# Anxiolytic-like Effects of *N,N*-Dialkyl-2-phenylindol-3-ylglyoxylamides by Modulation of Translocator Protein Promoting Neurosteroid Biosynthesis

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Novel *N,N*-disubstituted indol-3-ylglyoxylamides (**1**−**56**), bearing different combinations of substituents $R_1$−$R_5$, were synthesized and evaluated as ligands of the translocator protein (TSPO), the 18 kDa protein representing the minimal functional unit of the "peripheral-type benzodiazepine receptor" (PBR). Most of the new compounds showed a nanomolar/subnanomolar affinity for TSPO and stimulated steroid biosynthesis in rat C6 glioma cells with a potency similar to or higher than that of classic TSPO ligands such as PK 11195. Moreover, when evaluated in vivo by means of the elevated-plus-maze (EPM) paradigm in the rat, compound **32**, the best-performing derivative in terms of TSPO affinity and pregnenolone production, showed clear anxiolytic effects. The results of this study suggested that the novel *N,N*-disubstituted indol-3-ylglyoxylamides may represent a promising class of compounds potentially suited for the treatment of anxiety disorders.

## Introduction

Anxiety and sleep disorders can be controlled by the administration of benzodiazepines, such as diazepam and chlordiazepoxide, which produce their therapeutic effects by enhancing the affinity of $\gamma$-aminobutyric acid (GABA[a]) toward the central benzodiazepine receptor (BzR). The clinical usefulness of these relatively safe drugs is limited by unwanted side effects, including ataxia, ethanol potentiation, dizziness, amnesia, and the risk of dependence with long-term use.[1,2] Therefore, there is still a need for new and safer anxiolytic agents.

Several benzodiazepines bind to a receptor distinct from the BzR, originally identified outside the central nervous system (CNS) and therefore called "peripheral-type benzodiazepine receptor" (PBR).[3] The PBR was subsequently described as a multimeric complex made up of three subunits: a protein of 18 kDa hosting the diazepam binding site (PBR), a 32 kDa voltage-dependent anion channel (VDAC) and a 30 kDa adenine nucleotide carrier (ANC).[4,5] Although the PBR has a wide distribution in several peripheral tissues, especially those producing steroids,[4,5] it is also present in the CNS, where it is mainly expressed in glial cells and, at lower levels, in neurons.[6] Inside the cell, the PBR is primarily localized in the outer/inner mitochondrial membrane as a component of the megachannel responsible for mitochondrial permeability transition (MPT).[7]

The PBR is involved in a variety of biological processes, including calcium homeostasis, lipid metabolism, mitochondrial oxidation, cell growth and differentiation, apoptosis induction, and regulation of immune functions.[5,8] The basal expression of this receptor is up-regulated in a number of neuropathologies, including gliomas and neurodegenerative disorders, such as Alzheimer's disease, as well as in various forms of brain injury and inflammation.[9] Changes in the PBR receptor levels have also been found in patients affected by generalized anxiety, panic, post-traumatic stress, obsessive−compulsive disorders, and separation anxiety.[5,10,11]

The PBR is involved in the regulation of cholesterol translocation from the outer to the inner mitochondrial membrane, a process known to be the rate-determining step in steroid biosynthesis.[4] For this reason, Papadopoulos et al. proposed "translocator protein" (TSPO) as a new name for the 18 kDa protein representing the minimal functional unit of the PBR.[12] This term is used throughout the present paper.

High-affinity TSPO ligands, such as PK 11195,[13] alpidem,[14] and FGIN-1-27[15,16] (Chart 1), increase the levels of the so-called neurosteroids, such as pregnenolone and allopregnanolone, acting as positive allosteric modulators of GABA neurotransmission. Thus, neurosteroidogenic TSPO ligands may represent a significant advance in the development of novel pharmacological agents displaying an anxiolytic-like profile, without the unwanted side effects typical of classic benzodiazepines.[9]

We have recently described the *N,N*-dialkyl-2-phenylindol-3-ylglyoxylamides **I** (Chart 1)[17] as a new class of potent and selective TSPO ligands. The SARs of these compounds were rationalized in light of a pharmacophore/topological model made up of three lipophilic pockets (L1, L3, and L4) and an H-bond donor group (Figure 1).[18] The optimum binding affinity to TSPO requires two lipophilic substituents on the amide nitrogen ($R_1 = R_2 =$ *n*-propyl, *n*-butyl, *n*-hexyl, etc.). Moreover, substituents in the 4′ and 5 positions of the 2-phenylindole scaffold ($R_3$ and $R_4$, respectively) modulate potency in a manner dependent on the nature of the $R_1$ and $R_2$ substituents.[17] Several of our indole

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[a] Abbreviations: TSPO, translocator protein; PBR, peripheral-type benzodiazepine receptor; EPM, elevated plus-maze; GABA, $\gamma$-aminobutyric acid; MPT, mitochondrial permeability transition; VDAC, voltage-dependent anion channel; ANC, adenine nucleotide carrier.

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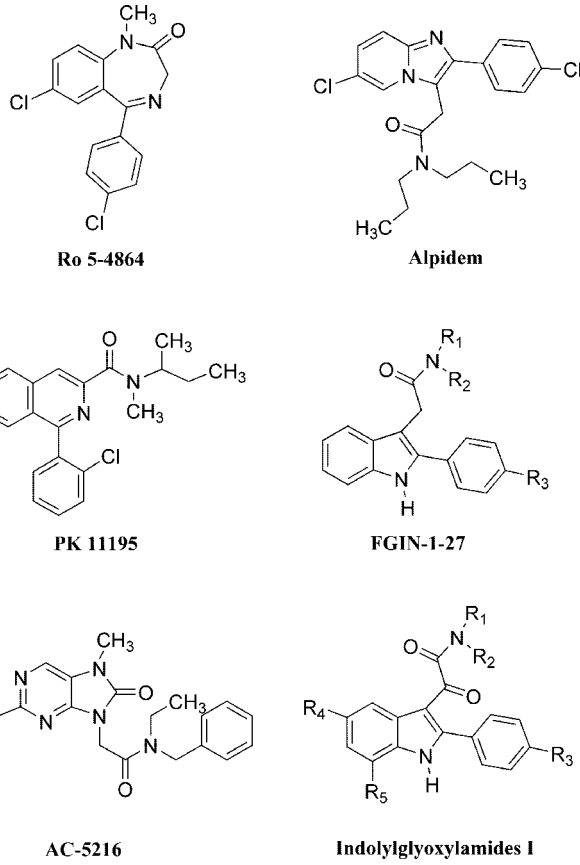
**Chart 1.** Structures of TSPO Ligands

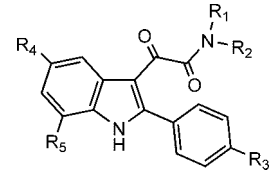



derivatives turned out to be more effective than standard TSPO ligands, such as PK 11195 and FGIN-1-27, in stimulating pregnenolone biosynthesis. Furthermore, the introduction of a fluorophore group linked to the 2-phenylindolylglyoxylamide scaffold gave fluorescent probes useful for investigating the localization and the expression level of TSPO.[19]

The present paper describes the refinement of our TSPO pharmacophore/topological model through the synthesis and the biological evaluation of novel indole derivatives with the general formula **I**, bearing different combinations of substituents $R_1-R_5$. Some of these compounds (**1**−**27**, Table 1) bear linear alkyl chains on the amide nitrogen ($R_1 = R_2 =$ *n*-propyl, *n*-butyl, *n*-hexyl) and modifications to the basic 2-phenylindol-3-ylglyoxylamide scaffold, consisting of the introduction of small groups in the para position of the 2-phenyl ring ($R_3$ = F, $NO_2$, $CF_3$) and the 5-position ($R_4$ = F, Cl, $NO_2$, $OCH_3$) and the 7-position ($R_5$ = Cl, $CH_3$) of the indole ring. A series of *N*-ethyl-*N*-benzyl substituted indoles (**28**−**42**, Table 2) was designed taking as a reference AC-5216 (Chart 1), a TSPO ligand that displays antianxiety activity without the undesirable effects caused by typical benzodiazepines.[20] We also investigated a subset of asymmetrical *N,N*-disubstituted indoles (**43**−**56**, Table 3) to probe the L3 and L4 lipophilic pockets of the TSPO binding site ($R_1$ = methyl, ethyl; $R_2$ = ethyl, *n*-butyl, *n*-pentyl; $R_3$ = H, Cl; $R_4$ = H, Cl).

Most of the compounds were evaluated for their ability to induce pregnenolone biosynthesis in rat C6 glioma cells. The best-performing ligands in terms of TSPO affinity and pregnenolone production were tested in a rat anxiety model to verify whether their ability to increase neurosteroid synthesis in vitro was associated with the production of anxiolytic effects in vivo.

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



| compd | $R_1 = R_2$ | $R_3$ | $R_4$ | $R_5$ | $K_i$ (nM)[a] | increase in pregnenolone production vs control (%)[b] |
|---|---|---|---|---|---|---|
| **1** | $(CH_2)_2CH_3$ | $NO_2$ | H | H | 0.95 ± 0.1 | 70 ± 7 |
| **2** | $(CH_2)_3CH_3$ | $NO_2$ | H | H | 0.27 ± 0.07 | 64 ± 2 |
| **3** | $(CH_2)_5CH_3$ | $NO_2$ | H | H | 0.23 ± 0.1 | 88 ± 7 |
| **4** | $(CH_2)_2CH_3$ | $CF_3$ | H | H | 1.69 ± 0.2 | 66 ± 7 |
| **5** | $(CH_2)_3CH_3$ | $CF_3$ | H | H | 1.16 ± 0.1 | 60 ± 6 |
| **6** | $(CH_2)_5CH_3$ | $CF_3$ | H | H | 1.0 ± 0.1 | 85 ± 5 |
| **7** | $(CH_2)_2CH_3$ | H | $NO_2$ | H | 20.2 ± 2.02 | 91 ± 2 |
| **8** | $(CH_2)_3CH_3$ | H | $NO_2$ | H | 21.6 ± 2.15 | 102 ± 10 |
| **9** | $(CH_2)_5CH_3$ | H | $NO_2$ | H | 30.3 ± 9.15 | 115 ± 10 |
| **10** | $(CH_2)_2CH_3$ | H | $OCH_3$ | H | 328 ± 45 | 45 ± 2 |
| **11** | $(CH_2)_3CH_3$ | H | $OCH_3$ | H | 65.2 ± 3.4 | 36 ± 3 |
| **12** | $(CH_2)_5CH_3$ | H | $OCH_3$ | H | 35.5 ± 8.7 | 87 ± 7 |
| **13** | $(CH_2)_2CH_3$ | H | F | H | 2.67 ± 0.48 | 66 ± 7 |
| **14** | $(CH_2)_3CH_3$ | H | F | H | 4.00 ± 0.15 | 88 ± 9 |
| **15** | $(CH_2)_5CH_3$ | H | F | H | 0.37 ± 0.12 | 36 ± 4 |
| **16** | $(CH_2)_2CH_3$ | F | F | H | 6.73 ± 1.39 | 17 ± 1 |
| **17** | $(CH_2)_3CH_3$ | F | F | H | 4.36 ± 0.05 | 91 ± 8 |
| **18** | $(CH_2)_5CH_3$ | F | F | H | 0.95 ± 0.1 | 40 ± 2 |
| **19** | $(CH_2)_2CH_3$ | F | Cl | H | 2.83 ± 0.08 | 42 ± 4 |
| **20** | $(CH_2)_3CH_3$ | F | Cl | H | 3.05 ± 0.45 | 37 ± 4 |
| **21** | $(CH_2)_5CH_3$ | F | Cl | H | 7.75 ± 1.55 | 135 ± 4 |
| **22** | $(CH_2)_2CH_3$ | H | H | Cl | 14.0 ± 1.5 | 0 |
| **23** | $(CH_2)_3CH_3$ | H | H | Cl | 3.40 ± 0.3 | 0 |
| **24** | $(CH_2)_5CH_3$ | H | H | Cl | 2.4 ± 0.3 | 0 |
| **25** | $(CH_2)_2CH_3$ | H | H | $CH_3$ | 25.0 ± 3.0 | 129 ± 13 |
| **26** | $(CH_2)_3CH_3$ | H | H | $CH_3$ | 6.0 ± 0.6 | 71 ± 8 |
| **27** | $(CH_2)_5CH_3$ | H | H | $CH_3$ | 1.90 ± 0.1 | 63 ± 5 |
| **Ia**[c] | $(CH_2)_2CH_3$ | H | H | H | 12.2 ± 1.0 | 8 ± 1 |
| **Ib**[c] | $(CH_2)_3CH_3$ | H | H | H | 7.5 ± 0.7 | 23 ± 3 |
| **Ic**[c] | $(CH_2)_5CH_3$ | H | H | H | 1.40 ± 0.2 | 30 ± 3 |
| PK 11195 | | | | | 9.3 ± 0.5 | 48 ± 5 |
| Ro5-4864 | | | | | 23 ± 3.1 | 41 ± 4 |
| alpidem | | | | | 0.5−7 | |

[a] The concentration of test 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] C6 glioma cells were incubated for 2 h at 37 °C in the presence of each compound. Pregnenolone was quantified by enzymatic immunoassay, 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. [c] Data taken from ref 17.

## Chemistry

The target *N,N*-dialkyl-2-phenylindol-3-ylglyoxylamides **1**−**56** were synthesized through the 2-phenylindoles **67**−**81**, which in turn were obtained, with the exception of the commercially available 2-phenylindole **67** and 2-(4-fluorophenyl)indole **68**, following experimental procedures reported in literature. The preparation of indoles **69**−**72** (see Experimental Section) was performed essentially as described in our previous work.[17]

2-(4-Nitrophenyl)indole **73** and 2-(4-trifluoromethylphenyl)indole **74** were prepared with a one-step Fischer indole synthesis by warming phenylhydrazine hydrochloride and 4′-nitro- or 4′-(trifluoromethyl)acetophenone with an excess of polyphosphoric

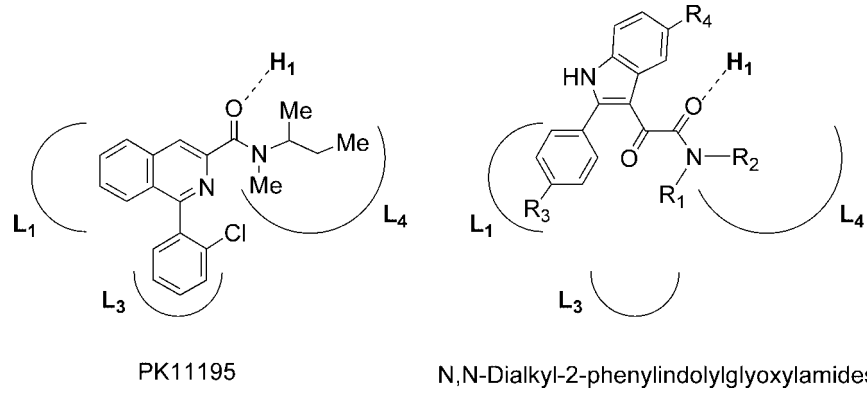
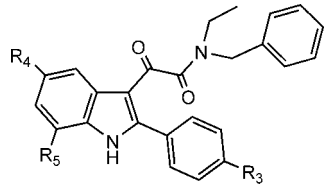



**Figure 1.** TSPO pharmacophore/topological model.[18]

**Table 2.** Receptor Binding Affinity of Compounds **28**−**42** for TSPO and Their Stimulatory Effects on Pregnenolone Biosynthesis




| compd | $R_3$ | $R_4$ | $R_5$ | $K_i$ (nM)[a] | increase in pregnenolone production vs control (%)[b] |
|---|---|---|---|---|---|
| **28** | H | H | H | 11 ± 1.0 | 20 ± 2 |
| **29** | F | H | H | 1.68 ± 0.12 | 96 ± 8 |
| **30** | Cl | H | H | 1.30 ± 0.05 | 136 ± 11 |
| **31** | H | Cl | H | 4.6 ± 0.5 | 85 ± 8 |
| **32** | Cl | Cl | H | 3.33 ± 0.3 | 171 ± 14 |
| **33** | $CH_3$ | H | H | 2.64 ± 0.1 | 86 ± 7 |
| **34** | $NO_2$ | H | H | 0.55 ± 0.02 | 103 ± 8 |
| **35** | $CF_3$ | H | H | 1.0 ± 0.1 | nd |
| **36** | H | $NO_2$ | H | 18.3 ± 0.15 | 0 |
| **37** | H | $OCH_3$ | H | 69.5 ± 3.6 | nd |
| **38** | H | F | H | 1.33 ± 0.2 | nd |
| **39** | F | F | H | 1.67 ± 0.37 | nd |
| **40** | F | Cl | H | 4.01 ± 0.26 | 0 |
| **41** | H | H | Cl | 5.0 ± 0.4 | 0 |
| **42** | H | H | $CH_3$ | 2.30 ± 0.2 | 0 |
| PK 11195 | | | | 9.3 ± 0.5 | 48 ± 5 |
| Ro5-4864 | | | | 23 ± 3.1 | 41 ± 4 |
| alpidem | | | | 0.5−7 | |

[a] The concentration of test 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] C6 glioma cells were incubated for 2 h at 37 °C in the presence of each compound. Pregnenolone was quantified by enzymatic immunoassay, 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.

acid (PPA) (Scheme 1). Nitration of 2-phenylindole **67** furnished the 5-nitro-2-phenylindole **75** (Scheme 1). The indole derivatives **76**−**81** (see Experimental Section) were obtained through a Houlihan[21] modification of the Madelung indole synthesis from the appropriate amides **61**−**66**, obtained by acylation of the corresponding 2-methylanilines (Scheme 1; all experimental data of amides **61**−**66** and indoles **73**−**75** are reported in the Supporting Information). In brief, amides **61**−**66** were transformed into the corresponding indole derivatives by reaction with 3 molar equiv of *n*-butyllithium in tetrahydrofuran at −20 °C and were then stirred at room temperature for 24−48 h. The crude indole derivatives recovered after workup were purified by flash chromatography and/or by recrystallization from the appropriate solvent (Supporting Information).

The general synthetic procedure used in the preparation of derivatives **1**−**56** is shown in Scheme 2 and involved the acylation of the appropriate 2-phenylindole **67**−**81** with oxalyl chloride, in anhydrous ethyl ether, at room temperature to obtain the corresponding 2-phenylindolylglyoxylyl chlorides **82**−**96**.[22] The acyl chlorides **88**−**92**, **94**−**96** have never been described before in literature and were characterized as their corresponding ethyl ester derivatives (Supporting Information). The indolylglyoxylyl chlorides **82**−**96** were allowed to react at room temperature with the appropriate dialkylamine or with *N*-ethyl-*N*-benzylamine in the presence of triethylamine in dry toluene solution to give products **1**−**56**, which were purified by recrystallization from the appropriate solvent or by flash chromatography. Their structures were confirmed by IR, $^1$H NMR, and elemental analysis (Supporting Information).

## Biological and Pharmacological Studies

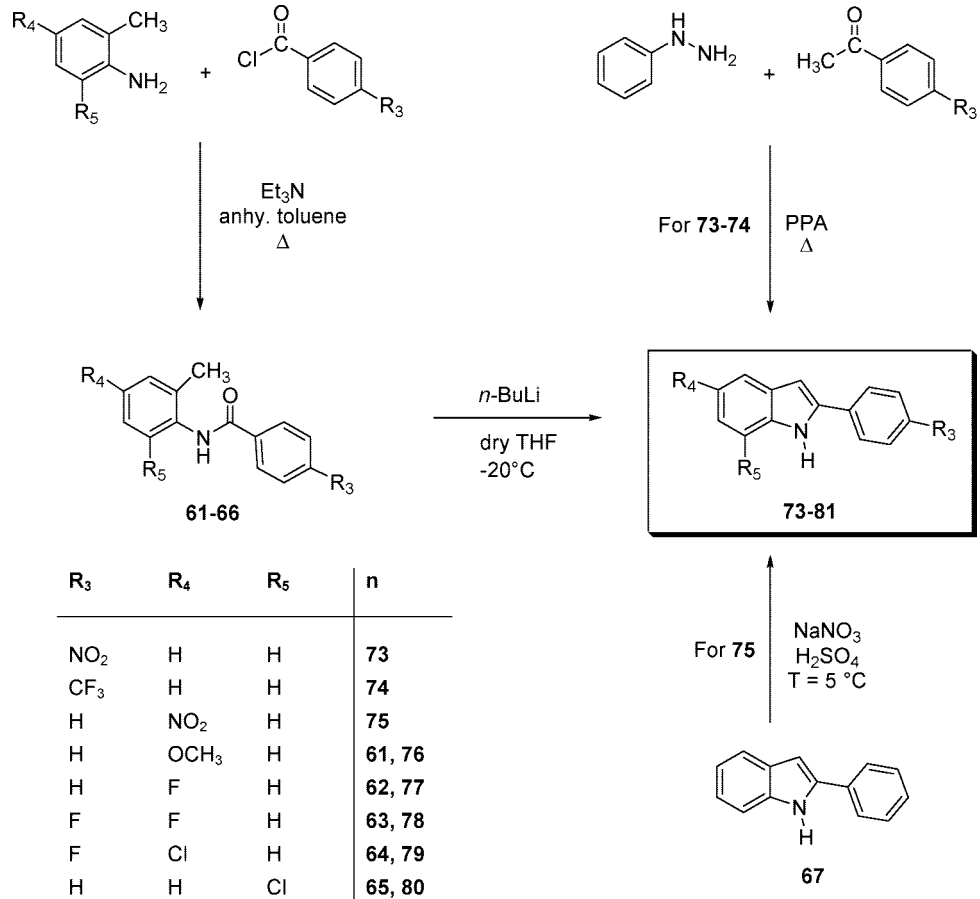
The binding affinity of all the newly synthesized indolylglyoxylamides **1**−**56** at the TSPO was determined in rat kidney membranes by competition experiments against [$^3$H]PK 11195.[17] Because of the well-established TSPO versus BzR selectivity of *N,N*-dialkyl-2-phenylindol-3-ylglyoxylamides,[17] a few randomly selected indole derivatives were evaluated for their BzR affinity, using membranes from rat brain tissues and [$^3$H]Ro15-1788 as the radioligand. The compounds tested showed no significant binding properties in this assay (data not shown).

A subset of TSPO high-affinity ligands was examined for the ligands' ability to stimulate pregnenolone formation from rat C6 glioma cells at 40 $\mu$M concentration. This high concentration of compounds with respect to their nanomolar affinity is necessary to induce pregnenolone production, as demonstrated by previously reported analogous studies.[17,18,23] The best-performing compounds, **21** and **32**, were evaluated for their potential in vivo anxiolytic activity by means of the elevated-plus-maze (EPM) paradigm in the rat. Both compounds were tested at a dose of 30 mg/kg (ip), prompted by similar in vivo experiments on 2-phenylimidazo[1,2-*a*]pyridine derivatives possessing TSPO affinity.[24] Additionally, the effects of compound **21** were also evaluated at a dose of 50 mg/kg (ip).

## Results and Discussion

The binding affinities of compounds **1**−**27**, **28**−**42**, and **43**−**56**, expressed as $K_i$ values, are given in Tables 1, 2, and 3, respectively, together with the $K_i$ values of the standard TSPO ligands PK 11195, Ro5-4864, and alpidem (Chart 1). The binding data of some of the previously investigated indole derivatives[17] are included for comparison at the bottom of Table 1 (**Ia**−**c**) and Table 3 (**Ia**,**d**−**f**). Tables 1−3 also include

**Scheme 1.** Synthesis of 2-Phenylindoles **73**−**81**




| $R_3$ | $R_4$ | $R_5$ | n |
|---|---|---|---|
| $NO_2$ | H | H | **73** |
| $CF_3$ | H | H | **74** |
| H | $NO_2$ | H | **75** |
| H | $OCH_3$ | H | **61, 76** |
| H | F | H | **62, 77** |
| F | F | H | **63, 78** |
| F | Cl | H | **64, 79** |
| H | H | Cl | **65, 80** |
| H | H | $CH_3$ | **66, 81** |

the neurosteroidogenic activity of most of the newly synthesized compounds, expressed as percent value vs control of the increase in pregnenolone production.

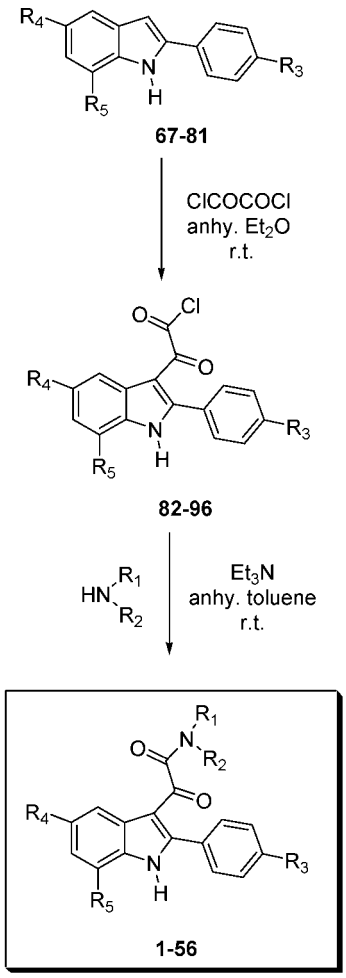
In series **1**−**27**, the amide nitrogen is symmetrically disubstituted ($R_1 = R_2$) with *n*-propyl, *n*-butyl, and *n*-hexyl chains. These compounds generally showed a high affinity for TSPO in the nanomolar/subnanomolar range. With the exception of derivatives **9** and **21**, the highest potency was achieved with $R_1 = R_2 =$ *n*-hexyl. Introduction of a 4′-$NO_2$ group on the 2-phenyl ring gave the most potent indolylglyoxylamides within this subset: **2** and **3** with a $K_i$ of 0.27 and 0.23 nM, respectively. A 4′-$CF_3$ group was likewise beneficial for potency (compare **4**−**6** with **Ia**−**c**), although to a lesser extent compared with the 4′-$NO_2$ group. These data confirmed the favorable influence on affinity exerted by an electron-withdrawing $R_3$ substituent. This effect was ascribed by us to a putative $\pi$-stacking interaction between the 2-phenyl and an electron-rich aromatic ring within the L1 pocket.[17] The affinity was lowered by an $NO_2$ or an $OCH_3$ group in the 5-position of the indole nucleus ($R_4$) (compare **7**−**12** with **Ia**−**c**) or, in contrast, enhanced by an F in the same position (compare **13**−**15** with **Ia**−**c**). These data suggest that $R_4$ has to be electron-withdrawing and also very small for optimal binding to TSPO, a combination of properties featured only by fluorine. Substitutions in the 4′- and 5-positions were merged to yield **16**−**18** ($R_3 = R_4 =$ F) and **19**−**21** ($R_3 =$ F, $R_4 =$ Cl). Two halogens in both the 4′- and 5-positions did not increase affinity in an additive manner, all the 4′,5-dihalo derivatives being almost as potent or slightly less potent than the 5-fluoro derivatives **13**−**15**. Insertion of an electron-withdrawing (Cl) or an electron-donating ($CH_3$) lipophilic group in the 7-position of the indole nucleus ($R_5$) did not produce any gain in affinity (compare **22**−**27** with their unsubstituted counterparts **Ia**−**c**).[17]

Compounds **1**−**21** displayed fair to high steroidogenic activity, with **21** performing best (135% increase in pregnenolone production under conditions in which the activities of PK 11195 and Ro 5-4864 were 48% and 41%, respectively). Consequently, **21** was selected for in vivo studies (vide infra). It is noted that TSPO affinity did not generally correlate with the steroidogenic activity (this holds true for all the indoles studied in the present paper, as well for those previously described[17]). On the other hand, the nature of the 7-substituent was decisive in the pregnenolone biosynthesis assay, as the 7-chloro derivatives **22**−**24** were devoid of any activity, whereas the 7-methyl derivatives **25**−**27** were good stimulators of pregnenolone production. The precise molecular determinants of the above differences in activity are unknown. At the moment, we can only speculate about an electronic-based triggering effect exerted by $R_5$ on the translocation function of the TSPO.

Further attempts to optimize potency focused on asymmetrically *N*,*N*-substituted indoles ($R_1 \neq R_2$).

A first subset of *N*-benzyl-*N*-ethyl derivatives (**28**−**42** in Table 2) revealed that this combination of $R_1$ and $R_2$ led to affinities roughly similar to those exhibited by *N*,*N*-di-*n*-butyl derivatives. Such a conclusion can be drawn by comparing the $K_i$ values of **28**−**42** with our previously reported symmetrically *N*,*N*-substituted indoles.[17] Thus, it is likely that the aromatic moiety of the *N*-benzyl chain is accommodated within the lipophilic pocket L3, which also hosts the phenyl ring of PK 11195, in agreement with our pharmacophore/topological model

**Scheme 2.** Synthesis of *N*,*N*-Dialkyl-2-phenylindol-3-ylglyoxylamides **1**−**56**




**Acyl chlorides 82-96**

| $R_3$ | $R_4$ | $R_5$ | n |
|---|---|---|---|
| H | H | H | **82** |
| F | H | H | **83** |
| Cl | H | H | **84** |
| H | Cl | H | **85** |
| Cl | Cl | H | **86** |
| $CH_3$ | H | H | **87** |
| $NO_2$ | H | H | **88** |
| $CF_3$ | H | H | **89** |
| H | $NO_2$ | H | **90** |
| H | $OCH_3$ | H | **91** |
| H | F | H | **92** |
| F | F | H | **93** |
| F | Cl | H | **94** |
| H | H | Cl | **95** |
| H | H | $CH_3$ | **96** |

**Substitution pattern of derivatives 1-27 (n)**

| $R_3$ | $R_4$ | $R_5$ | $R_1 = R_2$ $(CH_2)_2CH_3$ | $R_1 = R_2$ $(CH_2)_3CH_3$ | $R_1 = R_2$ $(CH_2)_5CH_3$ |
|---|---|---|---|---|---|
| $NO_2$ | H | H | **1** | **2** | **3** |
| $CF_3$ | H | H | **4** | **5** | **6** |
| H | $NO_2$ | H | **7** | **8** | **9** |
| H | $OCH_3$ | H | **10** | **11** | **12** |
| H | F | H | **13** | **14** | **15** |
| F | F | H | **16** | **17** | **18** |
| F | Cl | H | **19** | **20** | **21** |
| H | H | Cl | **22** | **23** | **24** |
| H | H | $CH_3$ | **25** | **26** | **27** |

**Substitution pattern of derivatives 28-42 (n)**

| $R_3$ | $R_4$ | $R_5$ | $R_1 = CH_2CH_3$ $R_2 = CH_2C_6H_5$ |
|---|---|---|---|
| H | H | H | **28** |
| F | H | H | **29** |
| Cl | H | H | **30** |
| H | Cl | H | **31** |
| Cl | Cl | H | **32** |
| $CH_3$ | H | H | **33** |
| $NO_2$ | H | H | **34** |
| $CF_3$ | H | H | **35** |
| H | $NO_2$ | H | **36** |
| H | $OCH_3$ | H | **37** |
| H | F | H | **38** |
| F | F | H | **39** |
| F | Cl | H | **40** |
| H | H | Cl | **41** |
| H | H | $CH_3$ | **42** |

**Substitution pattern of derivatives 43-56 (n)**

| $R_3$ | $R_4$ | $R_1 = CH_3$ $R_2 = CH_2CH_3$ | $R_1 = CH_3$ $R_2 = (CH_2)_3CH_3$ |
|---|---|---|---|
| H | H | **43** | **45** |
| Cl | Cl | **44** | **48** |
| Cl | H | | **51** |
| H | Cl | | **54** |

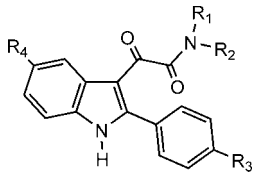
| $R_3$ | $R_4$ | $R_1 = CH_3$ $R_2 = (CH_2)_4CH_3$ | $R_1 = CH_2CH_3$ $R_2 = (CH_2)_2CH_3$ |
|---|---|---|---|
| H | H | **46** | **47** |
| Cl | Cl | **49** | **50** |
| Cl | H | **52** | **53** |
| H | Cl | **55** | **56** |

depicted in Figure 1.[18] Such a benzyl gives rise to the same type of hydrophobic interactions established by an aliphatic moiety of similar size. In this subset, the effects of $R_3$−$R_5$ on the affinity were similar to those observed in the series of symmetrical *N*,*N*-dialkyl derivatives **1**−**27** listed in Table 1. Thus, **34** ($R_3 = NO_2$, $R_4 = R_5 = H$) was the most potent ($K_i$ = 0.55 nM), whereas **37** ($R_3 = H$, $R_4 = 5\text{-}OCH_3$, $R_5 = H$) was the least potent ($K_i$ = 69.5 nM).

The highest levels of steroidogenic activity were displayed by **30**, **32**, and **34**. Among these three compounds, **32** was tested for its in vivo anxiolytic properties (vide infra).

Table 3 lists the binding data of asymmetrical *N*,*N*-dialkyl derivatives (**43**−**56**) designed to evaluate whether differences in the lengths of $R_1$ and $R_2$ could optimize their fit into the lipophilic pockets L3 and L4. For this purpose, the unsubstituted and the 4′,5-dichloro, the 4′-chloro, and the 5-chloro substitution patterns were chosen as representative within the previously investigated *N*,*N*-dialkylindoles (see for comparison **Ia**,**d**−**f** in Table 3).[17] Four combinations of $R_1$ and $R_2$ were selected: methy/ethyl (**43** and **44**), methyl/*n*-butyl (**45**, **48**, **51**, **54**), methyl/*n*-pentyl (**46**, **49**, **52**, **55**), and ethyl/*n*-butyl (**47**, **50**, **53**, **56**). The 4′,5-dichloro derivatives **48** ($R_1 = CH_3$, $R_2 = (CH_2)_3CH_3$, $K_i$ = 0.15 nM) and **49** ($R_1 = CH_3$, $R_2 = (CH_2)_4CH_3$, $K_i$ = 0.18 nM) turned out to be the most potent indolylglyoxylamides so far described by us.[17] These results indicate that the L3 and L4 pockets differ in their dimensions, and that $R_1$ and $R_2$ should

**Table 3.** Receptor Binding Affinity of Compounds **43**−**56** for TSPO and Their Stimulatory Effects on Pregnenolone Biosynthesis



| compd | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $K_i$ (nM)[a] | increase in pregnenolone production vs control (%)[b] |
|---|---|---|---|---|---|---|
| **43** | $CH_3$ | $CH_2CH_3$ | H | H | 940 ± 120 | nd |
| **44** | $CH_3$ | $CH_2CH_3$ | Cl | Cl | 9.54 ± 1.29 | 0 |
| **45** | $CH_3$ | $(CH_2)_3CH_3$ | H | H | 53.3 ± 4.0 | 32 ± 3 |
| **46** | $CH_3$ | $(CH_2)_4CH_3$ | H | H | 12.1 ± 1.0 | 36 ± 4 |
| **47** | $CH_2CH_3$ | $(CH_2)_3CH_3$ | H | H | 12.6 ± 1.0 | 43 ± 5 |
| **48** | $CH_3$ | $(CH_2)_3CH_3$ | Cl | Cl | 0.15 ± 0.02 | 67 ± 2 |
| **49** | $CH_3$ | $(CH_2)_4CH_3$ | Cl | Cl | 0.18 ± 0.02 | 31 ± 2 |
| **50** | $CH_2CH_3$ | $(CH_2)_3CH_3$ | Cl | Cl | 0.36 ± 0.04 | 11 ± 2 |
| **51** | $CH_3$ | $(CH_2)_3CH_3$ | Cl | H | 11 ± 1.0 | nd |
| **52** | $CH_3$ | $(CH_2)_4CH_3$ | Cl | H | 3.4 ± 0.4 | 65 ± 7 |
| **53** | $CH_2CH_3$ | $(CH_2)_3CH_3$ | Cl | H | 3.6 ± 0.4 | 32 ± 3 |
| **54** | $CH_3$ | $(CH_2)_3CH_3$ | H | Cl | 3.9 ± 0.5 | 70 ± 7 |
| **55** | $CH_3$ | $(CH_2)_4CH_3$ | H | Cl | 3.6 ± 0.5 | 76 ± 8 |
| **56** | $CH_2CH_3$ | $(CH_2)_3CH_3$ | H | Cl | 1.8 ± 0.2 | 72 ± 6 |
| **Ia**[c] | $(CH_2)_2CH_3$ | $(CH_2)_2CH_3$ | H | H | 12.2 ± 1.0 | 8 ± 1 |
| **Id**[c] | $(CH_2)_2CH_3$ | $(CH_2)_2CH_3$ | Cl | Cl | 0.62 ± 0.06 | 49 ± 6 |
| **Ie**[c] | $(CH_2)_2CH_3$ | $(CH_2)_2CH_3$ | Cl | H | 4.65 ± 0.52 | 40 ± 5 |
| **If**[c] | $(CH_2)_2CH_3$ | $(CH_2)_2CH_3$ | H | Cl | 2.80 ± 0.3 | 10 ± 2 |
| PK 11195 | | | | | 9.3 ± 0.5 | 48 ± 5 |
| Ro5-4864 | | | | | 23 ± 3.1 | 41 ± 4 |
| alpidem | | | | | 0.5−7 | |

[a] The concentration of test 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] C6 glioma cells were incubated for 2 h at 37 °C in the presence of each compound. Pregnenolone was quantified by enzymatic immunoassay, 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. [c] Data taken from ref 17.

be likewise different in their size. The 4′-chloro and 5-chloro derivatives (**51**−**56**) displayed $K_i$ values between those shown by their unsubstituted counterparts (**45**−**47**) and by the 4′,5-dichloro derivatives (**48**−**50**). Taken together, these data suggest that each chlorine favorably contributes to the affinity to a similar extent.

Most of the compounds in Table 3 showed a low/medium percentage increase in pregnenolone production vs control, despite their high affinities, thus confirming the lack of correlation between potency and steroidogenic activity.

The best-performing compounds in terms of pregnenolone production, **21** and **32**, were evaluated for their potential in vivo anxiolytic effects by means of the EPM paradigm in the rat, which employs an elevated four-arm maze with two open and two enclosed arms and measures the animal's preference for dark, enclosed places (closed arms) over bright, exposed places (open arms).[25] An increase in both number of entries and time spent by the rats in open arms was observed following administration of **32** (30 mg/kg, ip) with respect to rats treated with the vehicle (Figure 2). Notably, **32** was not found to influence the spontaneous exploration of the apparatus by the rats, as the total number of arm entries (in both open and closed arms) performed by rats treated with **32** did not significantly differ from that performed by control rats (9.29 ± 1 for **32** and 10.3 ± 0.7 for control rats, ns). Therefore, the modification in EPM performance observed seemed to be clearly attributable

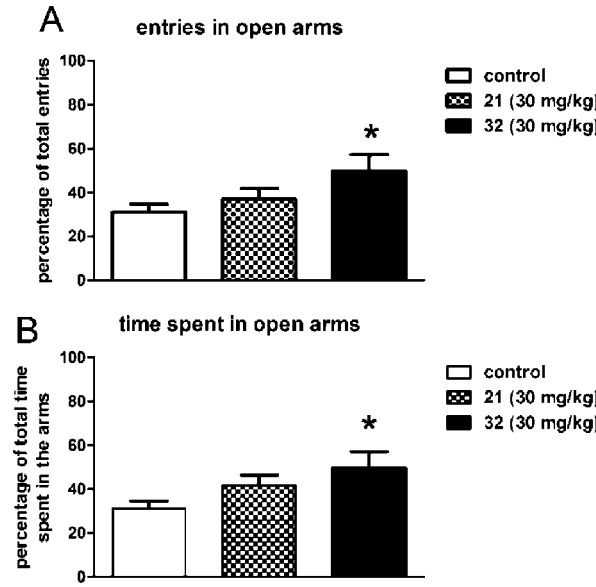



**Figure 2.** Effects of the administration of compounds **21** and **32** on EPM performance in rats. Administration of **32** (30 mg/kg ip) significantly modified rats' EPM performance by increasing both the entries in open arms (A) and the time spent by rats in the open arms (B). On the other hand, administration of **21** (30 mg/kg ip) elicited only a slight, but not significant, enhancement in both entries (A) and time spent by the rats (B) in the EPM open arms: (*) $P < 0.05$ compared with control rats; $N = 5-12$.

to an in vivo anxiolytic effect of the test compound. An increase in EPM open arm exploration is, in fact, a distinctive effect elicited by anxyolitic agents.[25] The effects observed here probably depend on the ability of **32** to increase the in vivo neurosteroid levels, as is suggested by its effect on pregnenolone production in C6 glioma cells and its lack of affinity for the BzR.

The administration of **21** to rats produced different results with respect to those observed following treatment with **32**. Indeed, at a dose of 30 mg/kg, ip, **21** modified neither the number of entries nor the time spent by rats in open arms of the EPM, and in addition, it did not influence rats' spontaneous exploratory activity, as revealed by the lack of difference in arm entries between **21**-treated and control rats (9.8 ± 1 for **21** and 10.3 ± 0.7 for control rats). On the other hand, when administered at the higher dose of 50 mg/kg ip, **21** suppressed the spontaneous exploratory behavior of rats, as shown by a significant reduction in the number of arm entries (2 ± 0.4 for **21** and 10.3 ± 0.7 for control rats, $P < 0.05$) performed by the animals over the 5 min test session. The results obtained following administration of **21** appear to be unexpected, since in vitro studies pointed out the effectiveness of this ligand in eliciting pregnenolone synthesis from C6 glioma cells, although at a lower level than that displayed by **32** (135% vs 171% increase in pregnenolone production vs control; see Tables 1 and 2). In this connection, however, it is emphasized that the in vivo effects of pharmacologically active substances are influenced by several factors and may diverge from those expected on the basis of in vitro experiments. For example, it might be possible that **21** and **32** are metabolized to different extents. Actually, AC-5216 (Chart 1), a TSPO ligand that displays antianxiety activity,[20] shares with **32** the same ethyl/benzyl substitution pattern on the amide nitrogen.

In conclusion, the SAR data deriving from the biological results of the newly synthesized *N,N*-dialkyl-2-phenylindol-3-ylglyoxylamide TSPO ligands **1**−**56** led to a refinement of our

TSPO pharmacophore/topological model: (1) the $R_3$ substituent has to be electron-withdrawing to reinforce a putative $\pi$-stacking interaction between the 2-phenyl and an electron-rich aromatic ring within the L1 pocket; (2) $R_4$ has to be both electron-withdrawing and very small for optimal binding, a combination of properties featured only by fluorine; (3) substitutions in the 7-position of the indole nucleus ($R_5$) do not produce any gain in affinity; (4) an aromatic moiety ($R_1$/$R_2$) is equivalent to an aliphatic moiety of similar size in interacting hydrophobically with the L3 or L4 lipophilic pocket; (5) the L3 and L4 pockets are probably different in their dimensions, as the best-performing substitution pattern on the amide nitrogen is obtained with $R_1$ and $R_2$ of different sizes.

Most of the new compounds exhibited a high affinity for TSPO, with $K_i$ values in the nanomolar/subnanomolar range, and stimulated steroid biosynthesis in rat C6 glioma cells to an extent similar to or higher than that of classic TSPO ligands such as PK 11195, Ro5-4864, and alpidem. Furthermore, in the EPM test, the indole derivative **32** elicited an anxiolytic activity. Taken together, the results of the present study suggest that the novel *N*,*N*-dialkyl-2-phenylindol-3-ylglyoxylamides may represent promising pharmacological tools suited for the treatment of anxiety disorders.

## Experimental Section

**Chemistry.** Melting points were determined using a Reichert Köfler hot-stage apparatus and are uncorrected. Infrared spectra were recorded with a Nicolet-Avatar 360 FT-IR spectrophotometer 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. Elemental analyses were performed by our analytical laboratory and agreed with theoretical values to within ±0.4%.

2-Phenylindole **67** was from Sigma-Aldrich; 2-(4-fluorophenyl)indole **68** was from Lancaster.

The literature data and synthetic procedures regarding the following compounds are described in our previous work:[17] 4-chloro-*N*-(2-methylphenyl)benzamide **57**; *N*-(4-chloro-2-methylphenyl)benzamide **58**; 4-chloro-*N*-(4-chloro-2-methylphenyl)benzamide **59**; 4-methyl-*N*-(2-methylphenyl)benzamide **60**; 2-(4-chlorophenyl)indole **69**; 5-chloro-2-phenylindole **70**; 5-chloro-2-(4-chlorophenyl)indole **71**; 2-(4-methylphenyl)indole **72**; (2-phenylindol-3-yl)glyoxylyl chloride **82**; [2-(4-fluorophenyl)indol-3-yl]glyoxylyl chloride **83**; [2-(4-chlorophenyl)indol-3-yl]glyoxylyl chloride **84**; (5-chloro-2-phenylindol-3-yl)glyoxylyl chloride **85**; [5-chloro-2-(4-chlorophenyl)indol-3-yl]glyoxylyl chloride **86**; [2-(4-methylphenyl)indol-3-yl]glyoxylyl chloride **87**.

The following compounds were prepared in accordance with reported procedures: *N*-(4-methoxy-2-methylphenyl)benzamide **61**; *N*-(4-fluoro-2-methylphenyl)benzamide **62**; 4-fluoro-*N*-(4-fluoro-2-methylphenyl)benzamide **63**; 4-fluoro-*N*-(4-chloro-2-methylphenyl)benzamide **64**; *N*-(2-chloro-6-methylphenyl)benzamide **65**; *N*-(2,6-dimethylphenyl)benzamide **66**; 2-(4-nitrophenyl)indole **73**; 2-(4-trifluoromethylphenyl)indole **74**; 5-nitro-2-phenylindole**75** (all experimental data of amides **61**−**66** and indoles **73**−**75** are reported in the Supporting Information).

**General Procedure for the Synthesis of Substituted 2-Phenylindole Derivatives 76−81.** *n*-Butyllithium 1.6 M solution in hexane (9.3 mL, 15 mmol) was added dropwise over 10−15 min to a stirred solution of the appropriate benzamide **61**−**66** (5.0 mmol) in 25 mL of dry THF and maintained under nitrogen at a temperature from −20 to 0 °C; then the stirred mixture was allowed to warm to room temperature over 1−2 h. The resulting dark-red solution was stirred at room temperature for 24−48 h (TLC analysis), cooled in an ice bath, and acidified to pH 5−6 with 2 N HCl solution. The organic layer was separated, and the aqueous phase was extracted with $CH_2Cl_2$. The combined organic layers were dried over $MgSO_4$, filtered, and evaporated to dryness, furnishing the crude indoles **76**−**81**, which were purified by flash chromatography (eluting system, cyclohexane−ethyl acetate or petroleum ether−dichloromethane in varying ratios) and/or by recrystallization from the appropriate solvent. Yields and melting points of indoles **76**−**81** are reported in the Supporting Information.

**General Procedure for the Synthesis of Substituted (2-Phenylindol-3-yl)glyoxylyl Chloride Derivatives 88−96.** Oxalyl chloride (0.65 mL, 7.5 mmol) was added dropwise, at 0 °C to a well-stirred mixture of the appropriate indole **73**−**81** (3.0 mmol) in 10 mL of freshly distilled ethyl ether. The mixture was maintained at room temperature for 2−24 h (TLC analysis). The precipitate formed was collected and washed with portions of petroleum ether 30−50 °C to give the acyl chlorides **88**−**96**, which were dried over $P_2O_5$ in vacuo and directly used in the subsequent reaction. All the acyl chloride derivatives, except for compound **93**, were characterized by conversion to their corresponding ethyl esters. Yields and melting points of derivatives **88**−**96** and analytical and spectral data of esters of compounds **88**−**92**, **94**−**96** are reported in the Supporting Information.

**General Procedure for the Synthesis of *N*,*N*-Dialkyl-2-(phenylindol-3-yl)glyoxylamide Derivatives 1−56.** A solution of the appropriate amine (2.5 mmol) in 5 mL of dry toluene was added dropwise to a stirred suspension, cooled at 0 °C, of indolylglyoxylyl chloride **82**−**96** (2.5 mmol) in 50 mL of the same solvent, followed by the addition of a solution of triethylamine (0.3 mL, 2.5 mmol) in 5 mL of dry toluene. The reaction mixture, allowed to warm to room temperature, was stirred for 2−24 h (TLC analysis) and then filtered to eliminate the triethylamine hydrochloride formed. The toluene solution was washed with water, dried with $MgSO_4$, and evaporated to dryness to yield the crude compounds **1**−**56**. In the case of the less soluble products, a first portion of the crude compound precipitated together with triethylamine hydrochloride and was collected after washing the solid with water. All products **1**−**56** were purified by recrystallization from the appropriate solvent or by flash chromatography (eluting system, petroleum ether−ethyl acetate or petroleum ether−dichloromethane in varying ratios). Yields, recrystallization solvents, melting points, and spectral data of compounds **1**−**56** are listed in the Supporting Information.

**Biological Methods. Materials.** [$^3$H]PK 11195 (S.A. 85.5 Ci/mmol) and [$^3$H]Ro15-1788 (S.A. 83.4 Ci/mmol) were purchased from Perkin-Elmer Life Sciences. Culture medium, fetal bovine serum (FBS), L-glutamine, and antibiotics were purchased from Cambrex Bio Science. PK 11195 and Ro5-4864 were obtained from Sigma-Aldrich. 1,2,3,4-Tetrahydro-4-oxo-7-chloro-2-naphthylpyridine (SU10603) and 2α,4α,5α,17β-4,5-epoxy-17-hydroxy-3-oxoandrostane-2-carbonitrile (Trilostane) were kindly given by Novartis Pharma Spa and Dr. Zister, University of Dublin. All reagents were obtained from commercial suppliers.

**[$^3$H]PK 11195 Binding to Rat Kidney Mitochondrial Membranes.** For binding studies, crude mitochondrial membranes were incubated with 0,6 nM [$^3$H]PK 11195 in the presence of a compound concentration range (0.1 nM to 10 $\mu$M) in 50 mM Tris-HCl, pH 7.4, as previously described.[17] For the active compounds, the $IC_{50}$ values were determined and $K_i$ values were derived in accordance with the equation of Cheng and Prusoff.[26]

**[$^3$H]Ro15-1788 Binding to Rat Cerebral Cortex Membranes.** Rat cerebral cortex membranes were prepared as previously described.[3] After differential centrifugation, the crude membrane fraction obtained was subjected to washing procedures to remove endogenous GABA.[27] The washed membranes were incubated with 0.4 nM [$^3$H]Ro15-1788 for 90 min at 0 °C in 500 $\mu$L of 50 mM Tris-citrate buffer, pH 7.4, as previously described.[28]

**Cell Culture.** Rat glioma C6 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% FBS, 2 mM L-glutamine, 100 units/mL penicillin, and 100 $\mu$g/mL streptomycin. Cultures were maintained in a humidified atmosphere of 5% $CO_2$/95% air at 37 °C.

**Steroid Biosynthesis.** The pregnenolone production measurement in C6 cells, exposed to the novel compounds or PK 11195 (40 $\mu$M), was performed by competitive enzyme-linked immunoassay (ELISA). Briefly, C6 cells were seeded in 96-well plates at a density of about 10 000 cells/well in a final volume of 100 $\mu$L and the pregnenolone secretion into the medium was performed as previously described.[29] The final concentration of DMSO and ethanol was constant for all the wells within each experiment and did not exceed 0.5% (v/v), a concentration that on its own had no effect on steroid production. At the end of the incubation period (2 h), the cell medium was used in an enzyme immunoassay for the direct quantitative determination of pregnenolone, under the conditions recommended by the supplier (Pregnenolone ELISA, the EiAsy Way, IBL Hamburg, Germany).

**Pharmacology. Subjects.** Male Sprague−Dawley rats (Charles River, Calco, Italy) weighing 180−200 g were used in this study. Animals were housed in groups of 6 per cage in a room with the temperature of 24 ± 1 °C and maintained under a 12 h light/dark cycle (lights on at 8:00 a.m.). Rats had free access to water and food except during the measurement of elevated-plus-maze performance.

All experiments were conducted in accordance with the guidelines for care and use of experimental animals of the European Communities Directive (86/609/EEC; D.L., 27.01.1992, number 116).

**Drugs. 21** and **32** were suspended in 0.5% methylcellulose + 1% Tween-80 in distilled water and were administered ip (1 mL/100 g of body weight).

**Elevated-Plus-Maze Performance.** The elevated plus-maze represents a reliable experimental tool to evaluate anxiety in rodents.[30,31] Briefly, the apparatus was made of white PVC and consisted of two opposite open arms (length 50 cm, width 10 cm) and two opposite closed arms (length 50 cm, width 10 cm), the latter enclosed by 40 cm high walls along their length. The four arms converged to a central square (10 cm × 10 cm), thus reproducing the shape of a plus sign. The apparatus was elevated 50 cm from the floor. Rats, having no prior experience of the elevated plus-maze, were placed in the central square and were left free to explore the whole apparatus for a single 5 min test session. Rat performance was videotaped, and percentages of arm entries as well as of time spent in open and closed arms were calculated with respect to the total number of entries and to the total amount of time spent in the arms, respectively. A rat was considered inside a specific arm when having all the four paws inside the arm. A reduced preference for the open arms is considered as an index of anxiety, and drugs bearing anxiolytic activity increase the preference of the rats for the open arms.[25]

**Statistical Analysis.** Values are reported as the mean ± SEM. Differences between experimental groups were evaluated by Student's *t* test. Significance was set at $p < 0.05$.

**Acknowledgment.** This work was supported by MIUR (cofin. 2004).

**Supporting Information Available:** General procedure for the synthesis of amides **61**−**66**; physical properties and spectral data of benzamides **61**−**66**, 2-phenylindole derivatives **73**−**81**, (2-phenylindol-3-yl)glyoxylyl chlorides **88**−**96**, and their corresponding ethyl esters **97**−**104**; Tables S1−S6 including yields, physical properties, and spectral data of indolylglyoxylamides **1**−**56**; analytical data of target compounds **1**−**56**. This material is available free of charge via the Internet at http://pubs.acs.org.

## References

(1) Costa, E.; Guidotti, A. Benzodiazepine on trial: a research strategy for their rehabilitation. *Trends Pharmacol. Sci.* **1996**, *17*, 192–200.
(2) Da Settimo, F.; Taliani, S.; Trincavelli, M. L.; Montali, M.; Martini, C. $GABA_A$/Bz receptor subtypes as targets for selective drugs. *Curr. Med. Chem.* **2007**, *14*, 2680–2701.
(3) Squires, R. F.; Braestrup, C. Benzodiazepine receptors in rat brain. *Nature* **1977**, *266*, 732–734.
(4) Papadopoulous, V. Peripheral-type benzodiazepine/diazepam binding inhibitor receptor: biological role in steroidogenic cell function. *Endocr. Rev.* **1993**, *14*, 222–240.
(5) Gavish, M.; Bachman, I.; Shoukrun, R.; Katz, Y.; Veenman, L.; Weisinger, G.; Weizman, A. Enigma of the peripheral benzodiazepine receptor. *Pharmacol. Rev.* **1999**, *51*, 619–640.
(6) Veenman, L.; Gavish, M. Peripheral-type benzodiazepine receptors: their implication in brain disease. *Drug Dev. Res.* **2000**, *50*, 355–370.
(7) Culty, M.; Li, H.; Boujrad, N.; Amri, H.; Vidic, B.; Bernassau, J. M.; Reversat, J. L.; Papadopoulos, V. In vitro studies on the role of the peripheral-type benzodiazepine receptor in steroidogenesis. *J. Steroid Biochem. Mol. Biol.* **1999**, *69*, 123–130.
(8) Casellas, P.; Galiegue, S.; Basile, A. S. Peripheral benzodiazepine receptors and mitochondrial function. *Neurochem. Int.* **2002**, *40*, 475–486.
(9) Papadopoulos, V.; Lecanu, L.; Brown, R. C.; Han, Z.; Yao, Z.-X. Peripheral-type benzodiazepine receptor in neurosteroid biosynthesis, neuropathology and neurological disorders. *Neuroscience* **2006**, *138*, 749–756.
(10) Chelli, B.; Pini, S.; Abelli, M.; Cardini, A.; Lari, L.; Muti, M.; Gesi, C.; Cassano, G. B.; Lucacchini, A.; Martini, C. Platelet 18 kDa translocator protein density is reduced in depressed patients with adult separation anxiety. *Eur. Neuropsychopharmacol.* **2008**, *18*, 249–254.
(11) Pini, S.; Martini, C.; Abelli, M.; Muti, M.; Gesi, C.; Montali, M.; Chelli, B.; Lucacchini, A.; Cassano, G. B. Peripheral-type benzodiazepine receptor binding sites in platelets of patients with panic disorder associated to separation anxiety symptoms. *Psycopharmacology (Berlin)* **2005**, *181*, 407–411.
(12) Papadopoulos, V.; Baraldi, M.; Guilarte, T. R.; Knudsen, T. B.; Lacapere, J.-J.; Lindemann, P.; Norenberg, M. D.; Nutt, D.; Weizman, A.; Zhang, M.-R.; Gavish, M. Translocator protein (18 kDa): new nomenclature for the peripheral-type benzodiazepine receptor based on its structure and molecular function. *Trends Pharmacol. Sci.* **2006**, *27*, 402–409.
(13) Le Fur, G.; Vaucher, N.; Perrier, M. L.; Flamier, A.; Benavides, J.; Renault, C.; Dubroeucq, M. C.; Guérémy, C.; Uzan, A. Differentiation between two ligands for peripheral benzodiazepine binding sites, [$^3$H]Ro5-4864 and [$^3$H]PK 11195, by thermodynamic studies. *Life Sci.* **1983**, *33*, 449–457.
(14) Costa, E.; Romeo, E.; Auta, J.; Papadopoulos, V.; Kozikowski, A. P.; Guidotti, A. Is There a Pharmacology of Brain Steroidogenesis? In *Neurosteroid and Brain Function*; Costa, E., Paul, S. M., Eds.; Fidia Research Foundation Symposium Series; Thieme Medical Publisher, Inc.: New York, 1991; pp 171−176.
(15) Romeo, E.; Auta, J.; Kozikowski, A. P.; Ma, D.; Papadopoulos, V.; Puia, G.; Costa, E.; Guidotti, A. 2-Aryl-3-indoleacetamides (FGIN-1): a new class of potent and specific ligands for the mitochondrial DBI receptor (MDR). *J. Pharmacol. Exp. Ther.* **1992**, *262*, 971–978.
(16) Kozikowski, A. P.; Ma, D.; Brewer, J.; Sun, S.; Costa, E.; Romeo, E.; Guidotti, A. Chemistry, binding affinity, and behavioral properties of a new class of "antineophobic" mitochondrial DBI receptor complex (mDRC) ligands. *J. Med. Chem.* **1993**, *36*, 2908–2920.
(17) Primofiore, G.; Da Settimo, F.; Taliani, S.; Simorini, F.; Patrizi, M. P.; Novellino, E.; Greco, G.; Abignente, E.; Costa, B.; Chelli, B.; Martini, C. *N,N*-Dialkyl-2-phenylindol-3-ylglyoxylamides. A new class of potent and selective ligands at the peripheral benzodiazepine receptor. *J. Med. Chem.* **2004**, *47*, 1852–1855.
(18) Campiani, G.; Nacci, V.; Fiorini, I.; De Filippis, M. P.; Garofalo, A.; Ciani, S. M.; Greco, G.; Novellino, E.; Williams, D. C.; Zisterer, D. M.; Woods, M. J.; Mihai, C.; Manzoni, C.; Mennini, T. Synthesis, biological activity, SARs of pyrrolobenzoxazepine derivatives, a new class of specific "peripheral-type" benzodiazepine receptor ligands. *J. Med. Chem.* **1996**, *39*, 3435–3450.
(19) Taliani, S.; Simorini, F.; Sergianni, V.; La Motta, C.; Da Settimo, F.; Cosimelli, B.; Abignente, E.; Greco, G.; Novellino, E.; Rossi, L.; Gremigni, V.; Spinetti, F.; Chelli, B.; Martini, C. New fluorescent 2-phenylindolglyoxylamide derivatives as probes targeting the peripheral-type benzodiazepine receptor: design, synthesis, and biological evaluation. *J. Med. Chem.* **2007**, *50*, 404–407.
(20) Kita, A.; Kohayakawa, H.; Kinoshita, T.; Ochi, Y.; Nakamichi, K.; Kurumiya, S.; Furukawa, K.; Oka, M. Antianxiety and antidepressant-like effects of AC-5216, a novel mitochondrial benzodiazepine receptor ligand. *Br. J. Pharmacol.* **2004**, *142*, 1059–1072.
(21) Houlihan, W. J.; Parrino, V. A.; Uike, Y. Lithiation of *N*-(2-alkylphenyl)alkanamides and related compounds. A modified Madelung indole synthesis. *J. Org. Chem.* **1981**, *46*, 4511–4515.
(22) Da Settimo, A.; Primofiore, G.; Marini, A. M.; Ferrarini, P. L.; Franzone, J. S.; Cirillo, R.; Reboani, M. C. *N*-(Indol-3-ylglyoxylyl) -methionine derivatives: preparation and gastric anti-secretory activity. *Eur. J. Med. Chem.* **1988**, *23*, 21–24.
(23) Selleri, S.; Bruni, F.; Costagli, C.; Costanzo, A.; Guerrini, G.; Ciciani, G.; Costa, B.; Martini, C. 2-Arylpyrazolo[1,5-*a*]pyrimidin-3-yl acetamides. New potent and selective peripheral benzodiazepine receptor ligands. *Bioorg. Med. Chem.* **2001**, *9*, 2661–2671.

(24) Serra, M.; Madau, P.; Chessa, M. F.; Caddeo, M.; Sanna, E.; Trapani, G.; Franco, M.; Liso, G.; Purdy, R. H.; Barbaccia, M. L.; Biggio, G. 2-Phenylimidazo[1,2-*a*]pyridine derivatives as ligands for peripheral benzodiazepine receptors stimulation of neurosteroid synthesis and anticonflict action in rats. *Br. J. Pharmacol.* **1999**, *127*, 177–187.

(25) Pellow, S.; Chopin, P.; File, S. E.; Briley, M. Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J. Neurosci. Methods* **1985**, *14*, 149–167.

(26) Cheng, Y.; Prusoff, W. H. Relationship between constant ($K_i$) and the concentration of inhibitor which causes 50% inhibition ($IC_{50}$) of an enzymatic reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.

(27) Karobath, M.; Sperk, G. Stimulation of benzodiazepine receptor binding by $\gamma$-aminobutyric acid. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 1004–1006.

(28) Costanzo, A.; Guerrini, G.; Ciciani, G.; Bruni, F.; Selleri, S.; Costa, B.; Martini, C.; Lucacchini, A.; Aiello, P. M.; Ipponi, A. Benzodiazepine receptor ligands. 4. Synthesis and pharmacological evaluation of 3-heteroaryl-8-chloropyrazolo[5,1-*c*][1,2,4]benzotriazine-5-oxides. *J. Med. Chem.* **1999**, *42*, 2218–2226.

(29) Selleri, S.; Gratteri, P.; Costagli, C.; Bonaccini, C.; Costanzo, A.; Melani, F.; Guerrini, G.; Ciciani, G.; Costa, B.; Spinetti, F.; Martini, C.; Bruni, F. Insight into 2-phenylpyrazolo[1,5-*a*]pyrimidin-3-yl acetamides as peripheral benzodiazepine receptor ligands: synthesis, biological evaluation and 3D-QSAR investigation. *Bioorg. Med. Chem.* **2005**, *13*, 4821–4834.

(30) Dawson, G. R.; Tricklebank, M. D. Use of the elevated plus-maze in the search for novel anxiolytic agents. *Trends Pharmacol. Sci.* **1995**, *16*, 33–36.

(31) Rodgers, R. J.; Dalvi, A. Anxiety, defence and the elevated plus-maze. *Neurosci. Biobehav. Rev.* **1997**, *21*, 801–810.

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