Novel N-Substituted Indol-3-ylglyoxylamides Probing the L_{Di} and L_1/L_2 Lipophilic Regions of the Benzodiazepine Receptor Site in Search for Subtype-Selective Ligands†

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Novel N-substituted indol-3-ylglyoxylamides (**10**−**37**) were synthesized and evaluated as ligands of the benzodiazepine receptor (BzR). In an effort to achieve affinity-based selectivity among BzR subtypes, these compounds were designed to probe the L_{Di} and L_2 lipophilic regions. Taking the α_1-selective benzylindolylglyoxylamides **Ia** and **Ib** as leads, we varied the substituent on the benzylamide phenyl ring (compounds **10**−**23**) or replaced the benzyl moiety with alkyl groups (compounds **24**−**37**). The above structural changes gave no shift of selectivity from the α_1 toward the α_2 or α_5 subtypes, thus confirming that a ligand which occupies the L_{Di} region probably exhibits α_1 selectivity, despite its interactions with other lipophilic areas in the receptor binding cleft. Compound **11** (*N*-(*p*-methylbenzyl)-5-nitroindol-3-ylglyoxylamide), which selectively binds with a full agonist efficacy at the α_1 receptor subtype and displays sedative action, can be regarded as an interesting potential zolpidem-like sedative−hypnotic agent.

Introduction

GABA (γ-aminobutyric acid), the major inhibitory neurotransmitter in the central nervous system (CNS), operates through three different receptor types: the ionotropic $GABA_A$ and $GABA_C$ receptors and the metabotropic $GABA_B$ receptor.[1,2] The $GABA_A$ receptor plays a fundamental role in several neurological functions including modulation of anxiety, sleep, and muscle tone, as well as in processes like memory and learning.[3] This membrane-bound heteropentameric protein receptor may be assembled from at least 21 subunits belonging to 8 different classes (6α, 4β, 4γ, 1ϵ, 1δ, 3ρ, 1θ, and 1π), which form a chloride channel.[1,3] Three types of subunits (α, β, and γ in a predominant 2:2:1 stoichiometry) are required for the construction of recombinant receptors which most closely mimic the biochemical, electrophysiological, and pharmacological functions of native $GABA_A$ receptors obtained from mammalian brain cells.[4]

The benzodiazepine receptor site (BzR) is located at the interface of the α and γ subunits of the $GABA_A$ complex and binds with a high affinity benzodiazepine derivatives, like diazepam, and other structurally different classes of compounds. BzR ligands can allosterically modulate the affinity of GABA for the $GABA_A$ receptor.[4] On the basis of their modulatory effects, they are classified as agonists (positive allosteric modulators, with anxiolytic, anticonvulsant, sedative−hypnotic, and myorelaxant activities) or inverse agonists (negative allosteric modulators, with anxiogenic, somnolytic, proconvulsant, or even convulsant activities), potentiating or decreasing the GABA-induced chloride influx, respectively. Spanning these efficacy extremes are partial agonists, antagonists (which bind to the BzR but have little or no effect on the chloride flux, being devoid of any appreciable intrinsic efficacy), and partial inverse agonists.[5,6]

The majority of benzodiazepine-sensitive $GABA_A$ receptor subtypes in the brain are $\alpha_1\beta_x\gamma_2$, $\alpha_2\beta_x\gamma_2$, $\alpha_3\beta_x\gamma_2$, and $\alpha_5\beta_x\gamma_2$, while the $\alpha_4\beta_x\gamma_2$ and $\alpha_6\beta_x\gamma_2$ subtypes do not respond to benzodiazepines and are therefore called benzodiazepine-insensitive receptors.[7,8] Since the γ_2 subunit does not vary, and the β subunit type does not seem to influence the pharmacology of benzodiazepines, the α subunit is the main determinant of affinity and efficacy of BzR ligands[7−9] (therefore BzR subtypes take their names from the α subunit).

The regional heterogeneity of the BzR subtypes (α_l, α_2, α_3, and α_5) has been suggested as the basis for the multiplicity of pharmacological properties of most agonists belonging to the class of benzodiazepines. In particular, α_1, which is the dominant subtype, is present in both cerebellum and cortex and thus mediates the sedative/muscle relaxant effects; α_2 is moderately abundant in the cortex and hippocampus and is related to the anxiolytic and anticonvulsant activities; the α_5 type is scarce and widely expressed only in the hippocampus and is associated with impairment of cognition and memory; finally, the role of α_3 remains unclear or, at least, it seems to be partly involved in mediating anxiety behavior.[7−10] Consequently, selective α_1 agonists represent ideal sedative and hypnotic agents devoid of side effects on cognition and memory performances; an agonist that selectively modulates α_2 and α_3 receptors, but does not affect the α_1 subtype, would represent a nonsedative anxiolytic; an inverse agonist selectively acting at the α_5 receptor may have therapeutic utility as a cognition-enhancing agent, lacking the unwanted side effects associated with inverse agonist activity

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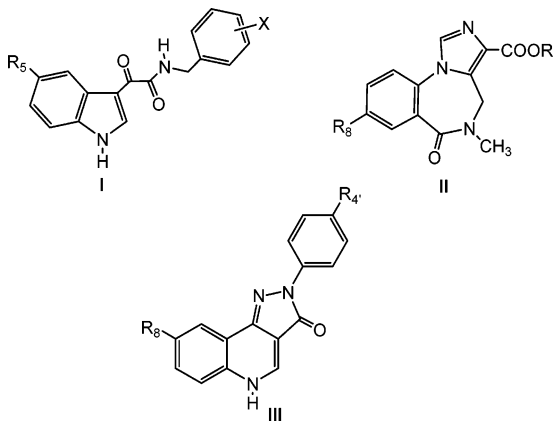
Chart 1. Structures of *N*-(Benzyl)indol-3-ylglyoxylamides **I**, Imidazobenzodiazepines **II**, and Pyrazoloquinolines **III**



at the other subtypes, including anxiogenesis or convulsant or proconvulsant activity.[8,11,12]

Structure−activity relationships (SARs) of widely different classes of BzR ligands were rationalized by Cook and co-workers through a pharmacophore/topological model made up of the following interaction sites within the receptor binding cleft: H_1 and H_2 as hydrogen bond donors, A_2 as a hydrogen bond acceptor, L_1, L_2, L_3, and L_{Di} as lipophilic regions, and S_1, S_2, and S_3 as sterically forbidden sites.[13]

Some years ago, we presented the *N*-(benzyl)indol-3-ylglyoxylamides **I** as a new class of BzR ligands (Chart 1).[14,15] These compounds display an enhanced affinity for the BzR α_1 subtype, compared with that of the α_2 and α_5 isoforms. SARs of indole derivatives soon revealed that the effects of the R_5 and X substituents on α_1 affinity are interdependent. Specifically, the affinity is favored by X = hydroxy/methoxy or halogens, depending on whether the 5-position of the indole nucleus is substituted (R_5 = Cl/NO_2) or not (R_5 = H), respectively. Thus, while affinity in the 5-Cl/NO_2 series is optimized by X = 3′,4′-$(OH)_2$ or 3′,4′-$(OMe)_2$, in the 5-H series the highest affinity is achieved with electron-attracting X = 4′-Cl. These puzzling data were interpreted by assuming that in their interaction with the receptor site, our ligands might adopt two alternative binding modes called A and B (Figure 1).[15] The 5-Cl/NO_2 indoles bind according to mode A, by interacting with the A_2 site (through the indole NH), with the H_1 and H_2 sites (through the C = O2 and C = O1), and with the L_1, L_2, and L_{Di} lipophilic regions (filled by the CH_2, the side chain phenyl ring, and the benzene moiety of the indole nucleus, respectively). In this orientation, the affinity is increased by hydroxy and methoxy X substituents, due to a hydrogen bond formed between the 3′-OH or 3′-OMe and a bifunctional hydrogen bond acceptor/donor (HBA/D) group located on the surface of the S_1 site, as recently proposed by our research group.[16]

Binding mode B requires R_5 = H (substituents bulkier than a hydrogen would be repelled by the sterically forbidden region S_2) and is favored by electron-attracting X groups. This binding mode relies on the following interactions: C = O2 and C = O1 are hydrogen-bound to the H_1 and H_2 sites; the L_1, L_2, and L_{Di} lipophilic regions are occupied by the pyrrole and benzene moieties of the indole nucleus, and the side chain phenyl ring, respectively; a hydrogen bond is donated by the indole NH to the HBA/D group belonging to the S_1 site.[16]

In an effort to identify novel ligands possessing selectivity at the BzR subtypes, we have now turned our attention to N-substituted indol-3-ylglyoxylamides filling the L_{Di} and L_2 lipophilic regions to different extents. Our approach was guided by research focusing on differences in the recognition properties of the BzR subtypes. Specifically, it has been suggested that the basic topology of these subtypes is highly conserved, with the exception of the L_2 and L_{Di} lipophilic pockets, whose different dimensions might play a role in determining selectivity profiles.[17] In particular, it has been proposed that the L_{Di} and L_2 regions are wider in the α_1 and α_5 binding sites, respectively, with respect to the other subtypes. Consequently, full occupation of L_{Di} or L_2 may account for α_1 and α_5 selectivity, respectively.[17,18] An excellent example of α_5-selective ligands is given by imidazobenzodiazepines **II** (Chart 1) featuring bulky sub-

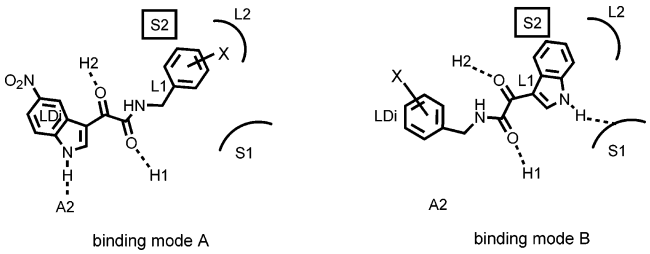
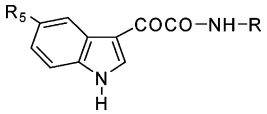


Figure 1. Hypothetical binding modes A and B of indole BzR ligands (ref 15) within the framework of Cook's pharmacophore/topological model (ref 13).

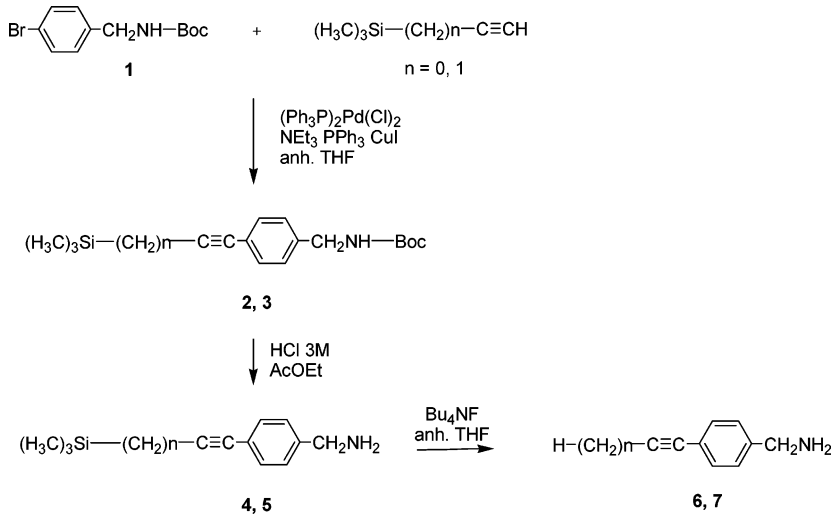
Table 1. Inhibition of [^{3}H]Flumazenil Specific Binding to Bovine Brain Membranes of Indol-3-ylglyoxylylamide Derivatives **Ia**,**b** and **10**−**37**



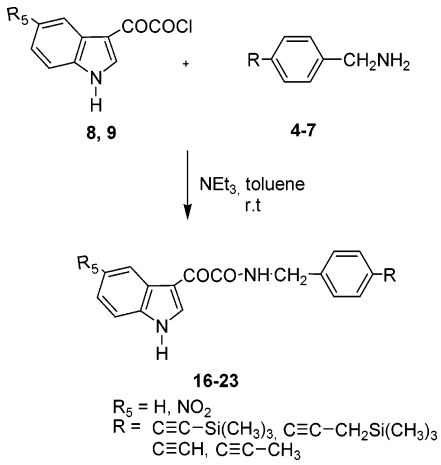
no.	R_5	R	K_i (nM) or % inhibition (10 μM)[a,b]
Ia[c]	H	$CH_2-C_6H_5$	120 ± 11
Ib[c]	NO_2	$CH_2-C_6H_5$	117 ± 12
10	H	$CH_2-C_6H_4-4'-CH_3$	606 ± 57
11	NO_2	$CH_2-C_6H_4-4'-CH_3$	88 ± 6
12	H	$CH_2-C_6H_4-4'-CH_2CH_3$	2160 ± 180
13	NO_2	$CH_2-C_6H_4-4'-CH_2CH_3$	42% ± 3
14	H	$CH_2-C_6H_4-4'-Br$	93 ± 8
15	NO_2	$CH_2-C_6H_4-4'-Br$	31% ± 3
16	H	$CH_2-C_6H_4-4'-C\equiv CSi(CH_3)_3$	29% ± 2
17	NO_2	$CH_2-C_6H_4-4'-C\equiv CSi(CH_3)_3$	21% ± 2
18	H	$CH_2-C_6H_4-4'-C\equiv C-CH_2Si(CH_3)_3$	3% ± 1
19	NO_2	$CH_2-C_6H_4-4'-C\equiv C-CH_2Si(CH_3)_3$	0% ± 2
20	H	$CH_2-C_6H_4-4'-C\equiv CH$	164 ± 10
21	NO_2	$CH_2-C_6H_4-4'-C\equiv CH$	28% ± 1
22	H	$CH_2-C_6H_4-4'-C\equiv C-CH_3$	3275 ± 299
23	NO_2	$CH_2-C_6H_4-4'-C\equiv C-CH_3$	0% ± 3
24	H	$(CH2)_3CH_3$	3720 ± 30
25	NO_2	$(CH2)_3CH_3$	110 ± 9
26	H	$(CH_2)_4CH_3$	1480 ± 11
27	NO_2	$(CH_2)_4CH_3$	4100 ± 40
28	H	$(CH_2)_5CH_3$	1650 ± 13
29	NO_2	$(CH_2)_5CH_3$	53% ± 4
30	H	$CH(CH_3)_2$	2690 ± 15
31	NO_2	$CH(CH_3)_2$	57.8 ± 4
32	H	$CH(CH_3)CH_2CH_3$	34% ± 4
33	NO_2	$CH(CH_3)CH_2CH_3$	412 ± 9
34	H	$C(CH_3)_3$	7% ± 2
35	NO_2	$C(CH_3)_3$	2800 ± 27
36	H	$CH_2CH(CH_3)_2$	43% ± 3
37	NO_2	$CH_2CH(CH_3)_2$	5% ± 2
diazepam			10 ± 1
flumazenil			0.90 ± 0.05
clonazepam			0.85 ± 0.02

[a] K_i values are means ± SEM of three determinations carried out in triplicate. [b] Percentage inhibition values of specific [^{3}H]flumazenil binding at 10 μM concentration are means ± three determinations carried out in triplicate. [c] Data taken from ref 14.

Scheme 1. Synthesis of 4-Substituted Benzylamine Derivatives **4**−**7**



Scheme 2. Synthesis of Indole Derivatives **16**−**23**



stituents in the 8-position, such as ethynyl and (trimethylsylyl)-ethynyl, which fit into the L_2 region.[17,18] Yu et al. have proposed that simultaneous occupation of L_2 and L_{Di} seems to promote α_2 selectivity, in accordance with the binding data of some pyrazoloquinolines **III** (Chart 1) substituted in both 4′- and 8-positions with lipophilic bulky groups.[19] However, the factors determining α_2 selectivity are not so clearly defined, as the same research group subsequently proposed that a BzR ligand which potently interacts with the L_{Di} region displays α_1 selectivity, despite occupation of other receptor lipophilic areas.[17,18]

Taking the benzylindolylglyoxylamides **Ia** and **Ib** as reference compounds (Table 1),[14] we decided to probe the steric recognition properties of the L_{Di} and L_2 pockets in two different ways: by varying the substituent on the benzylamide phenyl ring (compounds **10**−**23** in Table 1) or by replacing the benzyl moiety with alkyl groups (compounds **24**−**37** in Table 1). This paper describes the synthesis, biological evaluation, and SARs of the new indole derivatives **10**−**37**.

Chemistry. The synthetic procedure used for the preparation of compounds **10**−**15** and **24**−**37** involved the reaction in mild conditions of the indolylglyoxylyl chlorides **8** and **9**[20] with the appropriate amine in the presence of triethylamine in toluene solution (Supporting Information).

For the synthesis of indole derivatives **16**−**23**, we focused our attention on the palladium-catalyzed C−C bond forming process. Initially we took into consideration the palladium-catalyzed coupling of terminal alkynes with aryl or alkenyl halides described for the first time by Sonogashira et al. in 1975,[21] which is one of the most straightforward methods for the preparation of arylalkynes and conjugated enynes.[22]

On the basis of this evaluation, a possible route for the synthesis of derivatives **16**−**23** could consist of a coupling reaction between the appropriate, commercially available, terminal trimethylsilylalkynes and the 4′-bromo derivatives **14** and **15**, to give compounds **16**−**19**, which by subsequent removal of the protecting group could generate products **20**−**23**.

However, despite varying many parameters, such as solvent, catalyst, and base, no satisfactory results were obtained, as only traces of the final products **16**−**19** were isolated, probably due to the low solubility of the 4′-bromo derivatives **14** and **15** in the reaction solvent.

The key to the successful synthesis of derivatives **16**−**23** was the preparation of the amine intermediates **4**−**7** (Scheme 1), which possess good solubility properties, making the coupling reaction more efficient.

Synthesis of alkynes **6** and **7** was achieved in three steps starting from the commercially available 4-bromobenzylamine, which was first protected as its carbamate **1**,[23] and then coupled with the appropriate trimethylsilylalkyne, using an improved Sonogashira coupling procedure, reported by Thorand and Krause (Scheme 1).[24] It should be noted that a careful choice of the composition of the reaction mixture and the mode of addition of the reactants are crucial to obtain the intermediate products **2** and **3** with good to excellent yields. Following this method, the coupling reaction was carried out in THF instead of in an amine as the solvent; the side homocoupling reaction of the alkyne was prevented almost completely by slow addition of the alkyne; a slight excess of trimethylsilylalkyne (1.5 equiv) was used; the palladium catalyst (5 mol %) was stabilized by the addition of triphenylphosphine; finally, it turned out to be useful to employ a very small amount (ca. 1 mol %) of copper iodide and to add this as the last component to the reaction mixture.[24] The coupling reaction was carried out for 72−96 h at room temperature in a nitrogen atmosphere, following the reaction by GC analysis. The suspension obtained was worked up to yield the crude products **2** and **3**, which were purified by flash-chromatography (Scheme 1, Experimental Section). Simple deprotection of the Boc group, performed using 3 M HCl in ethyl acetate at room temperature for 96 h (GC analysis),[25] furnished good yields of the hydrochloride salts of trimethylsilylalkyne derivatives **4** and **5** (Experimental Section), which

Table 2. Inhibition of [^{3}H]Flumazenil Specific Binding of Selected Compounds on Rat $\alpha_1\beta_2\gamma_2$, $\alpha_2\beta_2\gamma_2$, and $\alpha_5\beta_3\gamma_2$ $GABA_A$/Bz Receptor Subtypes[a]

	K_i (nM) or % inhibition (10 μM)[b,c]		
no.	$\alpha_1\beta_2\gamma_2$	$\alpha_2\beta_2\gamma_2$	$\alpha_5\beta_3\gamma_2$
Ia	346 ± 29	39% ± 3	46% ± 5
Ib	65 ± 5	32% ± 3	44% ± 4
11	31.3 ± 2	0%	0%
12	559 ± 44	1600 ± 145	40% ± 3
14	17 ± 1	81 ± 7	1445 ± 120
20	250 ± 19	414 ± 31	1154 ± 99
22	881 ± 78	16% ± 2	13% ± 3
24	1175 ± 95	7% ± 1	28% ± 2
25	125 ± 10	553 ± 45	943 ± 80
31	15 ± 1	175 ± 12	498 ± 37
zolpidem	50 ± 3	765 ± 63	35% ± 3

[a] The ability of the compounds to displace [^{3}H]flumazenil was measured in membranes from HEK293 cells expressing the $\alpha_1\beta_2\gamma_2$, $\alpha_2\beta_2\gamma_2$, and $\alpha_5\beta_3\gamma_2$ subtypes, as described in the Experimental Section. [b] K_i values are means ± SEM of three determinations carried out in triplicate. [c] Percentage inhibition values of specific [^{3}H]flumazenil binding at 10 μM concentration are means ± SEM of three determinations carried out in triplicate.

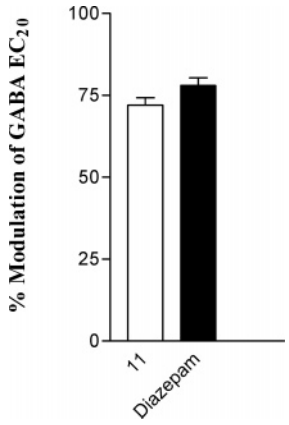


Figure 2. Maximal efficacy of compound **11** on rat recombinant $\alpha_1\beta_2\gamma_2$ $GABA_A$ receptor subtype. Results are expressed as percentages of increase in response to GABA at EC_{20} (mean ± SEM) from at least five independent experiments.

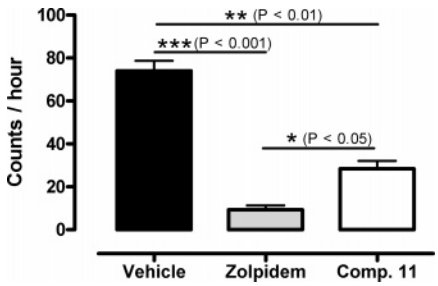


Figure 3. Histograms indicate the values of counts/h recorded in the groups of mice treated with vehicle, zolpidem, or compound **11**. The vertical bars indicate the standard errors. The asterisks represent the levels of statistical significance.

were utilized in the next reaction without further purification. Silyl cleavage was performed using tetrabutylammonium fluoride (TBAF) 0.1 M in THF at room temperature.[26] After 1 h (GC analysis) the reaction mixture was worked up to afford analytically pure amines **6** and **7**, which were characterized as hydrochloride salts (Experimental Section).

The condensation of amines **4**−**7** with the appropriate indolylglyoxylyl chloride **8** and **9** was achieved using the same experimental procedure utilized for the preparation of derivatives **10**−**15** and **24**−**37**, giving the desired compounds **16**−**23** with high yields (Scheme 2).

All target products **10**−**37** 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 analyses (Supporting Information).

Biological Studies. The binding affinity of the newly synthesized indolylglyoxylamide derivatives **10**−**37** at the BzR in bovine brain membranes was determined by competition experiments against the radiolabeled antagonist [^{3}H]flumazenil[27] and was expressed as the K_i value only for those compounds inhibiting radioligand binding by more than 80% at a fixed concentration of 10 μM (Table 1).

Subtype selectivities of selected compounds (**11**, **12**, **14**, **20**, **22**, **24**, **25**, and **31**) and benzyl derivatives **Ia** and **Ib** were evaluated by assaying their ability to displace [^{3}H]flumazenil in membranes from HEK293 cells expressing rat α_1, α_2, and α_5 BzR subtypes (Table 2).[15]

The functional efficacy of compound **11** was determined by measuring its modulatory effect on $^{36}Cl^-$ influx through the ion channel pore at a GABA concentration evoking 20% maximum influx (EC_{20}) in cloned HEK293 cells expressing the α_1 BzR subtype, as described by Harris et al.[28] A concentration 100 times greater than the compound K_i value provided the maximal effect on GABA-evoked $^{36}Cl^-$ influx.[29] Efficacy results are shown in Figure 2, in which the nonselective full agonist diazepam was included as a standard.

In order to evaluate a potential sedative activity, the pharmacodynamic profile of the selected compound **11** was examined by means of a behavioral test on mice. In particular, the influence of compound **11** (10 mg/kg ip) on the spontaneous motor activity of the animals was analyzed and compared with that induced by the same dose of the α_1-selective reference drug zolpidem, through the well-known test of "intermittent observations" (Figure 3).[30]

Results and Discussion

Table 1 lists the affinity values of the new indole derivatives **10**−**37** and those of the reference indoles **Ia** and **Ib**. These binding data show that the same amide side chain produces different effects on affinity, depending on whether the 5-position of the indole nucleus is substituted ($R_5 = NO_2$) or not ($R_5 = H$). This trend of the affinity confirms that 5-NO_2 indolylglyoxylamides bind to the BzR differently from their 5-H counterparts (according to modes A and B, respectively) (Figure 1).

The much higher affinity of the 5-H derivative **14** bearing a bromine in the 4′-position of the phenyl ring with respect to its 5-NO_2 analogue **15** is in agreement with previous findings that an electron-attracting group in the para position of the phenyl ring strongly disfavors binding mode A (compare **15** with **Ib**), while it slightly favors binding mode B (compare **14** with **Ia**).[15]

A methyl group in the 4′-position of the phenyl ring slightly increases affinity if $R_5 = NO_2$ (compare **11** with **Ib**), whereas it lowers affinity 5-fold if $R_5 = H$ (compare **10** with **Ia**). When the 4′-methyl is replaced by the bulkier 4′-ethyl, 4′-ethynyl, and 4′-(1-propynyl) groups, affinity is lost by the 5-NO_2 derivatives **13**, **21**, and **23** but is retained at a submicromolar or micromolar value by the 5-H derivatives **12**, **20**, and **22**. The excessive dimensions of the 4′-trimethylsilylalkynyl substituents are probably responsible for the inactivity of both 5-NO_2 and 5-H derivatives **16**−**19**.

According to the SARs so far outlined, the L_{Di} region appears to be longer than the L_2 one because the former can accom-

modate the lengthy 4′-substituents of 5-H indoles **12**, **20**, and **22** (oriented in binding mode B), whereas the latter cannot host the same 4′-substituents of the 5-NO_2 counterparts **13**, **21**, and **23** (oriented according to binding mode A). When the size of the 4′-substituent increases above a certain threshold, as in compounds **16**−**19**, both the L_{Di} and the L_2 pockets reveal their steric limits and affinity is lost in both 5-NO_2 and 5-H series.

In the same way as observed for *N*-benzyl compounds **11**−**23**, steric hindrance is a crucial determinant of affinity also for the *N*-alkyl derivatives **24**−**37**. However, in the latter subset, affinity seems to depend not only on the overall length and width but also on the shape of the side chain. Particularly, in the 5-NO_2 series (binding according to mode A) the *n*-butyl group perfectly replaces the benzyl one, as compound **25** is equipotent to **Ib**, but insertion of one or two additional methylene units in the alkyl chain lowers or completely disrupts affinity (see compounds **27** and **29**). The same N-substituents are still tolerated when included in the 5-H counterparts **24**, **26**, and **28** (binding with micromolar affinity according to mode B), confirming that the L_{Di} region is longer than the L_2 one, which should have a precise length, with the result that affinity is abolished when the amide side chain increases above a certain threshold.

α-Branching is tolerated within the L_1/L_2 pocket when included in the 5-NO_2 derivatives **31**, **33**, and **35** (binding with high or moderate affinity according to mode A), while it is sterically repelled by the L_{Di} region when included in the 5-H counterparts **30**, **32**, and **34** (binding with low or very poor affinity according to mode B), in agreement with earlier work on *N*-(α-methylbenzyl)indol-3-ylglyoxylamide derivatives.[15] Finally, the shape of the *β*-branched *N*-*i*-butyl groups of compounds **36** and **37** is unsuited for both L_{Di} and L_1/L_2 lipophilic cavities.

It is worth pointing out that the *N*-benzyl side chain can be successfully replaced by the *N*-alkyl moieties in the series of 5-NO_2 indoles (compare compounds **25**, **31**, and **33** with **Ib**) but not in the series of 5-H indoles, which are all less potent than the reference compound **Ia**. These data might be related to the different shapes and volumes of the L_1/L_2 and L_{Di} regions. As far as our indoles are concerned, the L_1/L_2 pocket might host either the aromatic or the aliphatic N-side chains of 5-NO_2 derivatives (oriented in binding mode A) as well as the fused benzene ring of the 5-H derivatives (oriented in binding mode B). By contrast, the L_{Di} cavity is able to accommodate the fused benzene ring of the 5-NO_2 indoles as well as the *N*-benzyl side chains of 5-H ligands but is not sterically suited for several *N*-alkyl chains of the 5-H indoles. In other words, besides being long (compared with the L_2 one), the L_{Di} region appears to be mainly flat and can be roughly described as a "benzyl-like" cavity. In fact, any deviation from this "best" substituent in the 5-H indoles so far investigated has unfavorable effects on affinity. Accordingly, several molecular modeling studies[17,18] have shown that most of the BzR ligands fill the L_{Di} region with aromatic moieties (e.g., a fused benzene ring of *β*-carbolines, CGS compounds, dihydropyridodiindoles) or small alkyl groups (e.g., a methyl of zolpidem and of the triazolopyrimidine CL 218872).

Selected representative indole derivatives (**11**, **12**, **14**, **20**, **22**, **24**, **25**, and **31**), together with the reference compounds **Ia** and **Ib**, were tested for their affinity at α_1, α_2, and α_5 BzR subtypes. All these ligands showed fair to high selectivity affinities for α_1 over α_2 and α_5 subtypes (Table 2). A good correlation exists between the affinities at wild-type and α_1-subtype receptors. The best result in terms of both α_1 affinity and selectivity is represented by compound **11**, which displays a K_i of 31.3 nM at the α_1 subtype and no appreciable binding at the α_2 or α_5 isoforms.

These data indicate that our indolylglyoxylamides, due to their strong interaction with the L_{Di} lipophilic region (in either of the binding modes A or B), maintain α_1 selectivity independently of the extent of the interaction with the L_1/L_2 lipophilic pockets. This is in agreement with literature reports, where the same effect was observed in several series of ligands, such as *β*-carbolines[31] and pyrazolo[4,3-c]quinolin-3-ones.[17,18,32]

Compound **11** was selected to be screened for functional efficacy, and as shown in Figure 2, its efficacy on coapplication with GABA at the BzR subtype was similar to that of standard diazepam (72% ± 2% and 78% ± 3% for compound **11** and diazepam, respectively), showing that the main feature of compound **11** is a full agonist efficacy profile at this receptor subtype.

Due to its efficacy profile, compound **11** should exert sedative effects in vivo. This hypothesis was preliminarily assessed by a behavioral model based on the observation of the spontaneous motor activity of mice,[30] employing the α_1-selective zolpidem[33] as the reference drug (Figure 3). The control groups of mice (vehicle) showed a spontaneous motor activity which was measured as 74 ± 5 counts/h. The spontaneous motor activity was found to decrease significantly and markedly in the groups of mice treated with zolpidem or compound **11** (9 ± 2 and 28 ± 4 counts/h, respectively). These results demonstrate that compound **11**, although less active than zolpidem, is a sedative−hypnotic agent.

In conclusion, none of the structural changes so far made in the indolylglyoxylamides have yielded any shift of selectivity from the α_1 subtype toward the α_2 or α_5 subtypes, thus confirming that a ligand which favorably occupies the L_{Di} region exhibits α_1 selectivity, despite its interactions with other lipophilic areas in the receptor binding cleft. Compound **11** has been identified as an affinity-based α_1-selective ligand displaying a full agonist efficacy profile in vitro and sedative−hypnotic activity in vivo.

Experimental Section

Chemistry. Melting points were determined using a Reichert Köfler hot-stage apparatus and are uncorrected. Infrared spectra were recorded with an FTIR spectrometer Nicolet/Avatar in Nujol mulls. Routine nuclear magnetic resonance spectra were recorded in DMSO-d_6 solution on a Varian Gemini 200 spectrometer operating at 200 MHz. Evaporation was performed in vacuo (rotary 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. GC analysis was performed with a Shimadzu 17A gas chromatograph equipped with a flame ionization detector, SPB-5 Supelco column (15 m × 0.53 mm); operating conditions: low isotherm 170 °C (5 min), high isotherm 280 °C (15 min), temperature scaled up 9 °C/min, injection temperature 250 °C, detector temperature 260 °C, carrier nitrogen 12 cm/s. Elemental analyses were performed by our analytical laboratory and agreed with theoretical values to within ±0.4%.

All 4-substituted benzylamines are commercially available products with the exception of products **4**−**7**. *N*-Boc-4′-bromobenzylamine **1** was prepared in accordance with ref 23.

General Procedure for the Synthesis of N-Substituted 5-Substituted Indol-3-ylglyoxylamide Derivatives 10−37. Triethylamine (3.0 mmol) was added dropwise to a stirred suspension, cooled at 0 °C, of indolylglyoxylyl chloride **8** and **9**[20] (2.5 mmol) and the appropriate amine (2.75 mmol) in 50 mL of dry toluene. The reaction mixture was left to warm to room temperature, stirred for 24−36 h (TLC analysis), and then filtered. The precipitate collected was triturated with a saturated $NaHCO_3$ aqueous solution, washed

with water, and collected again to give a first portion of crude product. The toluene solution was evaporated to dryness, and the residue was treated with saturated $NaHCO_3$ aqueous solution, washed with water, and collected to yield an additional amount of crude product. The quantities of amide derivatives obtained from the initial insoluble precipitate or from the toluene solution were variable, depending upon the solubility of the various compounds. All products **10**−**37** were finally purified by recrystallization from the appropriate solvent. Yields, recrystallization solvents, melting points, and analytical and spectral data are reported in the Supporting Information.

General Procedure for the Synthesis of (4-Trimethylsilylalkyn-1-ylbenzylamine)carbamic Acid *tert*-Butyl Ester Derivatives 2 and 3. Bis(triphenylphosphine)palladium(II) dichloride (0.182 g, 0.26 mmol), triphenylphosphine (0.034 g, 0.13 mmol), and triethylamine (1.10 mL, 7.9 mmol) were added to a stirred mixture of 1.50 g (5.25 mmol) of *N*-Boc-4-bromobenzylamine **1**[23] in 18 mL of dry THF under an atmosphere of nitrogen. A solution of 7.9 mmol of the appropriate alkyne (trimethylsilylacetylene, 3-trimethylsilyl-1-propyne) in 6 mL of dry THF was then added over 1 h. The mixture was stirred for 20 min at room temperature, and 0.012 g (0.063 mmol) of CuI was then added. After being stirred under an atmosphere of nitrogen and at room temperature for 72−96 h (GC analysis), the suspension obtained was filtered at reduced pressure, and the solvent was evaporated. The semisolid residue was then purified by flash-chromatography (eluting system: petroleum ether 60−80 °C/ethyl acetate:8/2), yielding analytically pure products **2** and **3**.

(4-Trimethylsilylethyn-1-ylbenzylamine)carbamic Acid *tert*-Butyl Ester 2 (yield 91%, mp = 91−92 °C). IR, ν cm^{-1}: 3320, 2160, 1680, 1520, 840. ^1H NMR, δ ppm: 0.26 (s, 9H, $Si(CH_3)_3$), 1.47 (s, 9H, $C(CH_3)_3$), 4.32 (d, 2H, CH_2), 4.84 (bs, 1H, CONH, exch with D_2O), 7.25 (d, 2H, 3-H, 5-H), 7.44 (d, 2H, 2-H, 6-H). Anal. ($C_{17}H_{25}NO_2Si$) C, H, N.

(4-Trimethylsilylprop-1-ynylbenzylamine)carbamic Acid *tert*-Butyl Ester 3 (yield 89%, mp = 30 °C). IR, ν cm^{-1}: 3340, 2210, 1710, 1500, 850. ^1H NMR, δ ppm: 0.16 (s, 9H, $Si(CH_3)_3$), 1.46 (s, 9H, $C(CH_3)_3$), 1.70 (s, 2H, CH_2Si), 4.13 (d, 2H, CH_2NH), 4.85 (bs, 1H, CONH, exch with D_2O), 7.27 (d, 2H, 3-H, 5-H), 7.32 (d, 2H, 2-H, 6-H). Anal. ($C_{18}H_{27}NO_2Si$) C, H, N.

General Procedure for the Synthesis of 4-(Trimethylsilylalkyn-1-yl)benzylamine Derivatives 4 and 5. Compounds **2** and **3** (0.48 mmol) were dissolved in 25 mL of ethyl acetate and treated with 10 mL of 3 M HCl. After being stirred at room temperature for 96 h (GC analysis), the mixture was treated with water, basified with solid K_2CO_3, and extracted with $CHCl_3$. The organic phase was dried over Na_2SO_4 and evaporated at reduced pressure, yielding practically pure amino derivatives **4** and **5** as hydrochloride salts (Supporting Information), which were utilized in the next reaction without further purification.

4-(Trimethylsilylethynyl)benzylamine Hydrochloride 4·HCl (yield 80%, mp = 238−240 °C). IR, ν cm^{-1}: 3320, 2160, 1245, 840. ^1H NMR, δ ppm: 0.25 (s, 9H, $3CH_3$), 3.94 (s, 2H, CH_2), 7.40 (d, 2H, 3-H, 5-H), 7.46 (d, 2H, 2-H, 6-H), 8.50 (bs, 3H, NH_3^+, exch with D_2O). Anal. ($C_{12}H_{18}ClNSi$) C, H, N.

4-(Trimethylsilylprop-1-ynyl)benzylamine Hydrochloride 5·HCl (yield 83%, mp = >300 °C). IR, ν cm^{-1}: 3390, 2060, 1250, 840. ^1H NMR, δ ppm: 0.17 (s, 9H, $3CH_3$), 1.76 (s, 2H, CH_2Si), 4.00 (s, 2H, $CH_2NH_3^+$), 7.29−7.43 (m, 4H, Ar−H), 8.44 (bs, 3H, NH_3^+, exch with D_2O). Anal. ($C_{13}H_{20}ClNSi$) C, H, N.

General Procedure for the Synthesis of 4-Alkyn-1-ylbenzylamine Derivatives 6 and 7. The trimethylsilyl compounds **4** and **5** (10.0 mmol) in 1.5 mL of dry THF were treated with 0.33 mL of 0.1 M Bu_4NF in THF (3.3 mmol). The suspension was stirred at room temperature for 1 h (GC analysis), after which the mixture was added to H_2O (10 mL) and extracted with $CHCl_3$. The organic phase was dried over Na_2SO_4 and evaporated at reduced pressure, furnishing the analytically pure derivatives **6** and **7**, which were utilized in the next reaction without further purification. Samples of **6** and **7** were characterized as hydrochloride salts.

4-Ethynylbenzylamine Hydrochloride 6·HCl (yield 81%, mp = 222−224 °C). IR, ν cm^{-1}: 3300, 2040, 1100, 840. ^1H NMR, δ ppm: 4.06 (s, 2H, CH_2), 4.26 (s, 1H, CH), 7.45 (d, 2H, 3-H, 5-H), 7.53 (d, 2H, 2-H, 6-H), 8.35 (bs, 3H, NH_3^+, exch with D_2O). Anal. ($C_9H_{10}ClN$) C, H, N.

4-(Prop-1-ynyl)benzylamine Hydrochloride 7·HCl (yield 86%, mp = 253−255 °C). IR, ν cm^{-1}: 3400, 2050, 1110, 830. ^1H NMR, δ ppm: 2.04 (s, 3H, CH_3), 4.00 (s, 2H, CH_2), 7.43−7.48 (m, 4H, Ar−H), 8.41 (bs, 3H, NH_3^+, exch with D_2O). Anal. ($C_{10}H_{12}ClN$) C, H, N.

Biological Procedures. 1. Radioligand 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 34. 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.[35]

[^3H]Flumazenil binding studies were performed as previously reported.[15]

HEK293 cells stably expressing rat $GABA_A$ receptor subtypes ($\alpha_1\beta_2\gamma_2$, $\alpha_3\beta_2\gamma_2$, $\alpha_5\beta_3\gamma_2$) were maintained, as previously described[36] in DMEM/nut mix F-12 with Glut-I (GIBCO), supplemented with 10% fetal bovine serum, L-glutamine (2 mM), penicillin (100 units/mL), and streptomycin (100 μg/mL) in a humidified atmosphere of 5% CO_2/95% air at 37 °C. Cells were harvested and then centrifuged at 500*g*. The crude membranes were prepared after homogenization in 10 mM potassium phosphate, pH 7.4, and differential centrifugation at 48 000*g* for 30 min at 4 °C. The pellets were washed twice in this manner before final resuspension in 10 mM potassium phosphate, pH 7.4, containing 100 mM potassium chloride.[36]

[^3H]Flumazenil binding assays to transfected cell membranes were carried out as previously described.[36] In brief, the cell line membranes were incubated in a volume of 500 μL which contained [^3H]flumazenil at a concentration of 1−2 nM and the test compound in the range of 10^{-9} to 10^{-5} M. Nonspecific binding was defined by 10^{-5} M diazepam. Assays were incubated to equilibrium for 1 h at 4 °C.

2. Functional Efficacy Studies. $^{36}Cl^-$ uptake was measured in transfected HEK293 cells as previously described with minor modifications.[37] In brief, cells were plated into each well at a density of 10×10^5 cells/well and were grown for 2 days at 37 °C with 5% CO_2 in DMEM/nut mix F-12 with Glut-I (GIBCO), supplemented with 10% fetal bovine serum, penicillin (100 units/mL), and streptomycin (100 μg/mL). Cells were initially washed at room temperature with buffer 136 mM NaCl, 5.4 mM KCl, 1.4 mM $MgCl_2$, 1.2 mM $CaCl_2$, 1 mM NaH_2PO_4, and 20 mM 4-(2-hydroxyethyl)-1-piperazin ethane sulfonic acid, adjusted to a pH of 7.4 with Tris base. Cells were then incubated in 0.2 mL of a $^{36}Cl^-$ solution (0.4 μCi/mL) containing the compound, with or without 100 nM GABA. Influx was terminated after 8 s by aspiration of incubation solution and immediate washing with 0.2 mL of ice-cold picrotoxin stop buffer. Then, 1.6 mL of 0.2 N NaOH was rapidly placed and left overnight. A 0.1 mL aliquot was removed and assayed for protein determination. The remaining 1.5 mL was neutralized with 0.3 mL of 1 N acetic acid, and 20 mL of BioSafe II was added for counting by liquid scintillation spectrometry. Values for $^{36}Cl^-$ influx were expressed as nanomol/mg protein.

3. Behavioral Test. The spontaneous motor activity was evaluated through a slight modification of the test of "intermittent observations".[30] Briefly, 27 adult male BalbC mice (about 40 g of body weight) were used for the pharmacological test, which was performed in three different sessions. In each session, nine mice (about 40 g) were divided into groups of three mice. Mice of the first group received the reference drug zolpidem (10 mg/kg ip), those of the second group received compound **11** (10 mg/kg ip), and those of the third group received the equivalent amount of vehicle (10 mL/kg of 10% DMSO, 60% PEG 400, 30% physiological saline solution). In order to avoid any possible influence

of the circadian rhythm, the experiments were performed only between 9:00 a.m. and 1:00 p.m. Each group was placed in a glass cage (20 cm × 40 cm). Twenty minutes after the ip injection, a video movie was recorded for 1 h by means of a video camera placed above the cages.

Data collection was performed with a blind procedure by three different observers. By means of the video movie, the observers looked at each cage for 1 s with 1 min intervals and counted if none, one, two, or all three mice showed any characteristic spontaneous motor activity (such as locomotion, rearing, grooming, or sniffing). Each observer collected the experimental results in the absence of the other observers and without knowing their findings. The data were obtained as counts/h and were expressed as means ± standard error, deriving from the results collected by the three observers in all the experimental sessions. The data were statistically analyzed by the two-way Student's *t*-test for unpaired data. A value of $P < 0.05$ was considered as representative of statistically significant differences.

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Supporting Information Available: Physical (Table 1), spectral (Table 2), and analytical data (Appendix) of **10**−**37**. This material is available free of charge via the Internet at http://pubs.acs.org.

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