Brief Articles

N,*N*-Dialkyl-2-phenylindol-3-ylglyoxylamides. A New Class of Potent and Selective Ligands at the Peripheral Benzodiazepine Receptor

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We report the synthesis and the affinity data at both the peripheral (PBR) and the central benzodiazepine receptors of a series of *N*,*N*-dialkyl-2-phenylindol-3-ylglyoxylamide derivatives **III**, designed as conformationally constrained analogues of 2-phenylindole-3-acetamides **II** such as FGIN-1-27. Most of the new compounds showed a high specificity and affinity for PBR, with K_i in the nanomolar to subnanomolar range. The most potent ligands (**4**−**7**, **9**, **13**−**27**) stimulated steroid biosynthesis in rat C6 glioma cells with a potency similar to or higher than that of classical ligands. The SARs of this new class of compounds are discussed.

Introduction

Benzodiazepines (Bz) are among the most widely prescribed drugs in the treatment of anxiety and sleep disorders, eliciting their action by binding to an allosteric site located on the γ-aminobutyric acid-A ($GABA_A$) receptor complex, the central benzodiazepine receptor (BzR).[1] Since Bz cause several unwanted side effects, an alternative approach to the identification of new anxiolytics has recently focused on ligands acting at other sites of the $GABA_A$ complex. Neurosteroids positively modulate GABA neurotransmission, eliciting nonsedative anxiolytic effects beneficial for the memory, learning, and emotional processes. In the central nervous system their concentration is increased by ligands at the peripheral benzodiazepine receptor (PBR) such as PK 11195, alpidem, Ro5-4864, and FGIN-1-27 (Figure 1), which facilitate the rate-limiting transport of cholesterol into mitochondria, where it is converted into pregnenolone, the parent molecule of endogenous steroids.[2−5] Being involved for several years in the search for new anxiolytics, we reported several indolylglyoxylamides **I** (Figure 1) as BzR ligands provided by agonist/partial agonist efficacy.[6] It is worth noting the structural similarity of indolylglyoxylamides **I** with the 2-arylindol-3-acetamides **II** such as FGIN-1-27 (Figure 1), reported by Kozikowski et al.[5] as PBR selective high-affinity ligands. Prompted by this observation, we prepared and tested a series of *N*,*N*-dialkyl-2-phenylindol-3-ylglyoxylamide derivatives **III** (Figure 1) designed as conformationally constrained analogues of the indoleacetamides **II** that could hopefully exhibit higher affinity at the PBR.

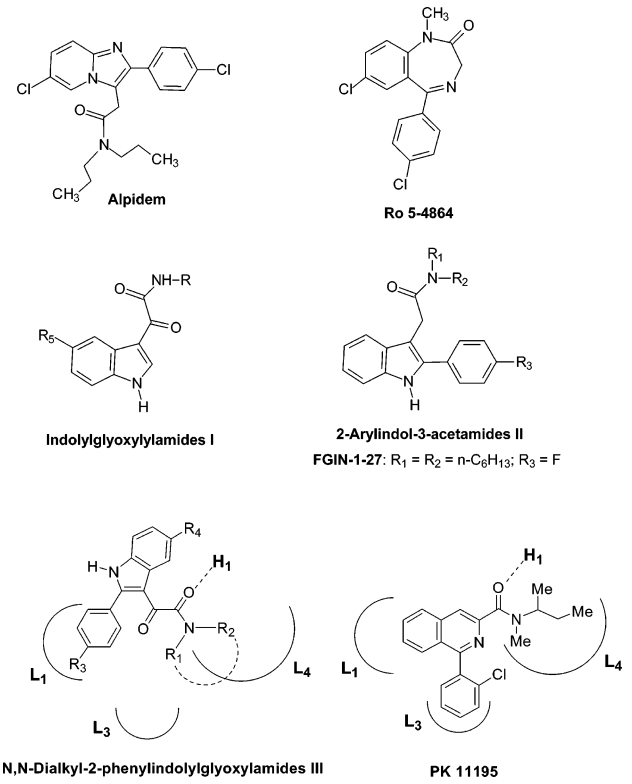

Figure 1. Structures of PBR ligands and pharmacophore/topological model.[8]

Chemistry

The intermediates for the synthesis of the *N*,*N*-dialkyl-2-phenylindol-3-ylglyoxylamides **1**−**27** were the 2-arylindoles **32**−**37**, of which **33** and **35**−**37** were obtained through a modified Madelung indole synthesis[7] (Scheme 1). The general procedure employed to prepare **1**−**27** involved the acylation of the 2-phenylindoles **32**−

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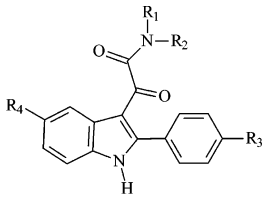
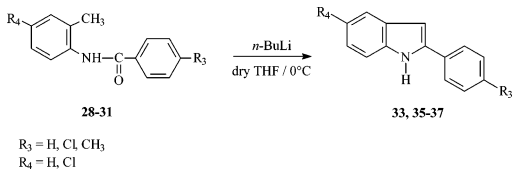
Table 1. Receptor Binding Affinity of Compounds **1**−**27** for PBR, BzR, and Their Stimulatory Effects on Pregnenolone Biosynthesis



compd	R_1	R_2	R_3	R_4	K_i (nM) or inhibition (%) PBR[a]	K_i (nM) or inhibition (%) BzR[b]	increase of pregnenolone production vs control (%)[c]
1	$(CH_2)_2CH_3$	H	H	H	815 ± 80	3929 ± 381	
2	$(CH_2)_3CH_3$	H	H	H	1167 ± 99	12%	
3	CH_2CH_3	CH_2CH_3	H	H	43.0 ± 4	6%	
4	$(CH_2)_2CH_3$	$(CH_2)_2CH_3$	H	H	12.2 ± 1.0	16%	8 ± 1
5	$(CH_2)_3CH_3$	$(CH_2)_3CH_3$	H	H	7.50 ± 0.7	3%	23 ± 3
6	$(CH_2)_4CH_3$	$(CH_2)_4CH_3$	H	H	16.0 ± 2.0	3%	35 ± 4
7	$(CH_2)_5CH_3$	$(CH_2)_5CH_3$	H	H	1.40 ± 0.2	10%	30 ± 3
8	$CH(CH_3)_2$	$CH(CH_3)_2$	H	H	103 ± 9.0	4%	
9	$CH(CH_3)CH_2CH_3$	$CH(CH_3)CH_2CH_3$	H	H	17.0 ± 2.0	5%	10 ± 2
10		$-(CH_2)_4-$	H	H	2400 ± 125	4%	
11		$-(CH_2)_5-$	H	H	665 ± 30	3%	
12		$-(CH_2)_6-$	H	H	33.0 ± 3.0	5%	
13	$(CH_2)_2CH_3$	$(CH_2)_2CH_3$	Cl	H	4.65 ± 0.52	14%	40 ± 5
14	$(CH_2)_3CH_3$	$(CH_2)_3CH_3$	Cl	H	1.00 ± 0.27	0%	55 ± 6
15	$(CH_2)_5CH_3$	$(CH_2)_5CH_3$	Cl	H	0.55 ± 0.19	4%	109 ± 9
16	$(CH_2)_2CH_3$	$(CH_2)_2CH_3$	F	H	4.28 ± 0.32	4%	20 ± 4
17	$(CH_2)_3CH_3$	$(CH_2)_3CH_3$	F	H	2.40 ± 0.81	0%	26 ± 3
18	$(CH_2)_5CH_3$	$(CH_2)_5CH_3$	F	H	0.37 ± 0.13	0%	62 ± 7
19	$(CH_2)_2CH_3$	$(CH_2)_2CH_3$	CH_3	H	5.50 ± 0.38	7%	148 ± 12
20	$(CH_2)_3CH_3$	$(CH_2)_3CH_3$	CH_3	H	3.80 ± 0.91	11%	58 ± 8
21	$(CH_2)_5CH_3$	$(CH_2)_5CH_3$	CH_3	H	1.60 ± 0.13	6%	77 ± 9
22	$(CH_2)_2CH_3$	$(CH_2)_2CH_3$	H	Cl	2.80 ± 0.3	10%	10 ± 2
23	$(CH_2)_3CH_3$	$(CH_2)_3CH_3$	H	Cl	4.91 ± 0.4	13%	5 ± 1
24	$(CH_2)_5CH_3$	$(CH_2)_5CH_3$	H	Cl	58.4 ± 6	3%	31 ± 3
25	$(CH_2)_2CH_3$	$(CH_2)_2CH_3$	Cl	Cl	0.62 ± 0.06	5%	49 ± 6
26	$(CH_2)_3CH_3$	$(CH_2)_3CH_3$	Cl	Cl	1.9 ± 0.2	0%	147 ± 13
27	$(CH_2)_5CH_3$	$(CH_2)_5CH_3$	Cl	Cl	5.8 ± 0.6	3%	166 ± 12
PK 11195					9.3 ± 0.5		48 ± 5
Ro 5-4864					23 ± 3.1		41 ± 4
alpidem					0.5−7	1−28	

[a] The concentration of tested compounds that inhibited [^{3}H]PK11195 binding to rat kidney mitochondrial membranes (IC_{50}) by 50% was determined with six concentrations of the displacers, each performed in triplicate. K_i values are the mean ± SEM of three determinations. [b] The inhibition percent of [^{3}H]flumazenil specific binding at 10 μM of the compound are the mean ± SEM of five determinations. K_i values are the mean ± SEM of three determinations. [c] C6 glioma cells were incubated for 2 h at 37 °C in the presence of each compound. Pregnenolone was quantified by radioimmunoassay as described in the text. The values represent the mean ± SEM of at least three determinations. For comparison, the effects of PK 11195 and Ro5-4864 on pregnenolone production are also included.

Scheme 1



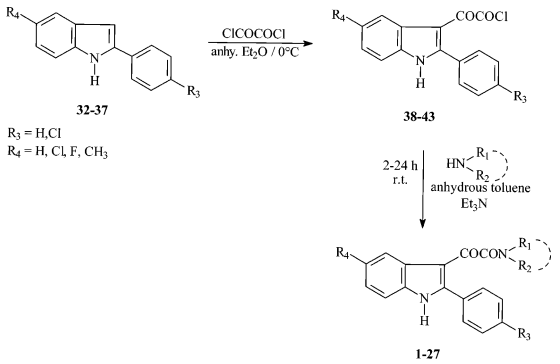
37 with oxalyl chloride to give the corresponding indolylglyoxylyl chlorides **38**−**43**, which were allowed to react with the appropriate amine in the presence of triethylamine in dry toluene solution (Scheme 2).

Biological Studies

The binding affinity of the indolylglyoxylamides **1**−**27** at the PBR was determined in rat kidney membranes by competition experiments against [^{3}H]PK 11195. The PBR/BzR selectivity of **1**−**27** was evaluated by binding studies using membranes from rat brain tissues and [^{3}H]flumazenil as radioligand.

Furthermore, a subset of high-affinity compounds was examined for their ability to stimulate pregnenolone formation from rat C6 glioma cells. All experimental details are given in Supporting Information.

Scheme 2



Results and Discussion

Most of the compounds **1**−**27** showed a high affinity for PBR in the nanomolar to subnanomolar range and a high selectivity for PBR over BzR because they inhibited the binding of [^{3}H]flumazenil at rat brain membranes to an extent varying from 0% to 16% at a fixed 10 μM concentration (Table 1). Noticeably, the indolylglyoxylamides displayed a gain in affinity values

of at least 1 order of magnitude with respect to their 2-phenylindole-3-acetamide counterparts **II**.[5]

SARs will be discussed in light of our pharmacophore/topological model of the PBR binding site[8] made up of three lipophilic pockets (L_1, L_3, and L_4) and a H-bond donor group (H_1) (see Figure 1).

Among the unsubstituted indolylglyoxylamides **1**−**12** ($R_3 = R_4 = H$), the least effective were the N-monosubstituted derivatives **1** and **2** probably because they cannot occupy both the L_3 and L_4 lipophilic pockets. Thus, we did not further examine this type of substitution.

The N,N-disubstituted derivatives **3**−**9** exhibited nanomolar affinity because they fill both the L_3 and L_4 sites. Compound **7**, bearing two *n*-hexyl groups, was the most potent one, with a K_i of 1.4 nM. A progressive enhancement of affinity was generally observed by increasing the length of the linear *N*-alkyl groups (**3**−**7**), due to the progressively better filling of both the L_3 and L_4 lipophilic pockets, with **6** being the only exception. Branching of the *N*,*N*-dialkyl groups (**8** and **9**) gave mixed results. Compound **8** (K_i = 103 nM) showed a surprising drop in affinity if compared with its unbranched counterpart **3** (K_i = 43 nM), while product **9** maintained the affinity with respect to **4**. This affinity difference between **8** and **9** does not find any easy rational explanation either from the point of view of the lipophilicity or from that of the steric hindrance. In any case, the affinity data of **3**−**9** suggest that interactions at the L_3 and L_4 sites are governed primarily by lipophilicity and that steric factors may also play some role.

Also, in the subseries of the cyclic amides **10**−**12**, the affinity was enhanced with increasing dimensions of the cycle. However, **10** and **11** showed a considerable drop in affinity with respect to the linear derivatives **3** and **4** probably because of their incapability of optimally filling both the L_3 and L_4 sites, which are separated in the binding site, while the more lipophilic and flexible **12** displayed an appreciable affinity (K_i = 33 nM), again confirming the role of lipophilicity in the interaction of these ligands with the receptor site.

The three most potent derivatives **4**, **5**, and **7** were selected as leads for further affinity optimization efforts. Insertion of the electron-donating lipophilic methyl group in the 4′-position of the 2-phenyl ring (R_3) left the affinity nearly unchanged because **19**−**21** were equipotent with the parent compounds **4**, **5**, and **7**. By introducing an electron-withdrawing substituent such as chlorine or fluorine into the 4′-position, we obtained **13**−**15** or **16**−**18**, respectively, which exhibited a gain in affinity of 2.5-fold to 7.5-fold. Among them, the *N*,*N*-di-*n*-hexyl derivative **18** stood out as the most potent of all the ligands, with a K_i of 0.37 nM, similar to that of alpidem. These results suggest that the phenyl ring of 2-phenylindoles might be involved in a π-stacking interaction with an electron-rich aromatic ring within the L_1 pocket and that this interaction is reinforced by 4′ electron-withdrawing substituents such as the halogens.

In the 4′-phenyl-substituted indoles, potency correlated with the *N*-alkyl length: *n*-hexyl > *n*-butyl > *n*-propyl. This affinity trend was reversed when a chlorine was introduced in the 5-position of the indole nucleus: the longer the side chains, the lower the potency. Among the 5-chloro-4′-phenyl-unsubstituted derivatives **22**−**24**, the highest potency was exhibited by **22** (K_i = 2.8 nM), which bears two *n*-propyl groups on the amide nitrogen. Insertion of a chlorine in both the 5 and 4′ positions improved potency with respect to the unsubstituted derivatives when the amide chain featured *n*-propyl or *n*-butyl groups (compare **25** and **26** vs **4** and **5**); otherwise, it was unfavorable (compare **27** vs **7**, both bearing *n*-hexyl groups). In other words, the effects on affinity of a chlorine in the 5 and 4′ positions are not additive but depend on the nature of the *N*,*N*-dialkyl chains. It is tempting to speculate that the length of the 5,4′-dichloro-2-phenylindole scaffold within the binding site restricts the mobility of the whole ligand within the binding site. Under these steric constraints, the L_3 and L_4 pockets are optimally filled by the smaller *n*-propyl or *n*-butyl, compared with the bulkier *n*-hexyl chains. To sum up, the lack of additive effects of substituents NR_1R_2, R_3, and R_4 is somehow related to a simultaneous increase in their dimensions, which makes each single interaction at the three lipophilic sites less effective.

The most potent indolylglyoxylamides (**4**−**7**, **9**, **13**−**27**) were tested for their ability to increase pregnenolone concentration in rat C6 glioma cells in comparison with PK 11195 and Ro5-4864 (Table 1). Products **19**, **26**, and **27** proved to be highly effective in increasing pregnenolone production by more than 145% vs controls. Several compounds (**13**, **14**, **18**, **20**, **21**, and **25**) increased pregnenolone accumulation with a potency similar to or slightly higher than that of PK 11195 and Ro5-4864. As a general trend, unsubstituted indolylglyoxylamides (**4**−**7**, **9**) did not produce a great effect on the pregnenolone level, whereas a better performance was displayed by the 4′-substituted derivatives **13**−**21** and **25**−**27**.

Experimental Section

Chemistry. General directions are in the Supporting Information.

General Procedure for the Synthesis of *N*,*N*-Dialkyl-[5-substituted-2-(4-substitutedphenyl)indol-3-yl]glyoxylamide Derivatives 1−27. A solution of the appropriate amine (2.75 mmol) in 50 mL of dry toluene was added dropwise to a stirred suspension, at 0 °C, of chloride **38**−**43** (2.5 mmol) and triethylamine (3.0 mmol) in 50 mL of the same solvent. The reaction mixture was left to warm to room temperature, stirred for 2−24 h (TLC analysis), and then filtered. In the case of products **1** and **2**, the precipitate was triturated with a saturated $NaHCO_3$ aqueous solution and washed with water to give the first portion of crude product. The toluene solution was washed with diluted HCl and then with a saturated $NaHCO_3$ aqueous solution and water, dried ($MgSO_4$), and evaporated to dryness to yield crude **3**−**27**.

All products **1**−**27** were purified by recrystallization from the appropriate solvent or by flash chromatography (eluting system: petroleum benzine (60−80 °C) and ethyl acetate in varying ratios). Yields, recrystallization solvents, melting points, and spectral data are listed in Tables 1 and 2 in the Supporting Information.

Supporting Information Available: General chemistry directions, synthesis and physical properties of compounds **33** and **35**−**43**, Tables 1 and 2 containing yields, recrystallization solvents, melting points, the IR and ^1H NMR spectral data of **1**−**27**, and biological methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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