N′-Phenylindol-3-ylglyoxylohydrazide Derivatives: Synthesis, Structure−Activity Relationships, Molecular Modeling Studies, and Pharmacological Action on Brain Benzodiazepine Receptors

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A series of *N′*-phenylindol-3-ylglyoxylohydrazides, isosters of the *N*-benzylindol-3-ylglyoxylamide derivatives previously described by us, were synthesized and tested for their ability to displace [^{3}H]Ro 15-1788 from bovine brain membranes. These compounds were designed with the aim of obtaining products which could exert an in vivo activity, thanks to a higher hydrosolubility and consequently a better bioavailability. Affinity was restricted to the derivatives unsubstituted in the 5 position of the indole nucleus (**1**, **6**, **9**, **12**, **15**, **18**, **23**, and **26**), with K_i values ranging from 510 to 11 nM. The most active compounds (**6**, **9**, **23**, and **29**) proved to be effective in antagonizing pentylenetetrazole-induced seizures. Molecular modeling studies were performed to rationalize the lack of affinity of hydrazides with a chloro or a nitro group in the 5 position of the indole nucleus. It was hypothesized that the conformational preference of the hydrazide side chain, characterized by a gauche disposition of lone pairs and substituents about the N−N bond, prevents all hydrazides from binding to the receptor similarly to other classes of indole analogues previously investigated. The potency of 5-H hydrazides was attributed to a binding mode which is not feasible for 5-Cl and 5-NO_2 counterparts. This theoretical model of ligand−receptor interaction permitted a more stringent interpretation of structure−affinity relationships of hydrazides and of recently described benzylamide derivatives (Da Settimo et al. *J. Med. Chem.* **1996**, *39*, 5083−5091).

Introduction

γ-Aminobutyric acid (GABA), one of the major inhibitory neurotransmitters in the brain, controls the excitability of many central nervous system (CNS) pathways by interacting with the $GABA_A$ chloride ion channel complex. This receptor is structurally constituted as macromolecular assemblies. The interaction of GABA with its receptor opens the ion channel so that the chloride influx is enhanced, the membrane is hyperpolarized, and the cell becomes less responsive to excitatory stimuli. This GABA-induced ion current can be allosterically regulated by diverse agents, which have specific binding sites on this macromolecular complex.[1,2] Of these, compounds which mediate their actions at the benzodiazepine receptor (BzR) are the most widely studied.[3−5] Benzodiazepines and several substances with a chemical structure different from that of classic 1,4-benzodiazepines (Bz) bind with a high affinity at the BzR and exhibit a wide variety of pharmacological actions spanning the entire efficacy spectrum.[6−14] Full agonists potentiate the GABA-induced chloride influx (positive modulation) decreasing the excitability of the neuron and have found widespread use in the treatment of anxiety and sleep disorders. Inverse agonists decrease the chloride ion influx (negative modulation) and produce proconvulsant, anti-inebriant, and anxiogenic effects. Antagonists, which have minimal or no effects on the chloride flux, have neutral efficacy and block the effects of both agonists and inverse agonists by competitive inhibition.[15,16] Partial agonists exist within this efficacy continuum and are of particular interest as they may display antianxiety properties devoid of the undesirable side effects typical of full agonist-type ligands, such as physical dependence, amnesia, oversedation, muscle relaxation, or ethanol potentiation, due to their lower intrinsic efficacy.[17,18] Moreover, molecular biology studies have confirmed the existence of several receptor subtypes, deriving from the combination of different subunits. Presently, a total of 16 subunits (6α, 4β, 3γ, 1δ, 2ρ) of the $GABA_A$/Bz receptor have been cloned and sequenced. Whereas the stoichiometric composition of the naturally existing GABA receptor is still unknown, recombinant $GABA_A$ receptors composed from $\alpha_x\beta_2\gamma_2$ (x = 1−6) most closely resemble the biochemical, electrophysiological, and pharmacological properties of those native receptors.[2] The development of subtype-selective ligands, which may show only anxiolytic or hypnotic activity and overt side effects, is one of the more

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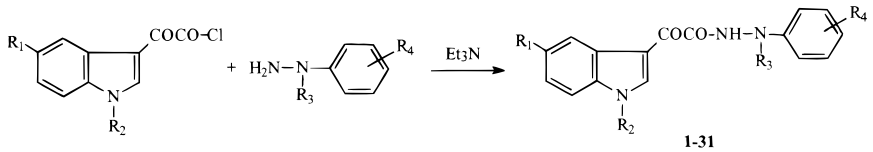
Scheme 1



R_1 = H, Cl, NO_2
R_2, R_3 = H, CH_3
R_4 = p-F, p-Cl, m- or p-CH_3, p-OCH_3, p-OH, p-NO_2

attractive consequences of these findings and constitutes a major advance in this field.

In the past few years, we have prepared several *N*-(arylalkyl)indolylglyoxylamide derivatives and reported their affinity data at the BzR and their activity profiles.[19,20] The affinity of these products depends directly on the distance between the side phenyl ring and the amide bond. In general, the most active compounds are those containing a benzylamine residue in the side chain, with K_i values in the nanomolar range. The phenylethylamide derivatives have an interesting pharmacological profile, spanning a continuum from inverse agonists to antagonists and partial agonists, depending on the nature of the substituents on the aryl group.[19] Similarly, the benzylamide derivatives show a varying in vitro efficacy profile, but they are devoid of any in vivo activity, probably because of an unfavorable pharmacokinetics.[20]

The good affinity of these compounds prompted us to search for new derivatives which could maintain a high affinity at the BzR and could reach the CNS, to exert, if possible, a pharmacological action. In the present paper, we report the synthesis, the affinity data, and the pharmacological profile of a number of indolylglyoxylohydrazide derivatives **1**−**31**. In this series of compounds, the methylene spacer of *N*-benzylindolylglyoxylamides[20] was substituted by an isoster NH group, which was intended to maintain a distance between the phenyl ring and the amide bond suitable for high affinity. Furthermore, since the lack of in vivo efficacy of benzylamide derivatives may be the result of poor absorption and bioavailability, the replacement of a CH_2 with an NH could circumvent this problem by increasing the water solubility of these indolylglyoxylohydrazide derivatives. The interaction of these hydrazide derivatives with the receptor site was also considered in the light of molecular modeling studies, which allowed us to better clarify the structure−activity relationships and the interaction mode of the previously described benzylamide derivatives.

Chemistry

The synthesis of the *N*′-phenyl(5-substituted indol-3-yl)glyoxylohydrazide derivatives **1**−**20** and **23**−**26** was carried out by adding a suspension of the appropriate glyoxylyl chloride[21] in anhydrous ethyl ether to a suspension of the appropriately substituted hydrazine hydrochloride in the same solvent, in the presence of triethylamine (Scheme 1). The *N*′-methyl-*N*′-phenyl(5-substituted indol-3-yl)glyoxylohydrazide derivatives **27**−**31** were prepared by adding a benzene solution of 1-methyl-1-phenylhydrazine to a suspension of the appropriate indolylglyoxylyl chloride and triethylamine in the same solvent (Scheme 1). The *p*-hydroxy compounds **21** and **22** were obtained by demethylation of the *p*-methoxy derivatives **18** and **19** with boron tribromide in anhydrous dichloromethane.[22] All products were purified by recrystallization from the appropriate solvent, after filtration, when necessary, through a silica gel column, and their structures were confirmed by IR, ^{1}H NMR, MS, and elemental analysis (Table 1). Spectral data of all newly synthesized compounds (**1**−**31**) are reported in the Supporting Information.

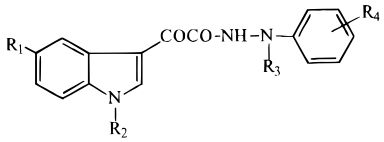
(1-Methyl-5-nitroindol-3-yl)glyoxylyl chloride, never previously described in the literature, was obtained by a method analogous to the one reported for the synthesis of (5-substituted indol-3-yl)glyoxylyl chlorides.[21] Its structure was confirmed by conversion into its methyl ester derivative (see Experimental Section).

Results

The binding affinity of all the hydrazide derivatives **1**−**31** at the BzR in bovine brain membranes was determined by competition experiments against the radiolabeled antagonist [^{3}H]Ro 15-1788.[23] The in vitro efficacy of the most active compounds was measured by two different methods: the GABA ratio, which, according to different authors, generally predicts the expected pharmacological properties of a BzR ligand,[24−26] and the [^{35}S]TBPS binding shift,[27−29] as the usefulness of the GABA ratio as a predictor of efficacy has sometimes been questioned.[30,31] The results of the in vitro assays are shown in Table 2.

In all the *N*′-phenylindolylglyoxylohydrazide series, with the exclusion of the *N*′-methyl-*N*′-phenylhydrazides **27**−**31** and the 1-methylindole derivatives **4**, **5**, and **26**, the most potent derivatives were those unsubstituted in the 5 position of the indole nucleus (compounds **1**, **6**, **9**, **12**, **15**, **18**, and **23**, with K_i values ranging from 510 to 11 nM, excluding **21** which showed an exceptionally weak affinity), whereas the 5-Cl- and 5-NO_2-substituted derivatives were inactive. The 1-unsubstituted *N*′-methyl-*N*′-phenylhydrazides (compounds **27**−**29**) showed weak affinity for the BzR. However, in this series, the highest affinity was shown by the compounds bearing a nitro group in the 5 position of the indole nucleus (**29**, K_i = 2400 nM), unlike the above-reported series and in agreement with other classes of indole derivatives acting via the BzR.[19,20] Regarding the affinity caused by the electronic features of the substituent on the side phenyl ring, an electron-withdrawing group in the para position, such as fluoro, chloro, or nitro, gave the most potent compounds (**6**, K_i = 57 nM; **9**, K_i = 62 nM; **23**, K_i = 11 nM), while the presence of an electron-donating group weakened the affinity (**12**, K_i = 290 nM; **18**, K_i = 430 nM; **21**, K_i = 3400 nM; compared with **1**, K_i = 203 nM). The shift of the substituent from the para to the meta position on the side phenyl ring led to a weaker

Table 1. Physical Properties of Hydrazide Derivatives **1**−**31**



no.	R_1	R_2	R_3	R_4	yield, %	recryst solvent	mp, °C	formula[a]
1	H	H	H	H	35	MeOH	217−219	$C_{16}H_{13}N_3O_2$
2	Cl	H	H	H	42	MeOH/H_2O	234−236	$C_{16}H_{12}ClN_3O_2$
3	NO_2	H	H	H	35	MeOH	235−237	$C_{16}H_{12}N_4O_4$
4	H	CH_3	H	H	38	benzene	233−235	$C_{17}H_{15}N_3O_2$
5	NO_2	CH_3	H	H	39	benzene	241−243	$C_{17}H_{14}N_4O_4$
6	H	H	H	4′-F	46	benzene	213−215	$C_{16}H_{12}FN_3O_2$
7	Cl	H	H	4′-F	42	benzene	225−227	$C_{16}H_{11}FClN_3O_2$
8	NO_2	H	H	4′-F	76	ethanol	235−237	$C_{16}H_{11}FN_4O_4$
9	H	H	H	4′-Cl	36	MeOH	239−240	$C_{16}H_{12}ClN_3O_2$
10	Cl	H	H	4′-Cl	46	MeOH/H_2O	232−233	$C_{16}H_{11}Cl_2N_3O_2$
11	NO_2	H	H	4′-Cl	31	MeOH	254−255	$C_{16}H_{11}ClN_4O_4$
12	H	H	H	4′-CH_3	26	benzene	172−175	$C_{17}H_{15}N_3O_2$
13	Cl	H	H	4′-CH_3	26	benzene	212−213	$C_{17}H_{14}ClN_3O_2$
14	NO_2	H	H	4′-CH_3	33	MeOH	242−243	$C_{17}H_{14}N_4O_4$
15	H	H	H	3′-CH_3	32	benzene	205−206	$C_{17}H_{15}N_3O_2$
16	Cl	H	H	3′-CH_3	38	benzene	210−211	$C_{17}H_{14}ClN_3O_2$
17	NO_2	H	H	3′-CH_3	30	MeOH	236−238	$C_{17}H_{14}N_4O_4$
18	H	H	H	4′-OCH_3	32	benzene	183−184	$C_{17}H_{15}N_3O_3$
19	Cl	H	H	4′-OCH_3	30	benzene	183−185	$C_{17}H_{14}ClN_3O_3$
20	NO_2	H	H	4′-OCH_3	33	AcOEt	233−234	$C_{17}H_{14}N_4O_5$
21	H	H	H	4′-OH	69	AcOEt	245−247d	$C_{16}H_{13}N_3O_3$
22	Cl	H	H	4′-OH	72	AcOEt	252−254	$C_{16}H_{13}ClN_3O_3$
23	H	H	H	4′-NO_2	31	MeOH	280−281	$C_{16}H_{12}N_4O_4$
24	Cl	H	H	4′-NO_2	32	MeOH	270−271	$C_{16}H_{11}ClN_4O_4$
25	NO_2	H	H	4′-NO_2	88	DMF/H_2O	301−303	$C_{16}H_{11}N_5O_6$
26	H	CH_3	H	4′-NO_2	94	DMF	269−271	$C_{17}H_{14}N_4O_4$
27	H	H	CH_3	H	68	benzene	200	$C_{17}H_{15}N_3O_2$
28	Cl	H	CH_3	H	42	benzene	224−225	$C_{17}H_{14}ClN_3O_2$
29	NO_2	H	CH_3	H	56	benzene	229−230	$C_{17}H_{14}N_4O_5$
30	H	CH_3	CH_3	H	65	benzene	141−143	$C_{18}H_{17}N_3O_2$
31	NO_2	CH_3	CH_3	H	72	ethanol	214−215	$C_{18}H_{16}N_4O_4$

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

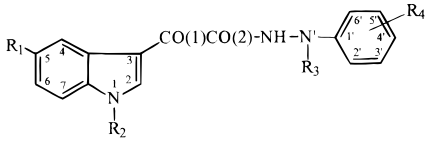
affinity (**12**, K_i = 290 nM; compared with **15**, K_i = 510 nM). Substitution of the indole NH with a methyl group caused a dramatic decrease in affinity for products **4** and **26** (compared with **1** and **23**, respectively). Methylation of both the indole and the aniline NH gave compounds **30** and **31**, which did not show any affinity for the BzR.

Using an exhaustively washed membrane preparation, the GABA ratio values of the most active compounds were evaluated. All the products tested, with the exclusion of **29**, showed values lower than, or close to, unity, predicting partial inverse agonist or antagonist properties. For the *N′*-methyl-*N′*-phenylhydrazide derivative **29**, the GABA ratio value higher than unity predicted a partial agonist profile.

The in vitro efficacy profile of compounds **1**, **6**, **9**, **12**, **15**, **18**, **23**, and **29** was also assayed by means of the [^{35}S]TBPS shift test.[32,33] BzR agonists enhance the [^{35}S]-TBPS binding shift, while antagonists have no effect, and inverse agonists reduce it.[27−29] The values obtained, reported in Table 2, confirmed the efficacy profile predicted by the GABA ratio values: the value of 53, which is intermediate between that of the agonist clonazepam (fixed here at a value of 100) and 15 observed for the antagonist Ro 15-1788, indicated partial agonist properties for compound **29**; and the values ranging between 9 and 20, almost equal to that of Ro 15-1788, or at least within the range of experimental error, predicted an antagonist profile for the remaining compounds.

The pharmacological profile of compounds **6**, **9**, **23**, and **29** was also checked by in vivo tests for anticonvulsant, proconvulsant, and diazepam antagonism action, essentially carried out as previously described.[19] The data reported in Table 3, showed for all the compounds, an anticonvulsant activity and a complete lack of diazepam antagonism or of proconvulsant action, even at the highest dose tested of 250 mg/kg. However, the moderately high ED_{50} values indicated a pharmacological profile of partial agonists for these compounds,[33] the highest activity being shown by **23** (ED_{50} = 10 mg/kg), which was also the most potent compound in vitro (K_i = 11 nM). The ED_{50} value of **29** was quite surprising, considering its weak in vitro affinity (K_i = 2400 nM): it antagonized the convulsant action of PTZ with an ED_{50} of 25 mg/kg, equal to that of the most active in vitro compound **9** (K_i = 62 nM). For compound **29**, the in vivo activity was consistent with the in vitro result, as the GABA ratio = 1.3 and the TBPS shift = 53 predicted an in vitro partial agonist efficacy. On the contrary, the in vivo results did not parallel the in vitro tests for compounds **6**, **9**, and **23**, as their GABA ratio values between 0.88 and 0.98 and the TBPS binding shift values between 16 and 20 indicated an in vitro antagonist efficacy. The lack of agreement between the GABA ratio and the [^{35}S]TBPS shift and the PTZ data may be due to metabolism factors, as the PTZ antagonism assay is an in vivo assay, and/or to differential binding to receptor subtypes.

Table 2. Inhibition of [^{3}H]Ro 15-1788 Specific Binding from Bovine Brain Membranes by Hydrazide Derivatives **1**−**31**



no.	R_1	R_2	R_3	R_4	K_i,[a] nM	GABA ratio[b]	[^{35}S]TBPS binding with GABA,[c] % clonazepam
1	H	H	H	H	203 ± 21	0.88	20 ± 5
2	Cl	H	H	H	ND[d]		
3	NO_2	H	H	H	ND		
4	H	CH_3	H	H	1200 ± 100	0.70	
5	NO_2	CH_3	H	H	ND		
6	H	H	H	4′-F	57 ± 2	0.98	18 ± 2
7	Cl	H	H	4′-F	ND		
8	NO_2	H	H	4′-F	ND		
9	H	H	H	4′-Cl	62 ± 5	0.88	16 ± 6
10	Cl	H	H	4′-Cl	ND		
11	NO_2	H	H	4′-Cl	ND		
12	H	H	H	4′-CH_3	290 ± 23	0.72	9 ± 5
13	Cl	H	H	4′-CH_3	ND		
14	NO_2	H	H	4′-CH_3	ND		
15	H	H	H	3′-CH_3	510 ± 40	1.00	20 ± 7
16	Cl	H	H	3′-CH_3	ND		
17	NO_2	H	H	3′-CH_3	ND		
18	H	H	H	4′-OCH_3	430 ± 39	0.84	17 ± 5
19	Cl	H	H	4′-OCH_3	ND		
20	NO_2	H	H	4′-OCH_3	ND		
21	H	H	H	4′-OH	3400 ± 280	1.03	
22	Cl	H	H	4′-OH	ND		
23	H	H	H	4′-NO_2	11 ± 3	0.90	20 ± 7
24	Cl	H	H	4′-NO_2	ND		
25	NO_2	H	H	4′-NO_2	ND		
26	H	CH_3	H	4′-NO_2	455 ± 20	0.87	
27	H	H	CH_3	H	ND		
28	Cl	H	CH_3	H	ND		
29	NO_2	H	CH_3	H	2400 ± 230	1.30	53 ± 10
30	H	CH_3	CH_3	H	ND		
31	NO_2	CH_3	CH_3	H	ND		
Ro 15-1788					0.90 ± 0.05	0.90	15 ± 5
clonazepam					0.85 ± 0.02	1.97	100 ± 10

[a] K_i values are means ± SEM of three determinations. [b] GABA ratio = (K_i without GABA)/(K_i with GABA). [c] The effects of the compounds at 0.5 μM on TBPS binding were normalized with respect to the corresponding action of clonazepam. The data represent the mean ± standard error of three separate experiments. [d] Not determined.

Table 3. Biological Activity of Selected Hydrazide Derivatives

no.	anticonvulsant action: ED_{50}, mg/kg[a]	proconvulsant action[b]	diazepam antagonism[c]
6	60[d]	no effect[e]	no effect[e]
9	25[d]	no effect[e]	no effect[e]
23	10[d]	no effect[e]	no effect[e]
29	25[d]	no effect[e]	no effect[e]
diazepam	0.4[d]		
Ro 15-1788	no effect [f]	no effect [f]	0.3[d]
β-CCM	no effect [f]	19.4[d]	0.02[d]

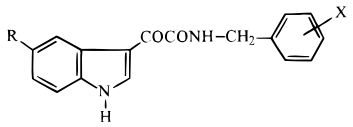
[a] Dose necessary to antagonize the convulsant action of PTZ (80 mg/kg, sc) in 50% of mice. [b] Dose necessary to induce convulsions in 50% of the mice that had previously been given a subconvulsant dose of PTZ (40 mg/kg, sc). [c] Dose necessary to antagonize the anticonvulsant effect of diazepam (2.5 mg/kg, ip) in mice that had been given a convulsant dose of PTZ (80 mg/kg, sc). [d] Values represent the mean of at least three determinations (≤20% differences between experiments). [e] The highest concentration of the compounds tested was 250 mg/kg. [f] The highest dose administered of Ro 15-1788 and β-CCM was 100 and 30 mg/kg, respectively.

Discussion

(A) SARs of Hydrazides and Recently Described Benzylamide Isosters. The inactivity of hydrazides substituted in the 5 position of the indole nucleus was unexpected, considering that a chlorine or nitro group in this position generally increased the potency in other classes of indole derivatives binding to the BzR.[19,20,34−36] In a recently reported series of *N*-benzylindol-3-ylglyoxylamides,[20] the effect of the 5-substituent on affinity depended directly on the type of substituent on the phenyl ring (a subset of these structures and binding data are listed in Table 4). Particularly, 5-Cl and 5-NO_2 compounds were associated with submicromolar−nanomolar affinity, provided that hydroxy/methoxy groups were also present on the phenyl (**5′**, **6′**, **8′**, and **9′**). Replacement of these electron-donating substituents with halogens abolished the potency of 5-Cl and 5-NO_2 derivatives (**11′**, **12′**, **14′**, and **15′**). Surprisingly, the binding of 5-H derivatives was much less sensitive to the nature of the substituents on the phenyl ring, since the affinity could be retained (**4′**) or enhanced (**7′**, **10′**, and **13′**) but was never lost.

In an attempt to rationalize some unclear aspects of the SARs outlined above, we resorted to molecular modeling approaches. The computational work was carried out by AM1 semiempirical calculations,[37] molecular superimposition, and graphics routines available within the SYBYL program.[38] The Cambridge Structural Database[39] was searched to investigate the conformational preference of hydrazides and benzylamides.

Table 4. In Vitro Data of *N*-Benzylindol-3-ylglyoxylamide Derivatives[a]



no.	R	X	K_i, nM
1′	H	H	120 ± 11
2′	Cl	H	490 ± 39
3′	NO_2	H	117 ± 12
4′	H	4′-OCH_3	163 ± 12
5′	Cl	4′-OCH_3	107 ± 10
6′	NO_2	4′-OCH_3	53 ± 5.3
7′	H	3′,4′-$(OCH_3)_2$	94 ± 10
8′	Cl	3′,4′-$(OCH_3)_2$	30 ± 5
9′	NO_2	3′,4′-$(OCH_3)_2$	11 ± 3
10′	H	Cl	67 ± 5
11′	Cl	Cl	ND[b]
12′	NO_2	Cl	ND
13′	H	F	52 ± 6
14′	Cl	F	ND
15′	NO_2	F	ND

[a] Data taken from ref 20. [b] Not determined.

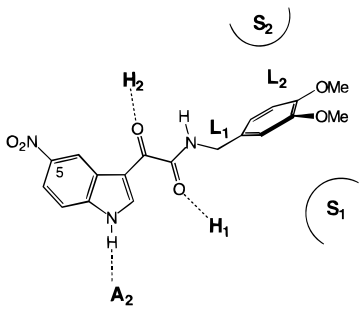


Figure 1. Binding mode "A" hypothesized for *N*-benzylindol-3-ylglyoxylamide derivatives[20] bearing electron-donating substituents on the phenyl ring. Labels refer to BzR subsites in accordance with Cook's topological model.[4]

Details of these procedures are given in the Experimental Section, whereas results are summarized below.

The pharmacophore scheme proposed by Cook et al.[4] for ligands of the BzR guided the modeling of hydrazides **1**−**31** (Table 2) and their isosteric benzylamide counterparts **1′**−**15′** (Table 4). Figure 1 illustrates the binding mode postulated in a previous work[20] for the most potent benzylamide derivative, **9′**. This compound adopts an overall coplanar geometry around the indolylglyoxylamide moiety, so as to engage interactions with the hydrogen bond acceptor site A_2 (through the indole NH hydrogen), the hydrogen bond donor sites H_1 and H_2 (through the C=O(2) and C=O(1) oxygens), and the L_1 and L_2 lipophilic regions (involving CH_2 and 3′,4′-dimethoxyphenyl). According to an earlier hypothesis,[35] an electron-withdrawing group in the 5 position of the indole nucleus, such as Cl or NO_2, facilitates the hydrogen bond between the indole NH and the protein heteroatom A_2. Consistently with the SARs of 5-Cl and 5-NO_2 derivatives, it is plausible that the 3′,4′-dimethoxyphenyl ring of **9′** interacts favorably with an electropositive function within the L_2 site. This latter interaction contributes significantly to the free binding energy, since the insertion of a halogen on the phenyl ring, as in compounds **11′**, **12′**, **14′**, and **15′**, destroys the affinity by decreasing the electron density above and below the plane of the aromatic π-system. Finally, none of our ligands fill the L_3 lipophilic pocket (not shown in Figure 1). Occupancy of this receptor site is not mandatory for the expression of nanomolar potency.[4]

Table 5. Values (deg) of Torsion Angles (O=)C−N−N−C1′ and N−N−C1′−X2′ [a] in Crystal Structures Featuring the CONHNHAr Moiety

ref code[b]	(O=)C−N−N−C1′	N−N−C1′−X2′
CLETHZ	67.8	21.4
DPCBAZ[c]	−89.5	−11.3
	95.8	−18.6
DPCBHZ	−98.6	10.0
FOWRAK	−123.4	27.9
LALYIG	122.8	23.6
VIZSIG	−94.4	15.6

[a] X was defined as any of C, N, O, S atoms. [b] Reference code of the entry stored in the Cambridge Structural Database (version 5.13). [c] Two molecules per cell.

(B) Conformational Preferences of Hydrazides and Benzylamides. It is conceivable that differences in the properties of the NHNHAr and $NHCH_2Ar$ side chains prevent 5-substituted hydrazides from binding to the receptor similarly to active 5-substituted benzylamides. In this respect, the conformational profiles of the two isosteric classes might play an important role. Several authors have provided evidence that repulsion between the vicinal lone pairs on two directly bound nitrogens stabilizes to a large extent conformations with N-substituents and N-lone pairs gauche to one another.[40] This conformational preference was confirmed by a substructure search in the Cambridge Structural Database (CSD).[39] Using CONHNHAr as a query, we identified six hits characterized by absolute values of the torsion angle (O=)C−N−N−C1′ between 67.8° and 123.4° (see data in Table 5), all implying an orientation of NNHAr out of the plane of CONHN. The NNHAr system appears to be quasi-coplanar, as the absolute values of the torsion angle N−N−C1′−X2′ fall between 10.0° and 27.9° (X was defined as any of the C, N, O, or S atoms attached to C1′ via an aromatic bond).

Compared with hydrazides, a wider conformational space is available for benzylamides through rotation about NH−CH_2 and CH_2−Ar bonds. A search in the CSD, using $CONHCH_2Ar$ as a query, yielded the crystal structures listed in Table 6. Most of the benzylamides retrieved (31 out of 36 hits) display a gauche arrangement about the torsion angle (O=)C−N−C−C1′ (absolute values between 69.3° and 129.5°). In five benzylamides, the same torsion angles reflect an anti conformation (absolute values between 151.3° and 176.2°). Finally, the values of the torsion angle N−C−C1′−X2′ span the full range of ±90°. The conformational differences between hydrazides and benzylamides are summarized in the Newman projections drawn in Figure 2.

In conclusion, we postulate that active 5-Cl and 5-NO_2 benzylamides assume a transoid active conformation about the C−N−C−C1′ torsion angle which is forbidden to inactive 5-Cl and 5-NO_2 hydrazides, forced into a gauche disposition about the C−N−N−C1′ torsion angle. This gauche disposition about the N−N bond might rather force the aryl group toward a sterically hindered region of the receptor, thus severely compromising the binding.

(C) Theoretical Model Accounting for the Effects of the 5-Substituent within the Hydrazide Series. While the inactivity of 5-Cl and 5-NO_2 hy-

Table 6. Values (deg) of Torsion Angles (O=)C−N−C−C1′ and N−C−C1′−X2′ [a] in Crystal Structures Featuring the $CONHCH_2Ar$ Moiety

ref code[b]	(O=)C−N−C−C1′	N−C−C1′−X2′
COYHUT10[c]	−93.6	−29.6
	−89.6	8.6
DOJPUN[c]	−73.2	−34.6
	96.8	30.8
FAMFER[c]	−95.5	80.6
	107.4	27.9
FATXUT	106.7	−34.5
FOWPUC	117.6	−63.4
FUPFUR[c]	−107.0	30.2
	113.9	89.9
GEHBUQ	151.3	−31.6
GENYED	−127.1	49.4
HEJCEE	−81.3	−81.0
HEMWUR	−113.2	32.7
HEMXAY	−106.9	−43.4
HEMXEC	−74.5	−58.5
HEMXIG	−122.6	−28.3
JEPHIV	−118.5	16.8
JOXXAV	164.8	63.2
KUBVIM	77.2	33.4
NIAMID	99.1	−1.8
PAGTIA	101.0	−3.9
PAGTOG	−69.3	−32.4
POLPIP	−129.5	47.0
SEYCUU	83.1	54.9
SUGZUP	169.6	67.5
TAXMIO	−82.3	−45.2
TEDTUR	−117.7	43.2
VUFZOL	−79.1	−74.1
WELWAL[c]	101.5	−87.9
	104.7	−16.8
YEFHUM[c]	−90.7	29.0
	127.2	−76.8
YEGWOW	93.2	−49.7
YIRXAY	−74.1	−35.5
YOVFIY[c]	176.2	68.2
	−95.3	−16.7
YULXIM	92.6	−85.1
ZAPYEU	−94.9	31.2
ZILHUX[c]	−97.8	87.2
	113.2	−9.9
ZIPBIJ[c]	−168.5	71.0
	−80.8	−17.3
ZOCRUE	−72.2	−10.5
ZUHMEU	−72.4	−33.9

[a] X was defined as any of the C, N, O, S atoms. [b] Reference code of the entry stored in the Cambridge Structural Database (version 5.13); entries associated with an anti conformation about (O=)C−N−C−C1′ are underlined. [c] Two molecules per cell or two substructures per molecule.

drazides seems to originate from unfavorable conformational properties, the potency of the corresponding 5-H derivatives might be related to an alternative binding mode (hereafter called mode B, whereas the one illustrated in Figure 1 will be referred to as mode A).

A theoretical model of binding mode B was sought, trying to satisfy three constraints: (i) the number of favorable interactions was not to be lower than that of binding mode A; (ii) the orientation of the NHNHAr chain had to be different from that of mode A; (iii) it had to be highly sensitive to the nature of the 5-substituent on the indole nucleus, so as to be feasible for 5-H but not for 5-Cl or 5-NO_2 derivatives.

Our hypothesis of binding mode B, shown in Figure 3 using hydrazide **23** as a reference ligand, can be described as follows: (i) the glyoxylic C=O(2) and C=O(1) oxygens are hydrogen-bound to the H_1 and H_2 sites, respectively; (ii) the lipophilic L_1 and L_2 regions are

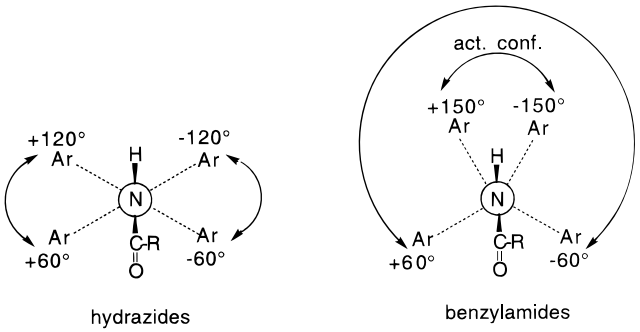


Figure 2. Newman projections showing ranges of torsion angles (O=)C−N−N−C1′(Ar) and (O=C)−NH−C−C1′(Ar) allowed for hydrazides and benzylamides. The extremes of the intervals correspond, approximately, to crystal structures retrieved from the Cambridge Structural Database. A transoid disposition of the side chain (−150° to 150° range) is supposed to be necessary for the binding mode of benzylamines schematized in Figure 1. Hydrogen(s) attached to the anilinic nitrogen or benzylic carbon is/are omitted for the sake of clarity.

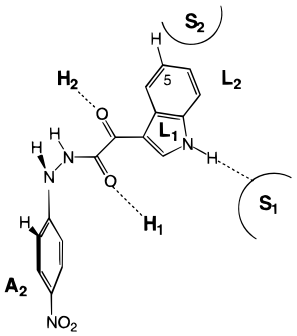


Figure 3. Binding mode "B" hypothesized for 5-unsubstituted hydrazides (5-unsubstituted benzylamides might anchor to the receptor similarly).

occupied, respectively, by the fused pyrrole and benzene rings of the indole moiety; (iii) the A_2 heteroatom no longer receives a hydrogen bond from the indole NH but faces a 2′-CH group of the phenyl moiety.

Electron-withdrawing substituents on the phenyl, such as the 4′-NO_2 group in the most potent hydrazide **23**, make the 2′-CH hydrogen more electropositive, thus favoring some interaction with the A_2 site. This model is consistent with the observation that the affinity roughly parallels the electron-withdrawing power of the 4′-substituent (see data in Table 2). However, although the 2′-CH is not electropositive enough to form a hydrogen bond with the A_2 site, it is still possible for a ligand to bind with high affinity as the interaction with this site is only accessory, and not mandatory, for high affinity at the receptor.[4] It is tempting to speculate that a significant contribution to the energy of binding mode B comes from a hydrogen bond formed between the indole NH group and a protein heteroatom belonging to the S_1 site. To our knowledge, the ability of this site to act as a hydrogen bond acceptor has never been addressed in the literature. However, this possibility does not contradict Cook's idea of S_1 being part of the steric boundaries of the receptor cavity.[4] The remarkable drop in affinity caused by methylation of the indole nitrogen (compare **4** vs **1**) is in agreement with binding mode B, although it does not argue against mode A from a logical viewpoint.

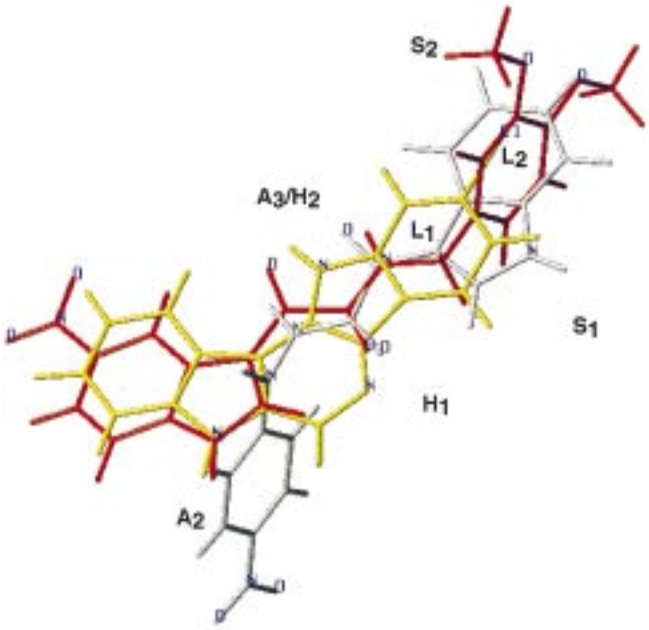


Figure 4. Superimposition of benzylamide **9′** (red) and hydrazide **23** (gray) on the extremely potent pyridodiindole **I**[4] (yellow). The H_2 site has been hypothesized to act also as a hydrogen bond acceptor (A_3).[4] The alignment of **9′** and **23** reflect binding modes A and B, respectively. Models were generated as described in the Experimental Section.

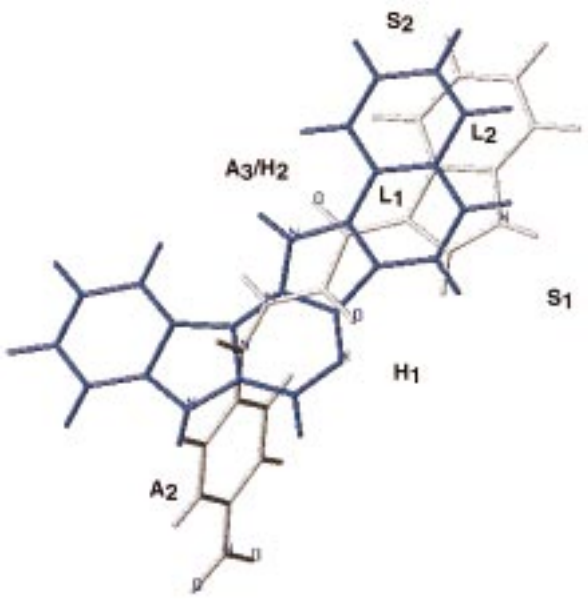


Figure 5. Superimposition of hydrazide **23** (gray) on the inactive benzopyridodiindole **III** (cyan). Substituents in the 5 position of the indole nucleus of hydrazides and the fused benzene ring F of **III** are close in space. The ring F of **III** is assumed by Cook et al.[4] to interact unfavorably with the sterically forbidden region of the receptor S_2. Models were generated as described in the Experimental Section.

Chart 1

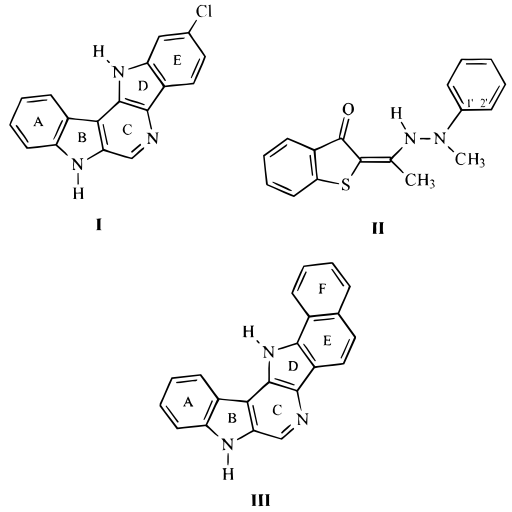


Figure 4[43] shows molecular models of hydrazide **23** and benzylamide **9′** superimposed on the crystal structure[41] of the highly potent pyridodiindole **I**[42] (Chart 1) as a rigid template. The two isosteric indole derivatives are aligned in accordance with binding modes B and A, respectively.

The weak affinity shown by the compounds bearing a methyl group on the anilinic nitrogen N′ (**27**−**31**) probably does not depend on changes in the side-chain conformation,[44] but might rather be determined by steric repulsion occurring between the *N′*-methyl and the receptor.

As already mentioned, binding mode B should justify the lack of affinity of 5-substituted hydrazides. Figure 5 shows the superimposed molecular models of hydrazide **23** (adopting binding mode B) and of the benzopyridodiindole **III**, reported to be much less potent than pyridodiindole **I**.[4] The fused benzene ring F of **III** has been claimed to disrupt binding through an unfavorable steric interaction with the receptor region S_2.[4,45] Notice that the 5 position of **23** points toward ring F of **III**. This suggests that alignment B is not feasible for 5-substituted hydrazides, due to a steric clash between the 5-Cl or 5-NO_2 group and the S_2 site (see also Figure 3). Additionally, mode B might not be ideal for 5-Cl and 5-NO_2 hydrazides because these substituents decrease the propensity of the benzene π-electrons to interact with an electropositive function of the L_2 receptor subsite (remember that the phenyl ring of benzylamides cannot fit into the L_2 subsite according to binding mode A if it bears electron-attracting substituents).

(D) Interpretation of SARs within the Benzylamide Series. The SARs relative to recently investigated benzylamides[20] (some of which are reported in Table 3) can now be reconsidered in the light of the alternative binding orientations A and B hypothesized for hydrazides.

Substituents on the phenyl ring of 5-H benzylamides might be either electron-donating or electron-attracting without any loss of affinity, whereas 5-Cl and 5-NO_2 benzylamides tolerate only electron-donating substituents on this ring. This might reflect the possibility for 5-H derivatives to dock the benzyl chain into one of two distinct receptor regions, each with its own recognition characteristics. In other words, there would be two alternative binding modes available for the more "versatile" 5-H compounds. The first one has already been described as binding mode A (Figure 1) and would take place whenever electron-donating substituents are borne by the phenyl moiety. The second possible orientation of 5-H benzylamides should be similar to that of 5-H hydrazides (binding mode B in Figure 3) and would be favored by halogens on the phenyl ring which disable

mode A. However, if these halogenated derivatives bear 5-Cl or 5-NO_2 substituents, their affinity is abolished for the same reasons which disallow binding mode B for 5-Cl and 5-NO_2 hydrazides. Finally, it is possible that modes A and B might both contribute to the binding of 5-H benzylamides, whose substituents on the phenyl ring have negligible electronic effects (i.e., compound **1′**).

Experimental Section

Chemistry. Melting points were determined using a Reichert Köfler hot-stage apparatus and are uncorrected. Infrared spectra were obtained on a PYE/UNICAM model PU 9561 spectrophotometer in Nujol mulls. Nuclear magnetic resonance spectra were recorded on a Varian CFT-20 spectrometer 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 (rotating evaporator). Analytical TLC was carried out on Merck 0.2-mm precoated silica gel aluminum sheets (60 F-254). Silica gel 60 (230−400 mesh) was used for column chromatography. Elemental analyses were performed by our Analytical Laboratory and agreed with theoretical values to within ±0.4%.

General Procedure for the Synthesis of *N′*-Phenyl(5-substituted indol-3-yl)glyoxylohydrazide Derivatives 1−20 and 23−26. A solution of triethylamine (5.5 mmol) in 10 mL of anhydrous ethyl ether was added dropwise, at 0 °C, under a nitrogen atmosphere, to a stirred suspension of the appropriate hydrazine hydrochloride (2.5 mmol) in 20 mL of the same solvent. The reaction mixture was left to stir at 0 °C for 30 min; then slowly added was a suspension of the appropriate glyoxylyl chloride (2.5 mmol) in 30 mL of dry ethyl ether. The suspension obtained was stirred for 15 min at 0 °C and then for 4−36 h at room temperature, monitoring the reaction by TLC analysis, and subsequently filtered. The precipitate collected was triturated with a saturated $NaHCO_3$ aqueous solution, washed with water, and collected again. The crude product was purified by recrystallization from the appropriate solvent, after filtration, when necessary, on a silica gel column. Yields, recrystallization solvents, and melting points are listed in Table 1. Spectral data are reported in the Supporting Information.

General Procedure for the Synthesis of *N′*-Methyl-*N′*-phenyl(5-substituted indol-3-yl)glyoxylohydrazide Derivatives 27−31. A solution of 1-methyl-1-phenylhydrazine (2.75 mmol) in 10 mL of dry benzene was added, dropwise, at 0 °C, to a suspension of the appropriate indolylglyoxylyl chloride (2.5 mmol) and triethylamine (3.0 mmol) in 40 mL of anhydrous benzene. The reaction mixture was left to warm at room temperature, stirred for 2 h, refluxed for 2 h, and then, after cooling, filtered. The precipitate collected was triturated with a saturated $NaHCO_3$ aqueous solution, washed with water, and collected again. The crude product was purified by recrystallization from the appropriate solvent. Yields, recrystallization solvents, and melting points are listed in Table 1. Spectral data are reported in the Supporting Information.

General Procedure for the Synthesis of *N′*-(4′-Hydroxyphenyl)(5-substituted indol-3-yl)glyoxylohydrazide Derivatives 21 and 22. A solution of 0.55 mL (5.68 mmol) of boron tribromide in 5 mL of anhydrous dichloromethane was added, dropwise, at −10 °C, to a suspension of the appropriate indole derivative **18** and **19** (0.87 mmol) in 8 mL of the same solvent. The dark reaction mixture was left to stir for 30 min at 0 °C and for 1 h at room temperature and was then poured slowly into ice. The solid obtained was collected, washed with water, and purified by recrystallization from the appropriate solvent. Yields, recrystallization solvents, and melting points are listed in Table 1. Spectral data are reported in the Supporting Information.

(1-Methyl-5-nitroindol-3-yl)glyoxylyl Chloride. Oxalyl chloride (0.7 mL, 8.0 mmol) was added dropwise, at 0 °C, to a well-stirred suspension of 1-methyl-5-nitroindole[46] (1.0 g, 5.7 mmol) in 30 mL of anhydrous ethyl ether. The reaction mixture was left to warm at room temperature and then refluxed for 24 h. The precipitate formed was collected and washed with small portions of dry ethyl ether. The acid chloride obtained (1.25 g, yield 82%) had mp 161−165 °C and was characterized by conversion into its methyl ester.

(1-Methyl-5-nitroindol-3-yl)glyoxylyl Methyl Ester. Yield: 91%. Mp: 210−212 °C (benzene). IR (Nujol): 1720, 1650, 1520, 1200, 760 cm^{-1}. ^1H NMR (DMSO-d_6): δ 3.92 (s, 3H, N−CH_3); 3.99 (s, 3H, O−CH_3); 7.75−8.98 (m, 4H, ArH). MS (EI): *m/e* 262 (M^+), 203, base.

Molecular Modeling. All molecular modeling was conducted using the software package SYBYL[38] running on a Silicon Graphics Indigo XS24 workstation. Model building and geometry optimization of the benzylamide derivative **9′** were performed with the semiempirical quantum-mechanics methods AM1[37] available in the MOPAC program[47] as described in a previous paper.[20]

The crystal structure of the pyridodiindole **I** was retrieved from the April 1997 release (3D graphics 5.13 version for UNIX platforms) of the Cambridge Structural Database (CSD)[39] with the reference code JOJHIZ.

A starting model of benzopyridodiindole **III** was obtained by modifying the crystal structure of **I** using the geometry of benzene taken from the SYBYL fragment library. The resulting model was fully energy-minimized with the AM1 method.

The crystal structures of hydrazides listed in Table 5 and of the hydrazide derivative **II** (reference code LELYUW) were retrieved through substructure queries formulated using the CSD/QUEST graphic routine (CONHNH−C(X)X and CONHNMe−C(X)X, respectively, where X is any atom attached to the carbon via an aromatic bond). Crystal structures of benzylamides were retrieved similarly using $CONHCH_2$−C(X)(X) as the substructure query.

Modeling of hydrazides is hampered by the poor performance of semiempirical quantum-mechanics methods[48] and the lack of parameters for molecular mechanics calculations. A model of hydrazide **23** was constructed by merging the AM1-calculated geometry of indol-3-yl-$COCONH_2$ ("MMOK" keyword used) with the geometry of the CONHNHPh moiety extracted from the crystal strucuture of 1,5-diphenylcarbonohydrazide listed in Table 5 (reference code DPCBHZ). The two structures were preliminarily fitted about the common CON group (rms distance = 0.021 Å). The amidic hydrogen of indolylglyoxylamide eclipsed with the carbonyl oxygen was replaced by the NHPh fragment of the hydrazide derivative using the SYBYL/MERGE command. Then, the N−N bond distance was given the same value measured in the hydrazide structure. Finally, a nitro group was added in the 4 position of the phenyl and geometry-optimized with the AM1 method, keeping the rest of the molecule fixed.

Molecular models were superimposed by minimizing the rms distance between selected atom pairs using the SYBYL/FIT command. The pseudoatoms H_1, H_2, A_2, and A_3 were added to the structures being compared to simulate hypothetical positions of receptor hydrogen bond functions. The positioning of these pseudoatoms and the overlay of the models shown in Figures 4 and 5 were accomplished following the procedures described by Cook et al.[49]

Binding Studies. [^{3}H]Ro 15-1788 (specific activity 83.2 Ci/mmol) and [^{35}S]TBPS (specific activity 80 Ci/mmol) were obtained from DuPont de Nemours, New England Nuclear Division (Dreieichenhaim, Germany). All other chemicals were of reagent grade and obtained from commercial suppliers.

Bovine cerebral cortex membranes were prepared in accordance with ref 50. 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.[51]

[^{3}H]Ro 15-1788 Binding Studies. These studies were performed using a filtration technique essentially as previously reported.[35] K_i values were calculated for the hydrazide derivatives (10 μM) showing percents of inhibition of specific [^{3}H]Ro 15-1788 binding ≥ 80%.

[^{35}S]TBPS Binding Studies. The membrane suspension was incubated together with 5 nM [^{35}S]TBPS for 90 min at 25 °C in 500 μL (final volume) of T1 buffer containing 200 mM KBr and 0.1 mM EDTA. The binding assay was performed using a filtration technique. After incubation, the samples were diluted with 5 mL of assay buffer, immediately filtered under reduced pressure through glass filter disks (Whatman GF/C), and then washed with 5 mL of the same buffer. The filter disks were then placed in polypropylene scintillation vials together with 8 mL of Ready Safe Beckman scintillation cocktail; the radioactivity of the filters was determined by a Beckman LS 1800 scintillometer. Drugs were added as concentrated ethanolic solutions (0.5 μM). The level of ethanol did not exceed 0.2% and was maintained constant in all tubes. Nonspecific [^{35}S]TBPS binding was estimated in the presence of 600 μM picrotoxinin and was subtracted to compute specific binding. The characterization of the actions of various drugs on [^{35}S]TBPS binding was performed as described elsewhere.[29]

In Vivo Studies: Proconvulsant, Anticonvulsant, and Diazepam Antagonism Action. Groups of 10 mice were injected intraperitoneally (0.1 mL) with graded doses of the compounds (up to the highest dose of 250 mg/kg), suspended in 20% dilute Emulphor−80% saline solution (vehicle) (dilute Emulphor is Emulphor diluted 1:1, w/w, with ethanol), or an equal volume of the vehicle, followed 30 min later by PTZ at 40 or 80 mg/kg to assess the proconvulsant and anticonvulsant actions, respectively, as described by Trudell et al.[52]

Antagonism of the anticonvulsant effects of diazepam was carried out as described by Cain et al.[53] Groups of 10 mice were injected with diazepam (2.5 mg/kg ip) followed 10 min later by administration of graded doses of the test compound or vehicle. Fifteen minutes after injection of the compound, animals were injected with PTZ (80 mg/kg).

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Supporting Information Available: Table containing the ^{1}H NMR, MS, and IR spectral data of compounds **1**−**31** (4 pages). Ordering information is given on any current masthead page.

References

(1) Macdonald, R. L.; Olsen, R. W. $GABA_A$ Receptor Channels. *Annu. Rev. Neurosci.* **1994**, *17*, 569−602.
(2) Sieghart, W. Structure and Pharmacology of γ-Aminobutyric $Acid_A$ Receptor Subtypes. *Pharmacol. Rev.* **1995**, *47*, 181−234.
(3) Villar, H. O.; Davies, M. F.; Loew, G. H.; Maguire, P. A. Molecular Models for Recognition and Activation at the Benzodiazepine Receptor: a Review. *Life Sci.* **1991**, *48*, 593−602.
(4) Zhang, W.; Koehler, K. F.; Zhang, P.; Cook, J. M. Development of a Comprehensive Pharmacophore Model for the Benzodiazepine Receptor. *Drug Des. Discovery* **1995**, *12*, 193−248.
(5) Gupta, S. P. Recent Advances in Benzodiazepine Receptor (BZR) Binding Studies. *Prog. Drug Res.* **1995**, *45*, 67−106.
(6) Allen, M. S.; Hagen, T. J.; Trudell, M. L.; Codding, 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.
(7) 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.
(8) 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.
(9) Tarzia, G.; Occelli, E.; Toja, E.; Barone, D.; Corsicò, N.; Gallico, L.; Luzzani, F. 6-(Alkylamino)-3-aryl-1,2,4-triazolo[3,4-a]-phthalazines. A New Class of Benzodiazepine Receptor Ligands. *J. Med. Chem.* **1988**, *31*, 1115−1123.
(10) Toja, E.; Tarzia, G.; Barone, D.; Luzzani, F.; Gallico, L. Benzodiazepine Receptor Binding and Anticonflict Activity in a Series of 3,6-Disubstituted Pyridazino[4,3-c]isoquinolines Devoid of Anticonvulsivant Properties. *J. Med. Chem.* **1985**, *28*, 1314−1319.
(11) Tanaka, H.; Kirihara, S.; Yasumatsu, H.; Yakushiji, T.; Nakao, T. Synthesis and Structure−Activity Analysis of 2-(4-Chlorophenyl)-5,6-dihydrothieno[2′, 3′: 2, 3]thiepino[4,5-c]pyridazin-3(2H)-ones as Ligands for Benzodiazepine Receptors. *Eur. J. Med. Chem.* **1995**, *30*, 859−868.
(12) Takada, S.; Sasatani, T.; Chomei, N.; Adachi, M.; Fujishita, T.; Eigyo, M.; Murata, S.; Kawasaki, K.; Matsushita, A. Synthesis and Structure−Activity Relationships of Fused Imidazopyridines: A New Series of Benzodiazepine Receptor Ligands. *J. Med. Chem.* **1996**, *39*, 2844−2851.
(13) Jacobsen, E. J.; Stelzer, L. S.; Belonga, K. L.; Carter, D. B.; Im, W. B.; Sethy, V. H.; Tang, A. H.; VonVoigtlander, P. F.; Petke, J. D. 3-Phenyl-Substituted Imidazo[1,5-a]quinoxalin-4-ones and Imidazo[1,5-a]quinoxaline Ureas That Have High Affinity at the $GABA_A$/Benzodiazepine Receptor Complex. *J. Med. Chem.* **1996**, *39*, 3820−3836.
(14) Liu, R.; Zhang, P.; Gan, T.; McKernan, R. M.; Cook, J. M. Evidence for the Conservation of Conformational Topography at Five Major $GABA_A$/Benzodiazepine Receptor Subsites. Potent Affinities of the (S)-Enantiomers of Framework-Constrained 4,5-Substituted Pyrroloimidazobenzodiazepines. *Med. Chem. Res.* **1997**, *7*, 25−35.
(15) Gardner, C. R. Interpretation of the Behavioral Effects of Benzodiazepine Receptor Ligands. *Drugs Future* **1989**, *14*, 51−67.
(16) 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 Agonist and Antagonist. *Adv. Drug Res.* **1985**, *14*, 166−322.
(17) Petersen, E. N.; Jensen, L. H.; Diejer, J.; Honore, T. New Perspectives in Benzodiazepine Receptor Pharmacology. *Pharmacopsychiatry* **1986**, *19*, 4−6.
(18) Haefely, W. Psychopharmacology of Anxiety. *Eur. Neuropsychopharmacol.* **1991**, *1*, 89−95.
(19) 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-yl)amine Derivatives. *J. Med. Chem.* **1992**, *35*, 2214−2220.
(20) 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.
(21) Da Settimo, A.; Primofiore, G.; Marini, A. M.; Ferrarini, P. L.; Franzone, J. S.; Cirillo, R.; Reboani, M. C. N-(Indol-3-ylglyoxyl-yl)methionine Derivatives: Preparation and gastric Anti-Secretory Activity. *Eur. J. Med. Chem.* **1988**, *23*, 21−24.
(22) Felix, A. M. Cleavage of Protecting Groups With Boron Tribromide. *J. Org. Chem.* **1974**, *39*, 1427−1429.
(23) Giannaccini, G.; Giacomelli, M.; Martini, C.; Lucacchini, A.; Piccolino, M. Binding of the Benzodiazepine Ligand [^{3}H]Ro 15-1788 to Membrane Preparations of the Rabbit and Turtle Retina. *Comp. Biochem. Physiol.* **1992**, *101C*, 337−342.
(24) Braestrup, C.; Nielsen, M.; Honorè, T.; Jensen, L. H.; Petersen, E. M. Benzodiazepine Receptor Ligands With Positive and Negative Efficacy. *Neuropharmacology* **1983**, *22*, 1451−1457.
(25) 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.
(26) 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.
(27) Squires, R. F.; Casida, J. E.; Richardson, M.; Saederup, E. [^{35}S]-*tert*-Butylbicyclophosphorothionate Binds with High Affinity to Brain-Specific Sites Coupled to γ-Aminobutyric Acid-A and Ion Recognition Sites. *Mol. Pharmacol.* **1983**, *23*, 326−336.
(28) Martini, C.; Pacini, R.; Giannaccini, G.; Lucacchini, A. [^{35}S]TBPS Binding to Purified Benzodiazepine-GABA-Ionophore Receptor Complex: the Effect of GABA on the Modulation Exerted by Benzodiazepine Site Ligands. *Neurochem. Int.* **1989**, *15*, 377−379.
(29) Im, W. B.; Blakeman, D. P. Correlation Between γ-Aminobutyric $Acid_A$ Receptor Ligand-Induced Changes in *tert*-Butylbicyclophosphoro[^{35}S]thionate Binding and $^{36}Cl^-$ Uptake in Rat Cerebrocortical Membranes. *Mol. Pharmacol.* **1991**, *39*, 394−398.

(30) Villar, H. O.; Uyeno, E. T.; Toll, L.; Polgar, W.; Davies, M. F.; Loew, G. H. Molecular Determinants of Benzodiazepine Receptor Affinities and Anticonvulsant Activities. *Mol. Pharmacol.* **1989**, *36*, 589−600.
(31) Ananthan, S.; Clayton, S. D.; Ealick, S. E.; Wong, G.; Evoniuk, G. E.; Skolnick, P. Synthesis and Structure−Activity Relationships of 3,5-Disubstituted 4,5-Dihydro-6H-imidazo[1,5-a][1,4]-benzodiazepin-6-ones at Diazepam-Sensitive and Diazepam-Insensitive Benzodiazepine Receptors. *J. Med. Chem.* **1993**, *36*, 479−490.
(32) Petke, J. D.; Haesook, K. I.; Im, W. B.; Blakeman, D. P.; Pregenzer, E. J.; Jacobsen, J.; Hamilton, B. J.; Carter, D. B. Characterization of Functional Interactions of Imidazoquinoxaline Derivatives with Benzodiazepine-γ-Aminobutyric $Acid_A$. *Mol. Pharmacol.* **1992**, *42*, 294−301.
(33) TenBrink, R. E.; Im, W. B.; Sethy, V. H.; Tang, A. H.; Carter, D. B. Antagonist, Partial Agonist, and Full Agonist Imidazo-[1,5-a]quinoxaline Amides and Carbamates Acting through the $GABA_A$/Benzodiazepine Receptor. *J. Med. Chem.* **1994**, *37*, 758−768.
(34) Martini, C.; Gervasio, T.; Lucacchini, A.; Da Settimo, A.; Primofiore, G.; Marini, A. M. Specific Inhibition of Benzodiazepine Receptor Binding Site by Some N-(Indol-3-ylglyoxylyl)-amino Acid Derivatives. *J. Med. Chem.* **1985**, *28*, 506−509.
(35) Primofiore, G.; Marini, A. M.; Da Settimo, F.; Martini, C.; Bardellini, A.; Giannaccini, G.; Lucacchini, A. Specific Inhibition of Benzodiazepine Receptor Binding Site by Some N-(Indol-3-ylglyoxylyl)amino Acid Derivatives: Stereoselective Interactions. *J. Med. Chem.* **1989**, *32*, 2514−2518.
(36) Primofiore, G.; Da Settimo, F.; Marini, A. M.; La Motta, C.; Martini, C.; Senatore, G.; Lucacchini, A. N-(Indol-3-ylglyoxylyl)-amino Acid Ester Derivatives. Synthesis and Interaction Properties at the Benzodiazepine Receptor. *Farmaco* **1995**, *50*, 5−12.
(37) 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.
(38) SYBYL Molecular Modeling System (version 6.2), TRIPOS Assoc., St. Louis, MO.
(39) Bernstein, F. C.; Koetzle, T. F.; Williams, G. J. B.; Meyer, E. F., Jr.; Brice, M. D.; Rodgers, J. R.; Kennard, O.; Shimanouchi, T.; Tasumi, T. The Protein Data Bank: A Computer Based Archival File for Macromolecular Structures. *J. Mol. Biol.* **1977**, *112*, 535−542.
(40) Dewar, M. J. S.; Jennings, W. B. Conformational Interchange in Acyclic Hydrazines. *J. Am. Chem. Soc.* **1973**, *95*, 1562−1569 and references therein.
(41) Szkaradzinska, M. B.; Roszak, A. W.; Trudel, M. L.; Cook, J. M.; Codding, P. W. Structure of 2-Chloro-7,11-dihydropyrido-[3,2-b:5,4-b′]diindole. *Acta Crystallogr. Sect. C: Cryst. Struct. Commun.* **1985**, *C41*, 107−110.
(42) Zhang, W.; Cook, J. M. Novel Organic Reactions in the Search for Anxioselective Anxiolytics at the Benzodiazepine Receptor. In *Drug Design for Neuroscience*; Kozikowski, A., Ed.; Raven Press: New York, 1993; pp 87−117.
(43) Considering that the +gauche and −gauche dispositions of the CONHNHAr moieties have similar energies, and in the absence of detailed structural information concerning the shape of the receptor pocket complementary to this part of the ligand, the −gauche conformation of **23** was selected arbitrarily for the superimposition illustrated in Figure 4. Benzylamide **9′** was modeled with torsion angles (O═)C−N−N−C1′ and N−C−C1′−C2′ of 171.4° and 95.1°, respectively (calculated by the AM1 wave function). The value of the former dihedral falls within the range of anti values (−150° to +150°) thought to be relevant for activity, whereas the value of the latter dihedral was selected arbitrarily. Work is in progress to deduce the bioactive orientation of the benzylamide aryl group more accurately through synthesis of conformationally constrained benzylamide analogues.
(44) The crystal geometry of the hydrazine derivative **II** (reference code LELYUG) was retrieved from the CSD as the entry structurally most closely related to hydrazides **27**−**31**. We assumed that the C═CNHNMePh moiety of **II** could represent a good model of the CONHNMePh side chain. The values of the torsion angles C−N−N−C1′ and N−N−C1′−C2′ measured on the crystal structure of **II** are −122.4° and 31.4°, respectively, which are close to those listed in Table 5 for hydrazides of type CONHNHAr.
(45) Martin, M. J.; Trudell, M. L.; Diaz-Arauzo, H.; Allen, M. S.; Deng, L.; Schultz, C. A.; Tan, Y.-C.; Bi, Y.; Narayanan, K.; Dorn, L. J.; Koehler, 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.
(46) Forbes, I. T.; Kennett, G. A.; Gadre, A.; Ham, P.; Hayward, C. J.; Martin, R. T.; Thompson, M.; Martyn, D. W.; Baxter, G. S.; Glen, A.; Murphy, O. E.; Stewart, B. A.; Blackburn, T. P. N-(1-Methyl-5-indolyl)-N′-(3-pyridyl)urea Hydrochloride: The First Selective 5-HT1C Receptor Antagonist. *J. Med. Chem.* **1993**, *36*, 1104−1107.
(47) MOPAC, version 6.0, Quantum Chemistry Program Exchange.
(48) Stewart, J. J. P. Semiempirical Molecular Orbital Methods. In *Reviews in Computational Chemistry*; Lipkowitz, K. B., Boyd, D. B., Eds.; VCH Publishers: New York, 1990; pp 45−81.
(49) Diaz-Arauzo, H.; Koehler, K. F.; Hagen, T. J.; Cook, J. M. Synthetic and Computer Assisted Analysis of the Pharmacophore for Agonists at Benzodiazepine Receptors. *Life Sci.* **1991**, *49*, 207−216.
(50) 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.
(51) 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.
(52) Trudell, M. L.; Lifer, S. L.; Tan, Y. C.; Martin, M. J.; Deng, L.; Skolnick, P.; Cook, J. M. Synthesis of Substituted 7,12-Dihydropyrido[3,2-b:5,4-b′]diindoles: Rigid Planar Benzodiazepine Ligands with Inverse Agonist/Antagonist Properties. *J. Med. Chem.* **1990**, *33*, 2412−2420.
(53) Cain, M.; Weber, R. W.; Guzman, F.; Cook, J. M.; Barker, S. A.; Rice, K. C.; Crawley, J. N.; Paul, S. M.; Skolnick, P. β-Carbolines: Synthesis and Neurochemical and Pharmacological Actions on Brain Benzodiazepine Receptors. *J. Med. Chem.* **1982**, *25*, 1081−1091.

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