
4m	Cl	H	CH ₃	S	+3.60	71	EtOH	169-171	C ₁₈ H ₁₅ ClN ₂ O ₂
4n	Cl	4'-CH ₃	CH ₃	R	+6.54	49	MeOH	186-187	C ₁₉ H ₁₇ ClN ₂ O ₂
4o	Cl	4'-CH ₃	CH ₃	S	-4.96	52	MeOH/H ₂ O	181-184	C ₁₉ H ₁₇ ClN ₂ O ₂
4p	Cl	4'-OCH ₃	CH ₃	R,S	-	51	EtOH	177-179	C ₁₉ H ₁₇ ClN ₂ O ₃
4q	Cl	3',4'-(OCH ₃) ₂	CH ₃	R,S	-	50	EtOH	150-151	C ₂₀ H ₁₉ ClN ₂ O ₄
4r	Cl	4'-NO ₂	CH ₃	R	-20.00	63	AcOH/H ₂ O	195-197	C ₁₈ H ₁₄ ClN ₃ O ₄
4s	Cl	4'-NO ₂	CH ₃	S	+19.41	60	AcOH/H ₂ O	198-199	C ₁₈ H ₁₄ ClN ₃ O ₄
4t	NO ₂	H	CH ₃	R	-45.00	81	EtOH	233-235	C ₁₈ H ₁₅ N ₃ O ₄
4u	NO ₂	H	CH ₃	S	+45.00	77	EtOH	258-260	C ₁₈ H ₁₅ N ₃ O ₄
4v	NO ₂	4'-CH ₃	CH ₃	R	-44.57	73	EtOH	235-237	C ₁₉ H ₁₇ N ₃ O ₄
4w	NO ₂	4'-CH ₃	CH ₃	S	+46.20	69	EtOH	232-235	C ₁₉ H ₁₇ N ₃ O ₄
4x	NO ₂	4'-OCH ₃	CH ₃	R,S	-	79	EtOH/H ₂ O	234-235	C ₁₉ H ₁₇ N ₃ O ₅
4y	NO ₂	3',4'-(OCH ₃) ₂	CH ₃	R,S	-	75	EtOH	209-211	C ₂₀ H ₁₉ N ₃ O ₆
4z	NO ₂	4'-NO ₂	CH ₃	R	-75.49	76	AcOH glac.	287-289	C ₁₈ H ₁₄ N ₄ O ₆

Table 1. (Continued)

no.	R ₅	X	R'	Config.	[α] _D	Yield, (%)	Recryst. Solvent	m.p., (°C)	Formula ^a
4aa	NO ₂	4'-NO ₂	CH ₃	S	+75.00	73	DMF/H ₂ O	278-280	C ₁₈ H ₁₄ N ₄ O ₆
4bb	NO ₂	H	CH ₂ OH	R,S	-	51	EtOH	199-201	C ₁₈ H ₁₅ N ₃ O ₅
4cc	NO ₂	H	CN	R,S	-	56	EtOH	243-246	C ₁₈ H ₁₂ N ₄ O ₄
4dd	NO ₂	H	CO ₂ C ₂ H ₅	R,S	-	84	EtOH	200-201	C ₂₀ H ₁₇ N ₃ O ₆
									
5a	H					61	EtOH	288-290	C ₁₈ H ₁₄ N ₂ O ₂
5b	Cl					57	DMF	>300	C ₁₈ H ₁₃ ClN ₂ O ₂
5c	NO ₂					52	EtOH	>300	C ₁₈ H ₁₃ N ₃ O ₄
									
6a	H					49	EtOH	222-223	C ₁₉ H ₁₆ N ₂ O ₂
6b	Cl					77	EtOH	254-255	C ₁₉ H ₁₅ ClN ₂ O ₂
6c	NO ₂					54	EtOH	298-300	C ₁₉ H ₁₅ N ₃ O ₄
									
7a	H			R	+108.43	65	EtOH	218-220	C ₁₉ H ₁₆ N ₂ O ₂
7b	Cl			R	+65.94	57	EtOH	229-231	C ₁₉ H ₁₅ ClN ₂ O ₂
7c	NO ₂			R	+37.22	69	EtOH	282-284	C ₁₉ H ₁₅ N ₃ O ₄
8a	H			S	-104.35	61	EtOH	220-222	C ₁₉ H ₁₆ N ₂ O ₂
8b	Cl			S	-67.29	59	EtOH	230-231	C ₁₉ H ₁₅ ClN ₂ O ₂
8c	NO ₂			S	-39.41	72	EtOH	285-287	C ₁₉ H ₁₅ N ₃ O ₄

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

drawing 4'-NO₂ 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'-NO₂ (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/NO₂ counterparts. Unsubstituted indoles tolerate the isoindolinyllamide side chain (**5a** being practically equipotent to **1a'**) but not the (R)-1-indanyllamide moiety (**7a** is 5.6-fold less potent than **1a'**). In contrast, 5-Cl/NO₂ indoles show no potency at the BzR when bearing the isoindolinyllamide side chain, while the (R)-1-indanyllamide residue produces an enhance-

ment 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/NO₂ indoles at the BzR. The binding data of the 1-indanyllamides **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-CH₂) fits into the same lipophilic pocket of the L₁ site hosting the (R)-α-Me group.

Isoindolinyllamides **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-NO₂ derivatives **5b** and **5c**. Actually, the inactivity of these two compounds

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

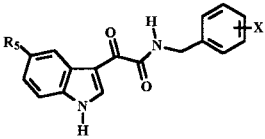
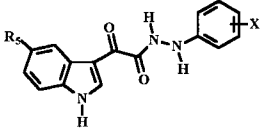
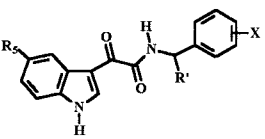
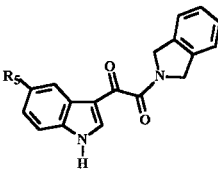
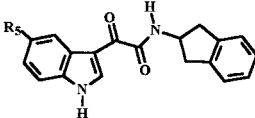
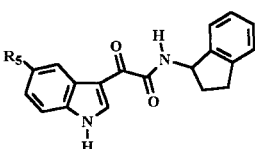
no.	R ₅	X	R'	Config.	K _i ^a (nM)	GABA ratio ^b
						
1a	H	4'-NO ₂			21 ± 2	0.75
1b	Cl	4'-NO ₂			1370 ± 128	0.73
1c	NO ₂	4'-NO ₂			620 ± 49	1.20
1d	OMe	4'-OMe			494 ± 39	1.15
1e	OMe	4'-Cl			ND ^c	
						
2a	OMe	4'-OMe			ND	
2b	OMe	4'-NO ₂			ND	
						
4a	H	H	Me	R	1307 ± 124	1.00
4b	H	H	Me	S	ND	
4c	H	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 ₂ OH	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 ₂ OH	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 [³H]flumazenil binding ≤80%.

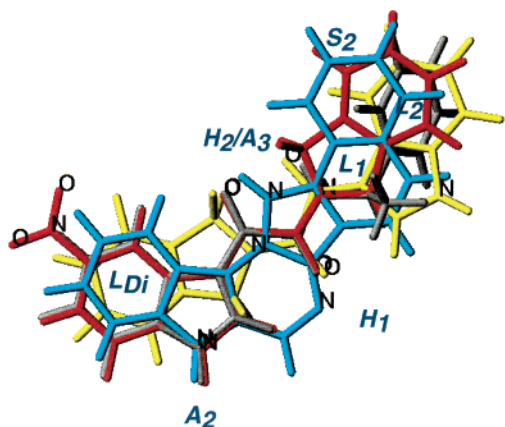
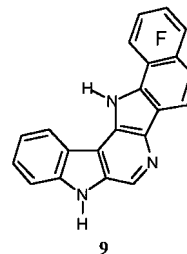


Figure 2. The isoindolylamides **5c** (inactive, in red) and **5a** (active, in yellow) oriented according to mode A and, respectively, mode B are aligned on the (R)-α-Me-benzylamide **4t** (active, in gray) and the benzopyridodiindole **9** (inactive, in cyan). The fused benzene ring F of **9** maps the sterically forbidden S₂ 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 S₂ 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 S₂ 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 isoindolylamides **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 L₂ site without contacting the S₂ site.



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/NO₂ (R)-1-indanylamides **7b** and **7c** are the only ones, among the newly investi-

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

no.	R ₅	X	R'	Config.	K _i (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-NO₂ 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 L₂ 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-CH₂ fragment of the (R)-1-indanylamide **7c**, supposed to enhance affinity through a hydrophobic interaction, is surrounded by a cartoon of the lipophilic pocket L₁. 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

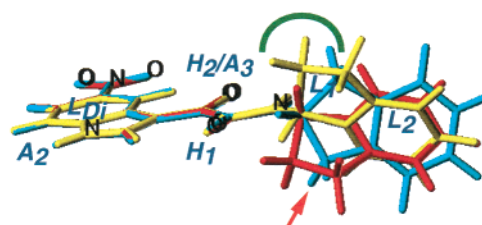


Figure 3. Superposition of the (R)-1-indanylamide **7c** (active, in yellow) on the (S)-1-indanylamide **8c** (inactive, in red) and the 2-indanylamide **6c** (inactive, in cyan), all oriented in accordance with mode A. Sterically unfavorable fragments of the inactive compounds **8c** and **6c** are marked by an arrow. A cartoon of the L₁ lipophilic pocket surrounds the favorable 2-CH₂ 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 [³H]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

no.	K _i (nM) ^b or % inhibition (10 μ M) ^c					
	$\alpha_1\beta_2\gamma_2$	GABA ratio ^d	$\alpha_3\beta_2\gamma_2$	GABA ratio ^d	$\alpha_5\beta_3\gamma_2$	GABA ratio ^d
1a	16 \pm 2	0.90	1600 \pm 110	0.91	58 \pm 6	0.73
4a	1150 \pm 86	0.92	5% \pm 0.4		5500 \pm 360	1.05
4t	14 \pm 2	1.17	283 \pm 19	1.30	239 \pm 21	1.30
5a	224 \pm 20	1.06	3200 \pm 150	1.10	43% \pm 4	
7a	225 \pm 13	1.13	28% \pm 3		2160 \pm 160	1.10
7c	9 \pm 0.6	1.13	1960 \pm 150	1.12	95 \pm 8	0.98
1j'	42 \pm 3	1.11	137 \pm 11	0.96	126 \pm 11	0.90
2c'	25 \pm 3	0.96	40 \pm 4	0.98	43 \pm 5	0.80
zolpidem	50 \pm 3		765 \pm 63		35% \pm 3	

^a The ability of the compounds to displace [³H]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 \pm SEM of three determinations. ^c Percentage inhibition values of specific [³H]flumazenil binding at 10 μ M concentration are means \pm 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 CH₂ 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 [³H]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 L₁ 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 Kofler hot-stage apparatus and are uncorrected. The [α]_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 $\pm 0.4\%$.

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-dihydroisindole.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 NaHCO₃ 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 NaHCO₃ 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, ¹H NMR, and MS spectral data are reported in the Supporting Information.

Binding Studies. [³H]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

[³H]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 described³⁴ in Minimum Essential Medium Eagle with EBSS, supplemented with 10% fetal calf serum, L-glutamine (2 mM), penicillin (100 units/mL), and streptomycin (100 μ g/mL) in a humidified atmosphere of 5% CO₂/95% air at 37 °C. Cells were harvested and then centrifuged at 500 \times g. The crude membranes were prepared after homogenization in 10 mM potassium phosphate, pH 7.4, and differential centrifugation at 48 000 \times 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

[³H]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 μ L which contained [³H]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 [³H]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 SYBYL²⁷ 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 field²⁶ 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 AM1²⁵ 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)C–N–C–C1'Ar = 159°, N–C–C1'Ar–C2'Ar = 60°; **5a** and **5c**: (O=C)C–N–C–CAr = 180°; **6c**: (O=C)N–C2–C1 = 108°; **7c**: (O=C)C–N–C–CAr = 155°; **8c**: (O=C)N–C–CAr = 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

Acknowledgment. This work was supported by grants from the Ministry of University and Scientific and Technological Research (MURST).

Supporting Information Available: Table containing the IR, ¹H 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>.

References

- Huang, Q.; He, X.; Ma, C.; Liu, R.; Yu, S.; Dayer, C. A.; Wenger, G. R.; McKernan, R.; Cook, J. M. Pharmacophore/Receptor Models for GABA_A/BzR Subtypes ($\alpha_1\beta_3\gamma_2$, $\alpha_5\beta_3\gamma_2$, and $\alpha_6\beta_3\gamma_2$) via a Comprehensive Ligand-Mapping Approach. *J. Med. Chem.* **2000**, *43*, 71–95.
- Sieghart, W. Structure and Pharmacology of γ -Aminobutyric Acid_A Receptor Subtypes. *Pharmacological Rev.* **1995**, *47*, 181–234.
- Haefely, W.; Martin, J. R.; Schoch, P. Novel Anxiolytics That Act as Partial Agonists at Benzodiazepine Receptors. *Trends Pharmacol. Sci.* **1990**, *11*, 452–456.
- Gardner, C. R. Interpretation of the Behavioral Effects of Benzodiazepine Receptor Ligands. *Drugs Future* **1989**, *14*, 51–67.
- Haefely, W. E.; Kyburz, E.; Gerecke, M.; Mohler, H. P. Recent Advances in the Molecular Pharmacology of Benzodiazepine Receptors and in the Structure–Activity Relationship of Their Agonists and Antagonists. *Adv. Drug Res.* **1985**, *14*, 166–322.
- Korpi, E. R.; Mattila, M. J.; Wisden, W.; Luddens, H. GABA_A-receptor Subtypes: Clinical Efficacy and Selectivity of Benzodiazepine Site Ligands. *Annals Med.* **1997**, *29*, 275–282.
- Gu, Z. Q.; Wong, G.; Dominguez, C.; de-Costa, B. R.; Rice, K. C.; Skolnick, P. Synthesis and Evaluation of Imidazo[1,5-a][1,4]-benzodiazepine Esters with High Affinities and Selectivities at Diazepam Insensitive (DI) Benzodiazepine Receptors. *J. Med. Chem.* **1993**, *36*, 1001–1006.
- Wong, G.; Koehler, K. F.; Skolnick, P.; Gu, Z. Q.; Ananthan, S.; Schonholzer, P.; Hunkeler, W.; Zhang, W.; Cook, J. M. Synthetic and Computer Assisted Analysis of the Structural Requirements for Selective, High Affinity Ligand Binding to “Diazepam-Insensitive” Benzodiazepine Receptors. *J. Med. Chem.* **1993**, *36*, 1820–1830.
- Liu, R.; Hu, R. J.; Zhang, P.; Skolnick, P.; Cook, J. M. Synthesis and Pharmacological Properties of Novel 8-Substituted Imidazobenzodiazepines: High-Affinity, Selective Probes for α_5 -Containing GABA_A Receptors. *J. Med. Chem.* **1996**, *39*, 1928–1934.
- Arbilla, S.; Depoortere, H.; George, P.; Langer, S. Z. Pharmacological Profile of the Imidazopyridine Zolpidem at Benzodiazepine Receptors and Electroencephalogram in Rats. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1985**, *330*, 248–251.
- Yokoyama, N.; Ritter, B.; Neubert, A. D. 2-Arylpyrazolo[4,3-c]quinolin-3-ones: Novel Agonist, Partial Agonist, and Antagonist of benzodiazepines. *J. Med. Chem.* **1982**, *25*, 337–339.
- Shindo, H.; Takada, S.; Murata, S.; Eigyo, M.; Matsushita, A. Thienylpyrazoloquinolines with High Affinity to Benzodiazepine Receptors: Continuous Shift from Inverse Agonist to Agonist Properties Depending on the Size of the Alkyl Substituent. *J. Med. Chem.* **1989**, *32*, 1213–1217.
- Allen, M. S.; Hagen, T. J.; Trudell, M. L.; Coddling, P. W.; Skolnick, P.; Cook, J. M. Syntheses of Novel 3-substituted β -Carbolines as Benzodiazepine Receptor Ligands: Probing the Benzodiazepine Receptor Pharmacophore. *J. Med. Chem.* **1988**, *31*, 1854–1861.
- Trudell, M. L.; Lifer, S. L.; Tan, Y. C.; Martin, M. J.; Deng, L.; Skolnick, P.; Cook, J. M. Synthesis of Substituted 7,12-Dihydro-pyrido[3,2-b:5,4-b']diindoles: Rigid Planar Benzodiazepine Ligands with Inverse Agonist/Antagonist properties. *J. Med. Chem.* **1990**, *33*, 2412–2420.
- Zhang, W.; Koehler, K. F.; Zhang, P.; Cook, J. M. Development of a Comprehensive Pharmacophore Model for the Benzodiazepine Receptor. *Drug Design Discovery* **1995**, *12*, 193–248.
- Da Settimo, A.; Primofiore, G.; Da Settimo, F.; Marini, A. M.; Novellino, E.; Greco, G.; Martini, C.; Giannaccini, G.; Lucacchini, A. Synthesis, Structure–Activity Relationships, and Molecular Modeling Studies of N–(Indol-3-ylglyoxylyl)benzylamine Derivatives Acting at the Benzodiazepine Receptor. *J. Med. Chem.* **1996**, *39*, 5083–5091.
- Da Settimo, A.; Primofiore, G.; Da Settimo, F.; Marini, A. M.; Novellino, E.; Greco, G.; Gesi, M.; Martini, C.; Giannaccini, G.; Lucacchini, A. N'-Phenylindol-3-ylglyoxylohydrazide Derivatives: Synthesis, Structure–Activity Relationships, Molecular Modeling Studies, and Pharmacological Action on Brain Benzodiazepine Receptors. *J. Med. Chem.* **1998**, *41*, 3821–3830.
- Martini, C.; Gervasio, T.; Lucacchini, A.; Da Settimo, A.; Primofiore, G.; Marini, A. M. Specific Inhibition of Benzodiazepine Receptor Binding by Some N–(Indol-3-ylglyoxylyl)amino Acid Derivatives. *J. Med. Chem.* **1985**, *28*, 506–509.
- Primofiore, G.; Marini, A. M.; Da Settimo, F.; Martini, C.; Bardellini, A.; Giannaccini, G.; Lucacchini, A. Specific Inhibition of Benzodiazepine Receptor Binding by Some N–(Indol-3-ylglyoxylyl)amino Acid Derivatives: Stereoselective Interactions. *J. Med. Chem.* **1989**, *32*, 2514–2518.
- Da Settimo, A.; Primofiore, G.; Marini, A. M.; Ferrarini, P. L.; Franzone, J. S.; Cirillo, L.; Reboani, M. C. N–(indol-3-ylglyoxylyl)-methionine Derivatives: Preparation and Gastric Anti-Secretory Activity. *Eur. J. Med. Chem.* **1988**, *23*, 21–24.
- Giannaccini, G.; Giacomelli, M.; Martini, C.; Lucacchini, A.; Piccolino, M. Binding of the Benzodiazepine Ligand [³H]Ro 15-1788 to Membrane Preparations of the Rabbit and Turtle Retina. *Comp. Biochem. Physiol.* **1992**, *101C*, 337–342.

- (22) Braestrup, C.; Nielsen, M.; Honoré, T.; Jensen, L. H.; Petersen, E. M. Benzodiazepine Receptor Ligands with Positive and Negative Efficacy. *Neuropharmacology* **1983**, *22*, 1451–1457.
- (23) Braestrup, C.; Nielsen, M. Benzodiazepine Receptors. In *Handbook of Psychopharmacology*; Iversen, L. L., Iversen, S. D., Snyder, S. H., Eds.; Plenum Press: New York, 1983; Vol. 17, pp 285–384.
- (24) Fryer, R. I.; Rios, R.; Zhang, P.; Gu, Z. Q.; Wong, G.; Basile, A. S. Skolnick, P. Structure Activity Relationships of 2-Phenylpyrazolo[4,3-c]quinolin-3-ones and their N- and O-Methyl Analogues at Benzodiazepine Receptors. *Med. Chem. Res.* **1993**, *3*, 122–130.
- (25) Dewar, M. J.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. AM1: A New General Purpose Quantum Mechanical Molecular Model. *J. Am. Chem. Soc.* **1985**, *107*, 3902–3909.
- (26) Clark, M.; Cramer, R. D., III.; van Opdenbosch, N. Validation of the General Purpose Tripos 5.2 Force Field. *J. Comput. Chem.* **1989**, *10*, 982–1012.
- (27) SYBYL Molecular Modeling System, version 6.5; Tripos Inc.: St. Louis, MO.
- (28) Martin, M. J.; Trudell, M. L.; Diaz-Araujo, H.; Allen, M. S.; Deng, L.; Schultz, C. A.; Tan, Y.-C.; Bi, Y.; Narayanan, K.; Dorn, L. J.; Koheler, K. F.; Skolnick, P.; Cook, J. M. Molecular Yardsticks: Rigid Probes to Define the Spatial Dimensions of the Benzodiazepine Receptor Binding Site. *J. Med. Chem.* **1992**, *35*, 4105–4117.
- (29) Bianucci, A. M.; Da Settimo, A.; Da Settimo, F.; Primofiore, G.; Martini, C.; Giannaccini, G.; Lucacchini, A. Benzodiazepine Receptor Affinity and Interaction of Some N-(Indol-3-ylglyoxyl-)amine Derivatives. *J. Med. Chem.* **1992**, *35*, 2214–2220.
- (30) Kametani, T.; Fukumoto, K. Cularine and Related Compounds. Part VI. A Total Synthesis of (±)-Cularine. *J. Chem. Soc.* **1963**, 4289–4296.
- (31) Bornstein, J.; Lashua, S. C.; Boisselle, A. P. Facile Synthesis of Dihydroisindole. *J. Org. Chem.* **1957**, *22*, 1255–1256.
- (32) Martini, C.; Lucacchini, A.; Ronca, G.; Hrelia, S.; Rossi, C. A. Isolation of Putative Benzodiazepine Receptors From Rat Brain Membranes by Affinity Chromatography. *J. Neurochem.* **1982**, *38*, 15–19.
- (33) Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein Measurement With the Folin Phenol Reagent. *J. Biol. Chem.* **1951**, *193*, 265–275.
- (34) Besnard, F.; Even, Y.; Itier, V.; Granger, P.; Partiseti, M.; Avenet, P.; Depoortere, H.; Graham, D. Development of Stable Cell Lines Expressing Different Subtypes of GABA_A Receptors. *J. Rec. Sign. Transd. Res.* **1997**, *17*, 99–113.
- (35) Gasteiger, J.; Marsili, M. Iterative Partial Equalization of Orbital Electronegativity. *Tetrahedron* **1980**, *36*, 3219–3228.
- (36) Purcel, V. P.; Singer, J. A. A Brief Review of Table and Semiempirical Parameters Used in the Hückel Molecular Orbital Method. *J. Chem. Eng. Data* **1967**, *12*, 235–246.
- (37) Head, J.; Zerner, M. C. A Broyden-Fletcher-Goldfarb-Shanno Optimization Procedure for Molecular Geometries. *Chem. Phys. Lett.* **1985**, *122*, 264–274.
- (38) Boström, J.; Norrby, P.-O.; Liljefors, T. Conformational Energy Penalties of Protein-Bound Ligands. *J. Comput.-Aided Mol. Design* **1998**, *12*, 383–396.
- (39) MOPAC, version 6.0; Quantum Chemistry Program Exchange. JM010827J