Chemistry, Binding Affinities, and Behavioral Properties of a New Class of "Antineophobic" Mitochondrial DBI Receptor Complex (mDRC) Ligands

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The mitochondrial DBI receptor complex (mDRC; previously called the peripheral benzodiazepine receptors) is linked to the production of neurosteroids such as pregnenolone sulfate, dehydroepiandrosterone sulfate, and others. In order to gain further information as to the function of the mDRC in the brain, we have constructed and tested both in vitro and in vivo a novel series of ligands, 2-arylindole-3-acetamides. The SAR studies detailed herein delineate some of the structural features required for high affinity binding to the mDRCs. In most cases the new ligands were prepared by use of the Fischer indole synthesis. Variations in the length and number of the alkyl groups on the amide nitrogen were probed together with the effects of halogen substituents on one or both of the aryl rings. Some ligands were also synthesized for study which represent conformationally constrained versions of the parent structure. Broad screening studies revealed these indoleacetamides to be highly selective for the mDRC, since they failed to bind with any significant affinity to other receptor systems. Some of the ligands were found to exhibit K_i values in the low nanomolar range for the mDRC as measured by the displacement of [^{3}H]4'-chlorodiazepam. A subset of these ligands was also shown to stimulate pregnenolone formation from the mitochondria of C6-2B glioma cells with an EC_{50} of about 3 nM. In animal experiments ligands selected for further study were found to exhibit antineophobic effects, in spite of the fact that they exhibit no direct action on $GABA_A$ receptors. Consequently, it is postulated that these ligands owe their action to an indirect modulation of $GABA_A$ receptor function, presumably by stimulation of neurosteroid production and release from glial cells, followed by neurosteroid modulation of GABA's action on the chloride ion channel conductance of $GABA_A$ receptors.

Introduction

The pioneering work of Baulieu and colleagues has provided several lines of evidence supporting the view that the brain can synthesize steroids.[1] These authors also showed that glial cells are probably the most important steroidogenic cells in the brain.[2] Glial cells produce pregnenolone sulfate, dehydroepiandrosterone sulfate, 3α-hydroxy-5α-pregnan-20-one (3α-OH-DHP), and 3α,21-dihydroxy-5α-pregnan-20-one (THDOC) which can bind with high affinity to sites probably located on the transmembrane domain of the heterooligomeric integral membrane protein functioning as the $GABA_A$ receptor and thereby modulate receptor responsiveness to GABA.[3] Since these steroids have as their target a neuronal structure functioning in neuron to neuron signaling, they have been termed neurosteroids. The term also helps to differentiate this mechanism of regulation from that of steroids binding to specific cytoplasmic receptors which form a steroid receptor complex that translocates to the nucleus to regulate gene expression.

Neurosteroid precursors are synthesized in the mitochondria under the regulation of recognition sites in the outer membrane which bind benzodiazepines, non-benzodiazepines such as the imidazopyridines (alpidem), and other ligands including the endogenous peptide DBI (diazepam binding inhibitor).[4,5] The mitochondrial receptor that controls steroidogenesis is a multimeric protein complex named initially the peripheral benzodiazepine receptor.[6] This receptor when occupied by the appropriate agonist facilitates the transport of cholesterol from the outer to the inner mitochondrial membrane where cytochrome $P450_{scc}$ produces pregnenolone. However, the "peripheral benzodiazepine receptor" terminology became unacceptable when it was shown that this receptor is abundant in the brain and that it binds non-benzodiazepines in addition to the benzodiazepines. In order to adhere to the classical nomenclature that names a receptor for its endogenous ligand, this receptor is more properly termed the mitochondrial DBI receptor complex (or mDRC).

As regards the pharmacological role of the mDRC in CNS function, it has been shown that the high affinity mDRC ligands, e.g., alpidem and 4'-chlorodiazepam, at nanomolar concentrations, stimulate pregnenolone synthesis in C6-2B glioma cell mitochondria[7,8] and C6-2B glioma cells,[9] and possibly through this mechanism produce behavioral actions in animals.[10] Some of the neurosteroids (DHP, THDOC, pregnenolone sulfate, and dehydroepiandrosterone) can in turn modulate in a negative or positive manner GABA's action on ion channels associated with $GABA_A$[11-14] receptors or glutamate's action on the NMDA subtype of glutamate receptors.[15] Taken together, it can be hypothesized that mDRC ligands may indirectly modulate GABAergic and glutamatergic transmission by virtue of their effects on glial cell steroid biosynthesis.

A problem associated with demonstrating this novel sequence of events relates to the fact that the most potent mDRC ligands 4'-chlorodiazepam[16] and alpidem[4] probably produce some of their effects by binding to $GABA_A$

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receptors directly, whereas the most selective mDRC ligand, PK 11195, fails to show marked behavioral effects in rats, either because it may act as a partial agonist of the mDRC,[17,18] or it may have some additional action in the biosynthesis or release of glial cell neurosteroids.

In this article we present the synthesis and structure–activity studies for a new class of ligands, 2-arylindole-3-acetamides, that bind with both high affinity and specificity to the mDRCs. The efficacy of a subset of these ligands in stimulating pregnenolone formation is shown to correlate with their binding affinities for the mDRC.[19] Additionally, data are provided herein which demonstrate the antineophobic properties of some of these agents as measured by their effects in animals in the elevated plus maze experiment.[20] The unique mechanism of action of the agents described herein coupled with their pronounced effects in behavioral experiments may herald an exciting new direction in the manipulation of brain function through glial cell-associated receptors.

Chemical Synthesis

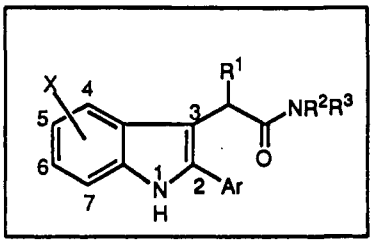
In designing compounds that might exhibit improved selectivity for the mDRCs, we chose to use alpidem and zolpidem as our structural leads. Specifically, from various electronic considerations, it appeared useful to examine the affinity of a class of derivatives related structurally to zolpidem and alpidem, but lacking the nitrogen atom at the ring fusion position. By this small structural change, one is afforded the opportunity of examining the activity of a class of 2-phenyl substituted indole-3-acetamide derivatives which can be easily procured by the Fischer indolization method.[21] Although the selected structural change would appear to be small, significant differences were observed in the pharmacology of these compounds vis-à-vis the less selective imidazopyridines (*vide supra*).

The synthesis of the majority of compounds shown in Table I was accomplished by the general strategy presented in Scheme I. The starting benzoylpropionic acid was prepared either by (i) cyanide ion catalyzed addition of aryl aldehyde to acrylonitrile or methacrylonitrile followed by hydrolysis[22] or (ii) Friedel–Crafts reaction of the requisite aryl compound with succinic anhydride. Next, the acid was converted to its mixed anhydride with ethyl chloroformate, and this intermediate reacted with the amine of choice to afford an amide. The amide was reacted in turn with the appropriate phenylhydrazine, and the resulting hydrazone heated with anhydrous zinc chloride at 170 °C for 5 min to provide the final 2-aryl substituted indole **2** upon workup.[23]

The indole-3-propionamide **3** (Table II) was prepared by Fischer indolization of the amide prepared from 4-chlorobenzoylbutanoic acid using conditions identical to those described above. The indole-3-propenamide **4** was prepared from 2-(4-chlorophenyl)indole by Vilsmeier formylation,[24] Wittig reaction with methyl triphenylphosphoranylidene acetate, and reaction with dimethylaluminum di-*n*-propylamide. Compound **5**, which contains an imidazoline ring as a possible amide surrogate was obtained by reacting ethyl 2-phenylindole-3-acetate with the aluminum reagent generated from the reaction of ethylenediamine with trimethylaluminum.[25] The *N*-methyl derivative **6** of compound **2f** was prepared by KOH/DMSO treatment followed by reaction with methyl iodide.

The conformationally constrained analogue **7** was prepared by the Fischer indolization of the α-keto lactam **16**.

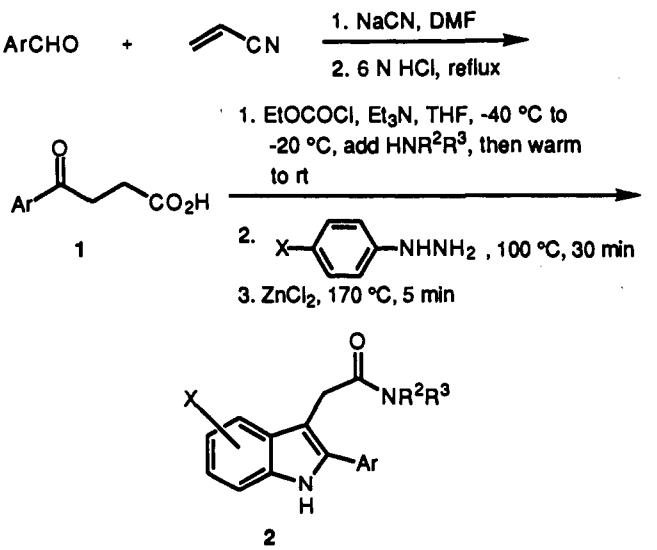
Table I. Structures and K_i Values of Indole-3-acetamides 2a–2ff



compd	R^1	R^2	R^3	X	Ar	K_i (nM)
2a	H	Me	Me	H	C_6H_5	170 ± 16
2b	H	Me	Me	5-Me	*p*-MeC_6H_4	150 ± 12
2c	H	Et	Et	H	C_6H_5	156 ± 12
2d	H	*n*-Pr	*n*-Pr	H	C_6H_5	100 ± 10
2e	H	*n*-Pr	*n*-Pr	H	*p*-FC_6H_4	100 ± 17
2f	H	*n*-Pr	*n*-Pr	H	*p*-ClC_6H_4	66 ± 7
2g	H	*n*-Pr	*n*-Pr	H	*m*-ClC_6H_4	678 ± 57
2h	H	*n*-Pr	*n*-Pr	H	*p*-BrC_6H_4	216 ± 16
2i	H	*n*-Pr	*n*-Pr	H	2-thienyl	304 ± 29
2j	H	*n*-Pr	*n*-Pr	H	*p*-IC_6H_4	216 ± 16
2k	Me	*n*-Pr	*n*-Pr	H	*p*-ClC_6H_4	800 ± 70
2l	H	*n*-Pr	*n*-Pr	6-F	C_6H_5	202 ± 28
2m	H	*n*-Pr	*n*-Pr	7-F	C_6H_5	211 ± 19
2n	H	*n*-Pr	*n*-Pr	5-Cl	2-thienyl	78 ± 7
2o	H	*n*-Pr	*n*-Pr	5-Cl	*p*-ClC_6H_4	3.9 ± 0.1
2p	H	*n*-Pr	*n*-Pr	5-Me	*p*-MeC_6H_4	66 ± 6
2q	H	-$(CH_2)_4$-		H	C_6H_5	338 ± 36
2r	H	-$(CH_2)_5$-		H	C_6H_5	350 ± 30
2s	H	*n*-Bu	*n*-Bu	H	*p*-FC_6H_4	45 ± 2
2t	H	*n*-C_5H_{11}	*n*-C_5H_{11}	H	*p*-FC_6H_4	15 ± 1
2u	H	H	o-BrC_6H_4	H	*p*-FC_6H_4	476 ± 41
2v	H	H	*n*-C_6H_{13}	H	C_6H_5	96 ± 17
2w	H	H	n-C_6H_{13}	H	p-FC_6H_4	323 ± 28
2x	H	Me	*n*-C_6H_{13}	H	*p*-FC_6H_4	135 ± 17
2y	H	*n*-C_6H_{13}	*n*-C_6H_{13}	H	C_6H_5	9.7 ± 0.6
2z	H	*n*-C_6H_{13}	*n*-C_6H_{13}	H	*p*-FC_6H_4	4.4 ± 0.1
2aa	H	*n*-C_6H_{13}	*n*-C_6H_{13}	5-Cl	C_6H_5	4.1 ± 0.1
2bb	H	*n*-C_6H_{13}	*n*-C_6H_{13}	5-Cl	*p*-FC_6H_4	2.3 ± 0.9
2cc	H	*n*-C_6H_{13}	*n*-C_6H_{13}	5-Cl	*p*-ClC_6H_4	3.6 ± 0.8
2dd	H	*n*-C_6H_{17}	*n*-C_6H_{17}	H	C_6H_5	>1000
2ee	H	H	*n*-$C_{10}H_{21}$	H	C_6H_5	>1000
2ff	H	H	*n*-$C_{16}H_{37}$	H	C_6H_5	ND[a]

[a] ND = no displacement.

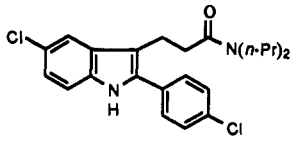
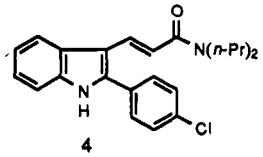
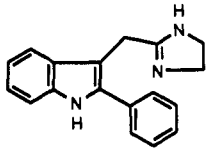
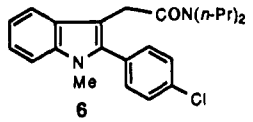
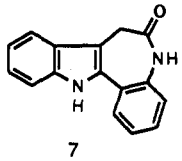
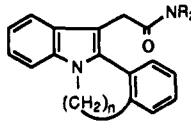
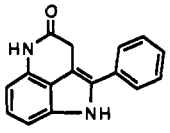
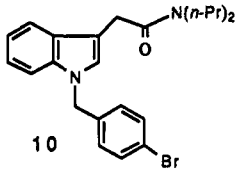
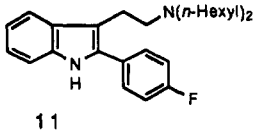
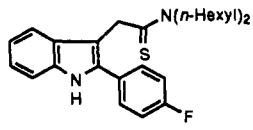
Scheme I



The α-keto lactam **16** was obtained by reaction of naphthoquinone with sodium azide in sulfuric acid followed by catalytic hydrogenation (Scheme II).[26]

The structurally rigidified tetracyclic structures **8a-c** were synthesized by haloarylalkylation of the indole nitrogen of the appropriate indole-3-acetamide followed

Table II. Structures and K_i Values of Compounds 3–14[a]

compound	K_i (nM)
 3	71 ± 6.7
 4	400 ± 35
 5	ND
 6	340 ± 47
 7	ND
 8a n = 1 R = n-Pr	20 ± 2
8b n = 3 R = n-Pr	328 ± 37
8c n = 1 R = n-C_6H_{13}	7.7 ± 0.9
 9	ND
 10	200 ± 38
 11	200 ± 16
 12	> 1000

by a palladium(0) mediated ring closure to the indole 2-position (Scheme III). This protocol has been described previously.[27]

The lactam 9, which embodies another mode of structural rigidification, was prepared starting from the indole 18, a compound easily obtained by the Bergman protocol.[28] Next, indole 18 was converted to its gramine derivative, and thence to the indole-3-acetonitrile 19 by reaction with sodium cyanide and dimethyl sulfate. The nitrile was converted in turn to its ethyl ester and the nitro group reduced to amine 21 by hydrogenation over Pd/C. The resulting aniline derivative 21 was heated in toluene at reflux to give lactam 9 (Scheme IV).

Compound 10 was synthesized from *N,N*-di-*n*-propyl indole-3-acetamide by *N*-benzylation employing potassium hydroxide in DMSO. Compounds 11 and 12 were prepared from indole-3-acetamide 2z (Table I) by LAH reduction or reaction with Lawesson's reagent, respectively.

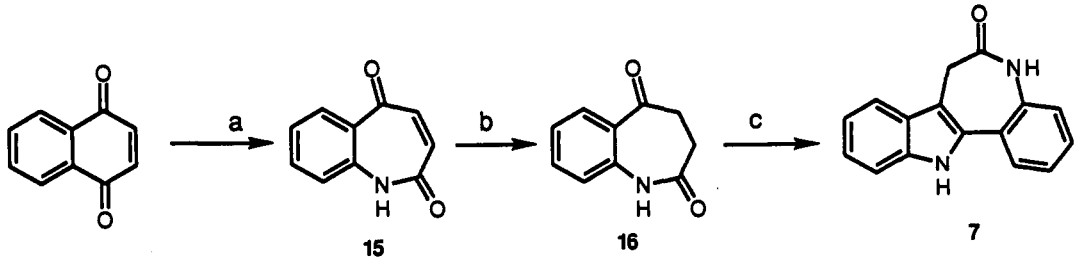
Biological Studies

Binding studies were carried out on all of the compounds reported herein using primary cultures of glial cells prepared from the cerebella of 8-day-old rat pups.[29] These cell cultures are comprised predominantly of astroglial cells, and they express a large number of mDRC which are specifically labeled by [^{3}H]4′-chlorodiazepam (4′CD). The B_{max} of [^{3}H]4′CD to glial cell mitochondria is 24–30 pmol/mg protein,and the affinity (K_D) is 2 to 3 nM. These sites are located mainly on mitochondrial outer membranes.[30] The binding data presented in Tables I and II represent the ability of our compounds to displace [^{3}H]4′CD binding from the mDRC. Since the Hill number calculated from the displacement curves of the compounds is close to unity, the assumption has been made that the compounds are competitive with 4′CD for the mDRC.[19] The data are expressed as K_i values which were calculated from the corresponding IC_{50} values according to the method of Bylund and Yamamura.[31] We further note here that the majority of these ligands have been examined for their receptor specificity by examining their ability to displace [^{3}H]4′-chlorodiazepam, [^{3}H]zolpidem, [^{3}H]fumazenil, [^{3}H]GABA, and [^{35}S]TBPS from $GABA_A$ receptors, [^{3}H]baclofen from $GABA_B$ receptors, [^{3}H]glycine from glycine receptors, [^{3}H]MK-801 from glutamate (NMDA) receptors, [^{3}H]AMPA from glutamate (non-NMDA) receptors, [^{3}H]naxolone and [^{3}H]3-PPP from opiate and σ receptors, [^{3}H]ketanserin from 5-HT_2 receptors, [^{3}H]spiperone from 5-HT_1 and dopamine receptors, [^{125}I]pindolol from β-adrenergic receptors, [^{3}H]CP-55,940 from cannabinoid receptors, and [^{3}H]L-365,260 from cholecystokinin receptors. None of the compounds were found to exhibit any significant binding affinity (K_i > 1000 nM) for the receptor systems listed (Table III).

A subset of the mDRC ligands have been examined previously for their ability to stimulate pregnenolone formation from the mitochondria of C6-2B glioma cells using the method of Papadopoulos *et al.*[8] In general a good correlation was found between binding affinity for the mDRC and the ability of the ligand to stimulate pregnenolone synthesis (Figure 1). While the reader is referred to ref 19 for complete details of the neurosteroid studies, we note here that compounds **2z** and **2bb** were among the most potent compounds tested with an EC_{50} around 3 nM; the percent maximal stimulation was 220–240% relative to control.

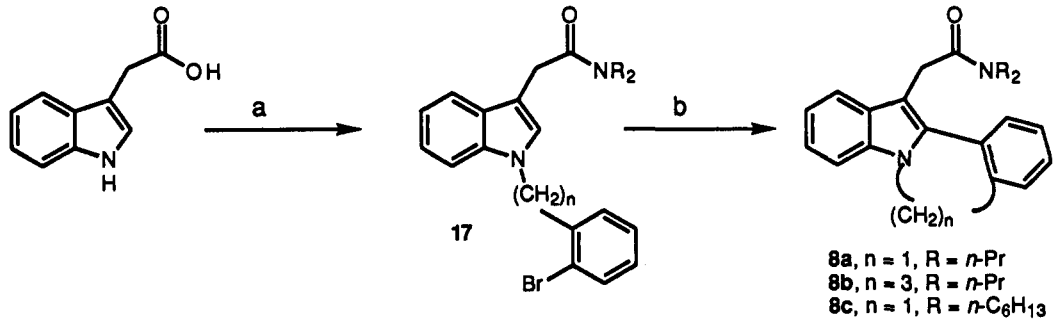
To further elucidate the role of mDRC in the steroidogenic action of 2-aryl-3-indoleacetamides, we investigated the effects of various concentrations of PK11195,

Scheme II[a]



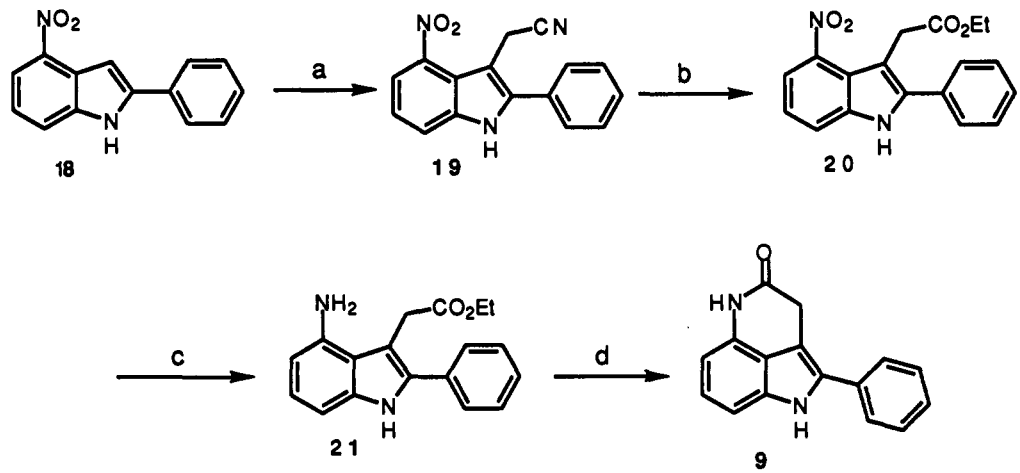
[a] Reagents and conditions: (a) NaN_3, H_2SO_4, 0–10 °C, 3 days; (b) H_2, Pd/C; (c) $PhNHNH_2$, then $ZnCl_2$.

Scheme III[a]



[a] Reagents and conditions: (a) 1. EtOCOCl, Et_3N, then R_2NH; 2. KOH, DMSO, then o-$BrC_6H_4(CH_2)_nX$; (c) $Pd(Ph_3P)_4$, KOAc, DMA, reflux, 6 h.

Scheme IV[a]



[a] Reagents and conditions: (a) 1. $CH_2{=}NMe_2^+$ Cl^-; 2. Me_2SO_4, NaCN, MeOH, room temperature, 18 h; (b) HCl, EtOH, H_2O, reflux, 18 h; (c) 1 atm H_2, Pd/C, EtOH; (d) $PhCH_3$, reflux.

an antagonist of mDRC modulated steroidogenesis. Although at 37 °C PK11195 binds to brain mitochondria mDRC with higher affinity ($K_D \cong 2$ nM) than **2z** (Table I), it failed to increase the rate of mitochondrial pregnenolone biosynthesis. At a concentration of 10^{-6} M it significantly inhibited the increase in pregnenolone accumulation elicited by **2z** (Figure 2) suggesting that PK11195 acts as a partial agonist of the steroidogenic action of our mDRC ligands.

Data are presented in Table IV which provide a measure of the action of some of our mDRC ligands against neophobia in rats as measured using the elevated plus maze test following per os administration.[19] This test is responsive to several classes of neuroactive drugs including serotonin and cholecystokinin antagonists and $GABA_A$ receptor modulators.[20] The data are recorded as the number of entries made by the animal into the open arms of the maze together with the percentage of time spent in the open arm.

To determine whether the antineophobic effect of the orally active ligands is mediated via stimulation of mDRC, we attempted to impair mDRC function with PK11195, a partial agonist at this receptor[19] that binds with high affinity to brain mDRC. Although per se PK11195 fails to modify the behavioral output of the animal, it attenuates the antineophobic effect of **2z** (Figure 3) and other compounds tested (compounds **2s** and **2bb**, data not shown here). The action of **2z** in the elevated plus maze test, unlike that of diazepam and alpidem, was flumazenil resistant (Figure 3). It is important to note that PK11195 also reduced the effect of alpidem, a very potent stimulant of mDRC and benzodiazepine binding sites at the $GABA_A$ receptor. On the other hand, PK11195 failed to block the effect of diazepam. Because mDRCs are present in the brain, but they are also abundant in peripheral tissues such as adrenal and testis, and they can stimulate steroid hormone production following occupation with the indoleacetamides, we also studied the antineophobic action

Table III. Indoleacetamide Derivatives Fail To Bind to Neurotransmitter Receptors in Crude Synaptic Membranes of Rat Brain[a]

receptor	ligand	indole derivative (2–12) IC_{50} (nM)
$GABA_A$	[^{3}H]zolpidem	>1000
	[^{3}H]flumazenil	>1000
	[^{3}H]GABA	>1000
	[^{35}S]TBPS	>1000
$GABA_B$	[^{3}H]baclofen	>1000
glycine	[^{3}H]glycine	>1000
glutamate (NMDA)	[^{3}H]MK-801	>1000
glutamate (non-NMDA)	[^{3}H]AMPA	>1000
opiate	[^{3}H]naxolone	>1000
	[^{3}H]3-PPP	>1000
serotonin	[^{3}H]ketanserin	>1000
dopamine-serotonin	[^{3}H]spiperone	>1000
cholecystokinin	[^{3}H]L-365,260	>1000
β-adrenergic	[^{125}I]pindolol	>1000
α-adrenergic	[^{3}H]WB-4101	>1000
	[^{3}H]clonidine	>1000
cannabinoid	[^{3}H]CP-55-940	>1000

[a] All indoles 2–12 tested for [^{3}H]4′CD binding (Tables I and II) were tested for their ability to displace all other tritiated ligands. For details of the binding protocols, see ref 19 and the articles cited therein.

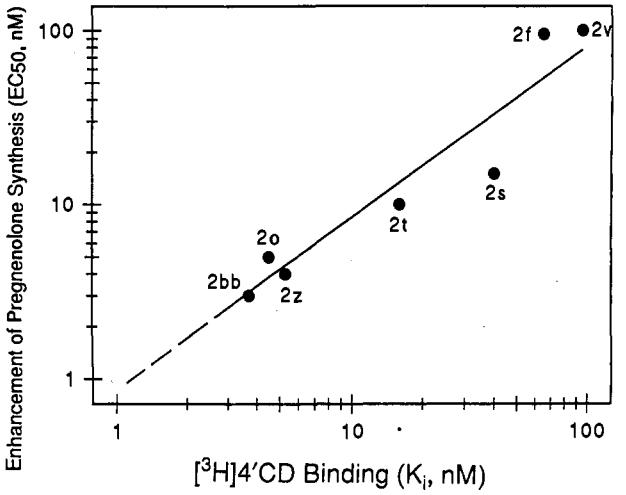


Figure 1. Effect of selected indoleacetamide derivatives on pregnenolone formation in C6-2B glioma cell mitochondria: correlation with the potency to displace the binding of [^{3}H]4′CD from glial mDRC (see Tables I and II).

of **2z** in adrenalectomized/castrated rats. Table V shows that the results are identical for both sets of animals suggesting that peripheral steroids and mDRC are not responsible for the behavioral effects of these mDRC ligands.

Molecular Mechanics Studies

While the results of more detailed molecular modeling studies will be reported after the functional properties of all the ligands reported herein are known, it was of immediate interest to gain some understanding as to the similar binding affinities of **2y** and its rigidified counterpart **8c**. Conformational analyses were thus performed using the systematic conformational search program SS1 in conjunction with the Tripos SYBYL program.[32] Energy minimization utilized the Tripos force field available in SYBYL, optimizing structures until the energy difference between subsequent conformations was less than 0.001 kcal/mol or until 1000 iterations had been completed.

The global minimum energy conformation of **2y** was determined by rotating 13 of its 14 rotatable bonds at 60°

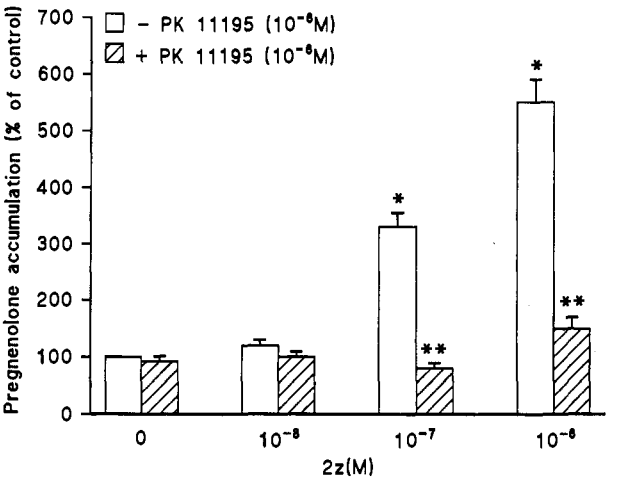


Figure 2. PK11195 antagonizes the steroidogenic action of **2z** in rat brain mitochondria. Values are mean ± SEM of at least three experiments. Control pregnenolone accumulation (100%) was the amount of pregnenolone accumulated in 5 min in the absence of drugs (12 ± 1.5 pmol/mg protein) after subtracting the pregnenolone present at 0 time (15 ± 2.5 pmol/mg protein). For details on the method see ref 19. * $p < 0.05$ versus control (Dunnett's test). ** $p < 0.01$ versus **2z** (Dunnett's test).

Table IV. Antineophobic Properties of mDRC Ligands Measured in the Elevated-Plus Maze

compd no.[a] (μmol/kg, po)	no. of open arm entries	% of time spent in the open arms
saline	0.4 ± 0.4	0.4 ± 0.4
2r (78)	2.3 ± 0.4[b]	5.7 ± 1.2[b]
2s (40)	3.6 ± 0.7[b]	8 ± 1.9[b]
2w (71)	0.6 ± 0.6	1.6 ± 1.6
2y (59)	1.6 ± 0.3	6.2 ± 1.2[b]
2z (57)	2.1 ± 0.4[b]	4.5 ± 0.8[b]
2aa (55)	2.3 ± 0.6[b]	4.3 ± 1[b]
2bb (53)	0.4 ± 0.4	0.4 ± 0.4
2cc (51)	2.3 ± 0.5[b]	5.3 ± 1.7[b]
8c (58)	2 ± 0.5[b]	6.4 ± 1.3[b]
10 (58)	0.6 ± 0.3	1 ± 0.5
11 (59)	2 ± 0.5[b]	4.1 ± 0.9[b]

[a] Animals were tested 45 min after receiving the drug. [b] $P > 0.01$ versus saline (analysis of variance, Dunnett's t-test).

increments and the one amide bond at 180° increments. Of the large number of initial conformations identified, 15 structures conformed to the energy constraints set to determine global and local minima. Six structures were found to differ from one another by at least one bond angle of at least 15°, and these were defined as "unique." The global minimum energy conformation (gmec) found for **2y** is shown in Figure 4.

The global minimum energy conformation of **8c** was determined by rotating 12 of its 13 rotatable bonds at 60° increments and the one amide bond at 180° increments. One hundred and twenty structures were identified which conformed to the energy constraints set to determine global and local minima. Thirty-six were found to differ from one another by at least one bond angle of at least 15°, and these were defined as "unique." The gmec of **8c** is shown in Figure 5.

In both of these structures, one of the *n*-hexyl carbon chains of the amide group and the aryl ring attached to the indole 2-position appear to be in steric contact. This specific interaction was found, however, to have little effect on the torsional angle between the indole ring and the aryl substituent of **2y**, for a conformational search performed on the *N,N*-dimethylamide derivative **2a** revealed a similar torsion angle (48.3°) to that found for **2y** (47.6°).

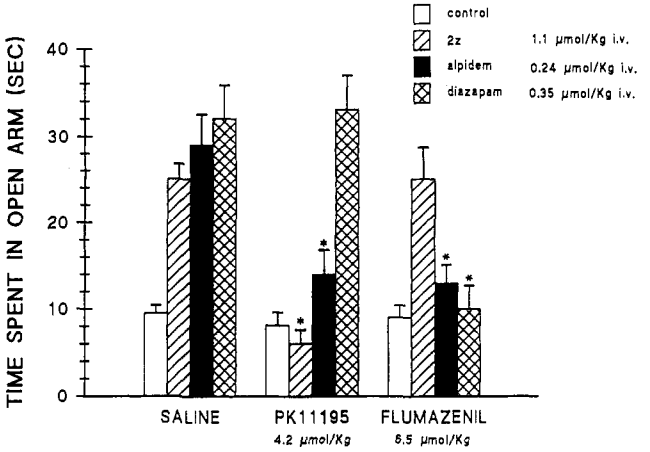


Figure 3. Effect of **2z**, diazepam, and alpidem on the time spent by rats in the open arm of an elevated plus maze. **2z**, diazepam, and alpidem were administered 10 min before the test. Control animals received vehicle (saline with 1% DMSO). Flumazenil and PK 11195 were administered iv 15 min before the test. Each value is the mean ± SEM of 10 animals. * $p < 0.05$ when PK11195 and flumazenil treated groups were compared with the respective saline treated groups. Diazepam was a gift from Hoffmann LaRoche, Nutley, NJ; Alpidem was a gift from Synthelabo, Paris, France, and PK11195 was from RBI, Boston, MA.

Table V. Adrenalectomy and Castration Do Not Abolish the Antineophobic Action of **2z**

	sham-operated		adrenalectomized/ castrated[b]	
treatment	no. of entries	time spent (s)	no. of entries	time spent (s)
vehicle (20 ml/kg, os)	1.6 ± 0.40	8.7 ± 3.3	0.98 ± 0.35	8.1 ± 2.6
2z (225 μmol/kg, os)[c]	4.9 ± 0.52[a]	22 ± 2.0[a]	5.5 ± 0.63[a]	25 ± 3.0[a]

[a] $P < 0.01$ when compared to control (vehicle treated rats). [b] Rats of 175–200 g were used in these experiments. Adrenalectomy and castration were performed 3 days before the test. [c] **2z** suspended in 1% DMSO and H_2O was administered per oral gavage 1 h prior to the test. Each value is the mean ± SEM of 10 rats.

Results and Discussion

As is apparent from an examination of the binding data presented in Tables I and II, the compounds that have been synthesized exhibit a broad range of binding affinities, from as low as 2.3 nM to inactive structures. The amide carbonyl group is seen to play an important role in binding to these mitochondrial receptors, for reduction of the amide to amine as in the case of **11** or conversion to the thioamide **12** leads to a large or complete loss in binding affinity. Likewise, the imidazoline group fails to serve as a surrogate for the amide group as revealed by compound **5**. The presence of the 2-aryl group on the indole ring is important, for attempts to mimic the basic 2-arylindole-3-acetamide structure by attachment of a benzyl group to the indole nitrogen of indole-3-acetamide as in compound **10** provided a structure of 200 nM affinity.

Additionally, substitution of the indole nitrogen leads to a reduction in binding affinity as observed from a comparison of the *N*-methyl derivative **6** (340 nM) and its unsubstituted counterpart **2f** (66 nM). Appendage of a methyl group α to the amide carbonyl group as in **2k** also reduces binding affinity as compared to the unsubstituted analogue **2f**. Attempts to explore rigidification of the amide group by linking the amide nitrogen to one of the neighboring rings as in structures **7** or **9** led to compounds that failed to exhibit any binding affinity; the lack of activity of these compounds may, however, also relate in part to the absence of an alkyl substituent on the amide nitrogen as discussed below. Rigidification of the indole-3-acetamide structure by linking the indole nitrogen to the 2-phenyl ring does, in contrast to amide group rigidification, lead to products of good binding affinity, especially in the case where the linker group is comprised of a single methylene group as in the case of **8a** or **8c**. The more flexible structure **8b** containing the three-carbon linker binds with 328 nM affinity. These results would appear to suggest that the mDRC binds ligands in the conformation in which a near planar relationship exists between the indole ring and the attached 2-aryl substituent. In this respect, it was interesting to find that the torsion angle between the aryl ring and the indole ring in **2y** in its calculated gmec is 47°. Further exploration of this point would, however, appear warranted.

The introduction of a second methylene group between the amide residue and the indole ring as in compound **3** leads to a reduction in binding (71 nM for **3** compared to 4 nM for **2o**). The propenamide analogue **4** also displays poorer binding (400 nM) relative to its acetamide counterpart **2f** (66 nM). Lastly, the length of the alkyl substituents on the amide nitrogen is seen to strongly influence binding to the mDRC. The presence of two *n*-propyl, *n*-butyl, or *n*-hexyl groups on the amide nitrogen generally leads to better binding affinities than observed for the short chain dimethyl or diethyl amides. As the alkyl chain on the amide nitrogen is increased in length, the binding affinity once again drops. For example, the dioctyl compound **2dd** fails to displace PK 11195 binding from glial cell mitochondria. Additionally, the presence of a single alkyl chain of 10 or 18 carbon atoms on the amide nitrogen leads to poorly active or inactive structures (e.g., **2ee** or **2ff**).

In summation, the optimal structure within the indole series for binding to the mDRC thus appears to be a 2-arylindole-3-acetamide whose amide nitrogen bears two *n*-hexyl groups. Additionally, the presence of one or more halogen substituents preferably located at either the 5-position of the indole ring or the para position of the 2-aryl ring is found to further increase binding affinity.

In reference to the behavioral data presented in Table IV, we note that those compounds found to exhibit the higher binding affinities were generally more effective in increasing the number of entries made by the animals into the open arms of the elevated plus maze as well as the percentage of time spent in the open arms. However, because of issues relating to drug solubility, uptake, distribution, metabolism, etc., this correlation is not perfect. For example, although compound **2s** exhibits poorer binding affinity for the mDRC *in vitro* than compound **2bb**, compound **2s** was found to be more active than **2bb** *in vivo*. While this apparent discrepancy may relate to compound **2s**'s better solubility and uptake, studies are currently underway using radiolabeled materials in order to gain an understanding of these issues.

Conclusions

In view of the high affinity and selectivity of members of the 2-arylindole-3-acetamide family for mitochondrial DBI receptors, together with their ability to stimulate the production of neurosteroids, these molecules would appear to serve as invaluable, new tools for probing the physiological function of mDRCs in the brain. Furthermore,

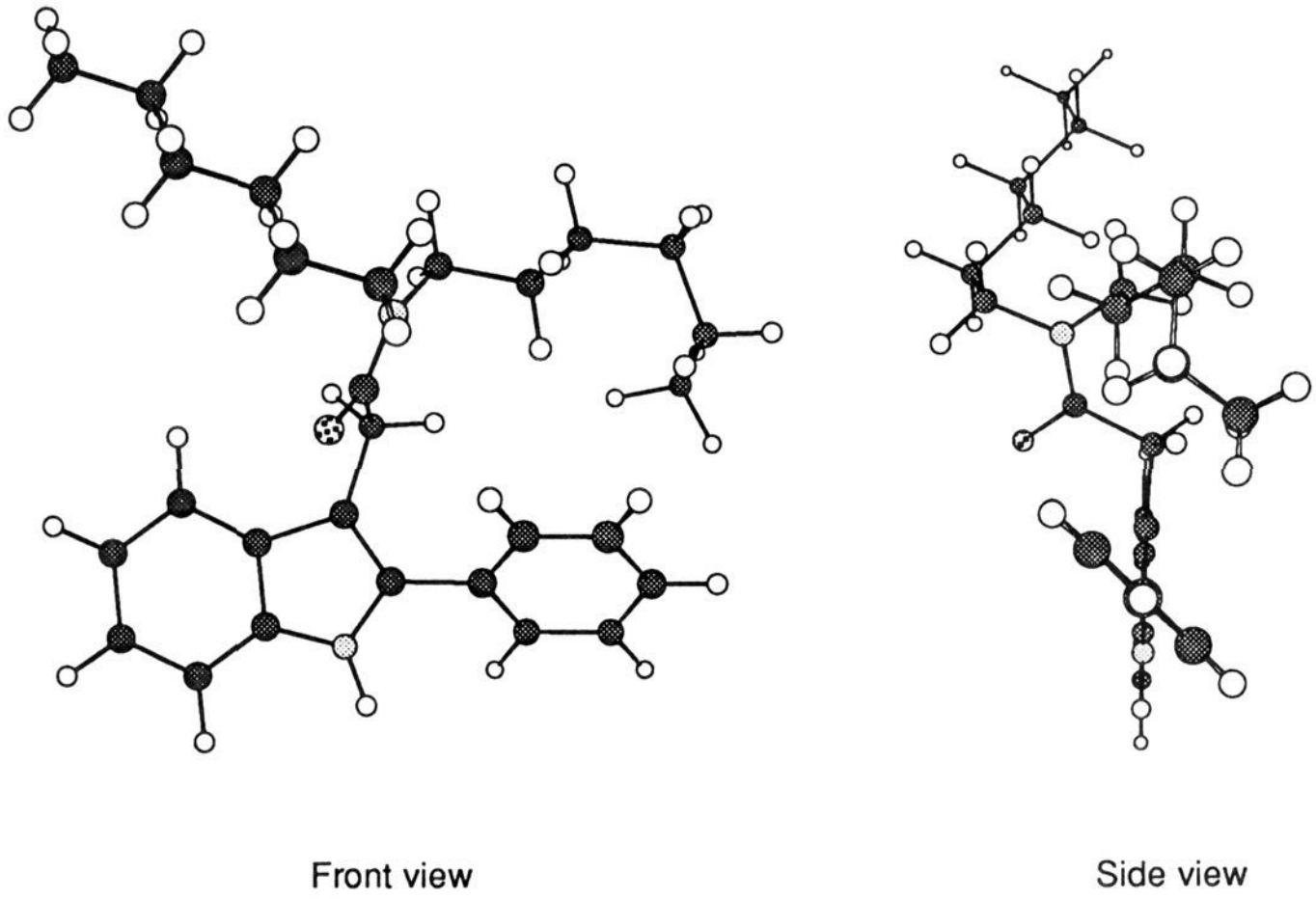


Figure 4. Ball and stick representation of the gmec of **2y**.

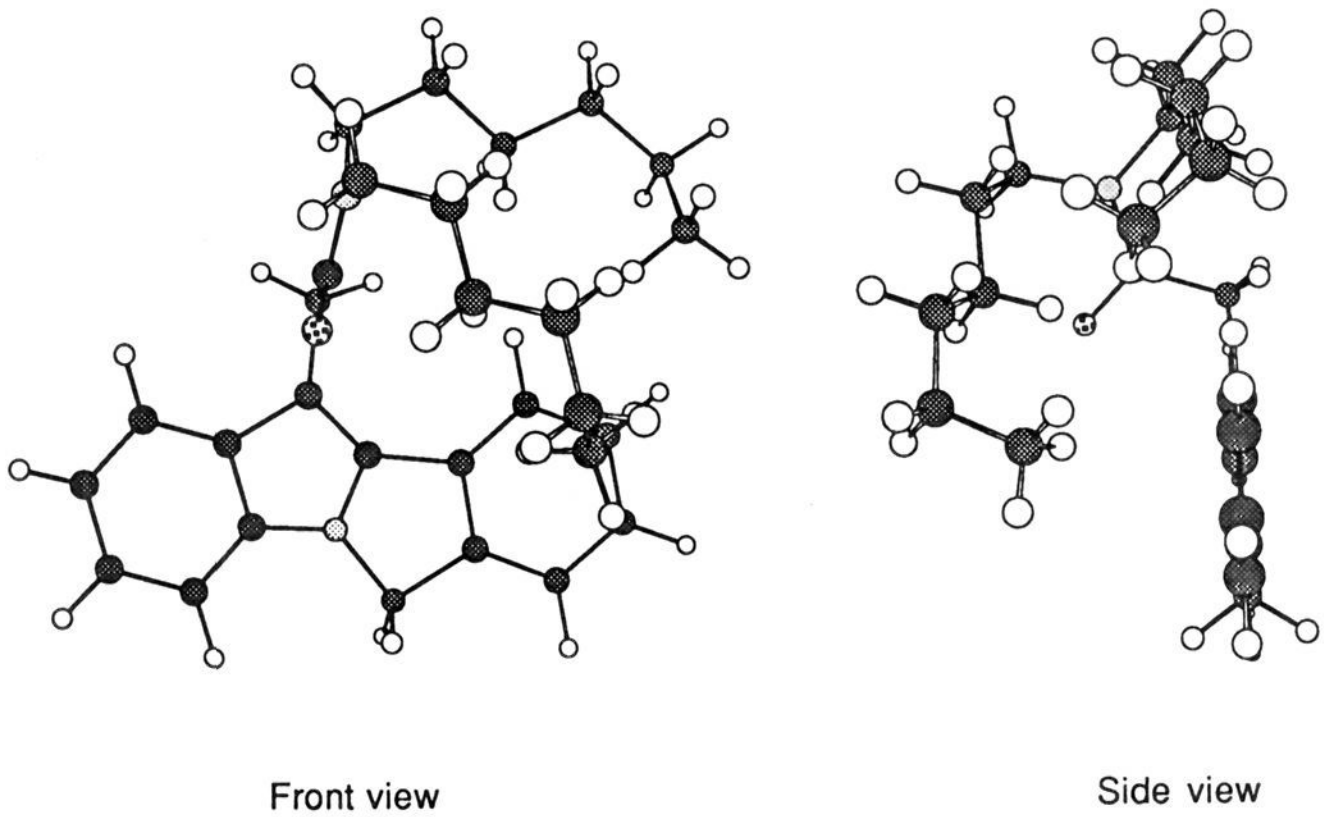


Figure 5. Ball and stick representation of the gmec of **8c**.

because of the demonstrated ability of some of these compounds to induce antineophobic effects in animals tested in the elevated plus maze, an action which is mimicked by the neurosteroid THDOC,[19] it is likely that the mechanism of action of these agents involves the stimulation of neurosteroid synthesis with the subsequent modulation of GABAergic or glutamatergic receptors located near the steroid synthesizing glial cells.[19] The effects of these compounds on neurosteroid production take on added interest in light of a recent report showing that pregnenolone and its metabolites possess memory enhancing effects.[33] Further studies of the biological properties of the compounds reported herein together with an expansion of their SAR and computer modeling efforts aimed at delineating the topography of the mDRC are underway.

Experimental Section

All reactions were carried out in oven- or flame-dried glassware under a dry argon atmosphere unless otherwise stated. Distilled reagent-grade solvents were used for chromatography and extraction. Tetrahydrofuran (THF) was distilled over sodium benzophenone ketyl. Benzene and toluene were distilled over calcium hydride. Methylene chloride was distilled over calcium hydride and stored over molecular sieves (4 Å). Dimethylformamide (DMF) was distilled over calcium hydride under reduced pressure and stored over molecular sieves (4 Å). Triethylamine (TEA) was distilled over calcium hydride and stored over potassium hydroxide. Dimethyl sulfoxide (DMSO) was distilled over calcium hydride and stored under argon. All other reagents were used as supplied unless otherwise stated.

Infrared spectra (neat film) were obtained on an Mattson 2020 FT-IR spectrometer. ^{1}H and ^{13}C NMR were recorded at 300 MHz and 75.46 MHz (Bruker AC-300), respectively, in the solvent(s) noted. ^{1}H chemical shifts (δ) were reported with

Me_4Si(δ = 0.00 ppm) or $CHCl_3$ (δ = 7.26 ppm) as internal standards. ^{13}C chemical shifts (δ) were reported with $CHCl_3$ (central peak, δ = 77.00 ppm) as internal standard. The following abbreviations are used: br = broad, d = doublet, m = multiplet, q = quartet, s = singlet, and t = triplet. Low-resolution mass spectra were determined on a Hewlett-Packard 5971A spectrometer, and high-resolution mass spectra on a VG 70-SE double focussing magnetic sector spectrometer. Elemental analyses were obtained from Oneida Research Services, Inc., Whitesboro, NY.

Silica gel 60 (Merck, 70–230 mesh, 230–400 mesh for flash chromatography) was used for column chromatography. Thin-layer chromatography (TLC) was performed on Merck silica gel 60 F-254 (0.25 mm, precoated on glass). Visualization of compounds on TLC was accomplished by UV illumination, or by staining with a solution prepared from 25 g of ammonium molybdate and 1 g of ceric sulfate in 500 mL of 10% sulfuric acid, followed by heating.

General Procedure for the Conversion of 3-Benzoylpropionic Acids 1 to Indole-3-acetamides 2. The 3-benzoylpropionic acid (1) (25 mmol) and triethylamine (87.5 mmol) were dissolved in 150 mL of THF and cooled to −40 °C. To this stirring solution, was added ethyl chloroformate (27.5 mmol) dropwise, and then the reaction suspension was stirred for 30 min at −20 °C before the addition of 27.5 mmol of di-*n*-hexylamine. The suspension was allowed to warm to ambient temperature, and stirring was continued for another 1 h. The reaction was quenched by introducing 100 mL of H_2O, and the resulting mixture was extracted with ether (400 mL). The ethereal extracts were washed successively with aqueous 5% HCl (100 mL) and saturated brine (2 × 100 mL) and dried over Na_2SO_4. The crude amide 2 was obtained by removing the solvent under reduced pressure, then mixing the residue with the corresponding hydrazine (25 mmol) and heating at 100 °C for 30 min. The water was removed under vacuum, and the hydrazone was treated with 5 equiv of powdered anhydrous zinc chloride. The mixture was heated at 170 °C for 5 min stirring vigorously by hand. The cooled mixture was dissolved in 100 mL of acetone and diluted with 500 mL of ether and 100 mL of H_2O. The organic layer was washed sequentially with 5% aqueous HCl solution (100 mL) and saturated brine (2 × 100 mL) and dried over Na_2SO_4. After evaporation to dryness, the pale yellow solid was recrystallized from ethyl acetate and *n*-hexane (0:1–1:1) to afford pure 2 as a white crystalline solid. The yield, melting point and spectral data for compounds **2a–2ff** are as follows.

2a: 78%, mp 173.5–174.5 °C; IR (KBr) 3482, 3057, 1637, 1494, 1448 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.29 (s, 1H), 7.68 (d, 1H), 7.54 (d, 2H), 7.45 (m, 2H), 7.32–7.37 (m, 2H), 7.09–7.18 (m, 2H), 3.91 (s, 2H), 2.90 (s, 3H), 2.82 (s, 3H); ^{13}C NMR ($CDCl_3$, 75.46 MHz) δ 171.3, 136.4, 135.7, 135.1, 129.3, 128.8, 128.5, 127.9, 122.4, 120.0, 119.6, 110.9, 106.9, 37.4, 35.8, 31.2; mass spectrum (15 eV), *m/z* 278 (M^+), 206, 178, 102, 72. Anal. ($C_{18}H_{18}N_2O$) C, H, N.

2b: 52%, mp 181–182 °C; IR (KBr) 3579, 1623, 1584, 1483 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.99 (s, 1H), 7.41–7.46 (m, 3H), 7.24–7.29 (m, 3H), 7.02 (d, 1H), 3.89 (s, 3H), 2.92 (s, 3H), 2.83 (s, 3H), 2.46 (s, 3H), 2.42 (s, 3H); ^{13}C NMR ($CDCl_3$, 75.46 MHz) δ 171.5, 137.7, 135.8, 134.3, 130.1, 129.6 129.4, 129.2, 128.2, 123.9, 119.1, 110.5, 105.7, 37.5, 35.8, 31.3, 21.6, 21.3; mass spectrum (15 eV) *m/z* 306 (M^+) 234, 228, 204, 176, 115, 91, 72.

2c: 80%, mp 179–180 °C; IR (KBr), 3283, 3061, 2972, 1630, 1452 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.13 (s, 1H), 7.70 (d, 1H), 7.54 (d, 2H), 7.46 (dd, 2H), 7.34–7.40 (m, 2H), 7.20 (dd, 1H), 7.14 (dd, 1H), 3.91 (s, 2H), 3.38 (q, 2H), 3.17 (q, 2H), 1.06 (t, 3H), 0.90 (t, 3H); ^{13}C NMR ($CDCl_3$, 75.46 MHz) δ 170.4, 136.1, 135.5, 132.8, 129.1, 128.9, 128.3, 127.9, 122.4, 110.9, 107.0, 42.2, 40.5, 31.2, 14.0, 13.1; mass spectrum *m/z* 306 (M^+), 206, 178, 128, 100, 72.

2d: 83%, mp 123.5–124.5 °C; IR (KBr) 3269, 3058, 1623, 1429 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.18 (s, 1H), 7.76 (d, 1H), 7.56 (d, 2H), 7.43 (dd, 2H), 7.37 (d, 1H), 7.33 (d, 1H), 7.08–7.20 (m, 2H), 3.91 (s, 2H), 3.25 (t, 2H), 3.06 (t, 2H), 1.23–1.55 (m, 4H), 0.79 (t, 3H), 0.62 (t, 3H); ^{13}C NMR ($CDCl_3$, 75.46 MHz) δ 170.8, 136.1, 135.5, 132.8, 129.1, 128.9, 128.4, 127.9, 122.4, 119.9, 119.8, 110.8, 107.1, 49.8, 47.9, 31.2, 22.2, 20.9, 11.4, 11.0; mass spectrum (15 eV) *m/z* 334 (M^+), 206, 203, 55; exact mass calcd for $C_{22}H_{26}N_2O$ 334.2045, found 334.2045.

2e: 73%; mp 140–141 °C; IR (KBr) 3570, 1621, 1554, 1453 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.11 (s, 1H), 7.66 (d, 1H), 7.55 (m, 2H), 7.33 (d, 1H), 6.90–7.21 (m, 4H), 3.85 (s, 2H), 3.27 (t, 2H), 3.15 (t, 2H), 1.46 (m, 4H), 0.82 (t, 3H), 0.70 (t, 3H); ^{13}C NMR ($CDCl_3$, 75.46 MHz) δ 170.8, 162.6 (d, J = 246.2 Hz), 136.0, 134.7, 130.1 (d, J = 6.8 Hz), 129.1, 129.0, 122.5, 120.1, 119.5, 115.9 (d, J = 21.8 Hz), 110.9, 107.2, 49.9, 48.0, 30.8, 22.3, 21.0, 11.4, 11.0; mass spectrum (15 eV) *m/z* 352 (M^+), 224, 128, 43; exact mass calcd for $C_{22}H_{25}FN_2O$ 352.1951; found 352.1951. Anal. ($C_{22}H_{25}FN_2O$) C, H, N.

2f: 86%, mp 177.5–178.5 °C; IR (KBr) 3273, 2964, 1627, 1486, 1455 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) 8.32 (s, 1H), 7.61 (d, 1H), 7.46 (d, 2H), 7.37 (d, 2H), 7.20–7.23 (m, 1H), 6.06–7.16 (m, 2H), 3.85 (s, 2H), 3.29 (t, 2H), 3.15 (t, 2H), 1.35–1.58 (m, 4H), 0.83 (t, 3H), 0.73 (t, 3H): ^{13}C NMR ($CDCl_3$, 75.46 MHz) δ 169.8, 136.0, 134.0, 132.2, 131.5, 129.6, 128.8, 128.6, 121.7, 119.0, 118.8, 111.1, 106.9, 48.9, 46.9, 30.0, 21.8, 21.5, 11.2, 10.8; mass spectrum (15 eV), *m/z* 370 (M^+, ^{37}Cl), 368 (M^+, ^{35}Cl), 240, 205, 177, 149, 128, 99, 86, 69, 55; exact mass calcd for $C_{22}H_{25}ClN_2O$ 368.1655, found 368.1655. Anal. ($C_{22}H_{25}ClN_2O$) C, H, N.

2g: 79%, mp 160–161 °C; IR (KBr) 3586, 1624, 1558, 1456 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.19 (s, 1H), 7.66 (d, 1H), 7.60 (s, 1H), 7.38 (d, 1H) , 7.30–7.35 (m, 3H), 7.18 (dd, 1H), 7.11 (dd, 1H), 3.88 (s, 2H), 3.29 (t, 2H), 3.15 (t, 2H), 1.50 (m, 4H), 0.84 (t, 3H), 0.73 (t, 3H); ^{13}C NMR ($CDCl_3$, 75.46 MHz) δ 170.7, 136.3, 134.7, 134.6, 134.1, 130.1, 129.0, 127.9, 127.7, 126.3, 122.8, 120.0, 119.4, 111.1, 107.9, 49.9, 48.1, 30.6, 22.3, 21.0, 11.5, 11.2; mass spectrum (15 eV), *m/z* 370 (M^+, ^{37}Cl), 368 (M^+, ^{35}Cl), , 240, 205, 128, 86; exact mass calcd for $C_{22}H_{25}ClN_2O$ 368.1655, found 368.1655. Anal. ($C_{22}H_{25}ClN_2O$) C, H, N.

2h: 81%; mp 188–189 °C; IR (KBr) 3586, 1624, 1560, 1456 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.15 (s, 1H), 7.64 (d, 1H), 7.58 (d, 2H) 7.43 (d, 2H), 7.32 (d, 1H), 7.18 (dd, 1H), 7.11 (d, 1H), 3.85 (s, 2H), 3.28 (t, 2H), 3.15 (t, 2H), 1.45 (m, 4H), 0.84 (t, 3H), 0.73 (t, 3H); ^{13}C NMR ($CDCl_3$, 75.46 MHz) δ 170.8, 136.7, 134.5, 132.0, 131.7, 129.9, 129.1, 122.7, 121.9, 119.9, 119.2, 111.1, 107.6, 50.0, 48.0, 30.8, 22.3, 22.0, 11.5, 11.2; mass spectrum (15 eV), *m/z* 414 (M^+, ^{81}Br), 412 (M^+, ^{79}Br), 284, 205, 128, 102, 86, 77; exact mass calcd for $C_{22}H_{25}BrN_2O$ 412.1150, found 412.1150.

2i: 50%, mp 153–154 °C; IR (KBr) 3582, 1625, 1561, 1452 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.11 (s, 1H), 7.70 (d, 1H), 7.38 (d, 1H), 7.32 (d, 1H), 7.25 (m, 1H), 7.08–7.19 (m, 3H), 3.98 (s, 2H), 3.26 (t, 2H), 3.12 (t, 2H), 1.37–1.54 (m, 4H), 0.82 (t, 3H), 0.70 (t, 3H); ^{13}C NMR ($CDCl_3$, 75.46 MHz) δ 170.5, 136.1, 134.2, 129.2, 129.0, 127.8, 125.6, 125.5, 122.7, 119.9, 119.6, 110.8, 107.6, 49.8, 47.9, 31.5, 22.2, 20.9, 11.4, 11.1; mass spectrum (15 eV) *m/z* 340 (M^+), 212, 194, 167, 55; exact mass calcd for $C_{20}H_{24}N_2OS$ 340,1609, found 340.1609.

2j: mp 195–196 °C; IR (KBr) 3561, 3051, 1642, 1489 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.32 (s, 1H), 7.74 (d, 2H), 7.61 (d, 1H), 7.27 (d, 2H), 7.24 (m, 1H), 7.09–7.18 (m, 2H), 3.82 (s, 2H), 3.34 (t, 2H), 3.18 (t, 2H), 1.42–1.56 (m, 4H), 0.83 (t, 3H), 0.78 (t, 3H); ^{13}C NMR ($CDCl_3$, 75.46 MHz) δ 170.7, 138.1, 136.5, 134.6, 132.7, 130.1, 128.9, 122.8, 120.2, 119.7, 111.0, 108.2, 93.5, 46.4, 42.5, 30.9, 22.4, 21.1, 11.3, 11.2; mass spectrum (15 eV) *m/z* 460 (M^+), 358, 332, 249, 204, 176, 128, 86. Anal. ($C_{22}H_{25}IN_2O$) C, H, N.

2k: 41%, mp 260–261 °C; IR (KBr) 3580, 1624, 1599, 1558, 1461 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.03 (s, 1H), 7.98 (d, 1H), 7.45–7.52 (m, 4H), 7.35 (d, 1H), 7.18 (dd, 1H), 7.10 (dd, 1H), 4.08 (q, 1H), 2.49–3.35 (m, 4H), 1.72 (d, 3H), 1.31–1.47 (m, 2H), 1.00–1.14 (m, 2H), 0.70 (t, 3H), 0.32 (t, 3H); ^{13}C NMR ($CDCl_3$, 75.46 MHz) δ 172.5, 136.2, 134.3, 132.9, 131.4, 129.4, 129.3, 127.7, 122.8, 121.5, 120.2, 114.3, 110.7, 49.0, 48.0, 34.7, 22.0, 20.4, 19.4, 11.4, 10.6; mass spectrum (15 eV) *m/z* 384 (M^+, ^{37}Cl), 382 (M^+, ^{35}Cl), 256, 254, 204, 126, 43; exact mass calcd for $C_{23}H_{27}ClN_2O$ 382.1812; found 382.1812. Anal. ($C_{23}H_{27}ClN_2O$) C, H, N.

2l: 21%, mp 134–135 °C; IR (KBr) 3231, 3045, 1628, 1460, 1234, 1138, 962, 873, 767 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.40 (s, 1H), 7.58 (dd, J = 8.7, 5.6, 1H), 7.34–7.48 (m, 5H), 6.93 (dd, J = 9.6, 2.2, 1H), 6.64 (m, 1H), 3.86 (s, 2H), 3.27 (t, 2H), 3.05 (t, 2H), 1.39–1.51 (m, 4H), 0.82 (t, 3H), 0.64 (t, 3H); ^{13}C NMR ($CDCl_3$, 75.46 MHz) δ 170.9, 160.1 (d, J = 234.5 Hz), 136.1 (d, J = 12.2 Hz), 135.9, 132.6, 128.7, 128.1, 127.8, 125.7, 120.4 (d, J = 9.9 Hz), 108.5 (d, J = 23.8 Hz) 107.0, 97.3 (d, J = 26.3 Hz), 49.8, 47.9, 31.0, 22.2, 21.0, 11.4, 11.0; mass spectrum (15 eV) *m/z* 352 (M^+), 251, 224, 196, 128, 86. Anal. ($C_{22}H_{25}FN_2O$) C, H, N.

2m: 37%, mp 147–148 °C; IR (KBr) 3254, 3053, 2958, 1631, 1458, 1236, 1140, 827, 769 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.37 (s, 1H), 7.57 (d, 2H), 7.39–7.49 (m, 4H), 6.99 (ddd, 1H), 6.68 (dd, J = 9.1, 7.3, 1H), 3.88 (s, 2H) 3.28 (t, 2H), 3.07 (t, 2H), 1.37–1.54 (m, 4H), 0.87 (t, 3H), 0.66 (t, 3H); ^{13}C NMR ($CDCl_3$, 75.46 MHz), δ 170.6, 149.4 (d, J = 241.7 Hz), 138.4, 132.8 (d, J = 4.1 Hz) 132.3, 128.9, 128.4, 128.3, 124.3 (d, J = 13.7 Hz) 120.2 (d, J = 5.7 Hz), 115.6, 107.9, 107.3 (d, J = 16.4 Hz), 49.8, 47.9, 31.1, 22.2, 20.9, 11.4, 11.1; mass spectrum (15 eV) m/z 352 (M$^+$), 251, 224, 177, 128, 86. Anal. ($C_{22}H_{25}FN_2O$) C, H, N.

2n: 51%, mp 161–162 °C; IR (KBr) 3586, 1624, 1558, 1454 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.47 (s, 1H), 7.55 (m, 1H), 7.39 (d, 1H), 7.15 (d, 1H), 7.02–7.12 (m, 3H), 3.91 (s, 2H), 3.33 (t, 2H), 3.21 (t, 2H), 1.48–1.63 (m, 4H), 0.87 (t, 3H), 0.80 (t, 3H); ^{13}C NMR ($CDCl_3$, 75.46 MHz) δ 170.6, 134.7, 134.0, 131.4, 127.6, 125.2, 125.1, 122.3, 117.7, 112.2, 106.3, 50.0, 48.2, 30.4, 22.3, 21.1, 11.5, 11.3; mass spectrum (15 eV) m/z 376 (M$^+$, ^{37}Cl), 374 (M$^+$, ^{35}Cl), 319, 274, 246, 210, 128, 86, 69, 59, 43; exact mass calcd for $C_{20}H_{23}ClN_2OS$ 374.1220, found 374.1220. Anal. ($C_{20}H_{24}N_2OS$) C, H, N.

2o: 85%, mp 178–180 °C; IR (KBr) 3283, 1625, 1482, 1435 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.83 (s, 1H), 7.41 (s, 1H), 7.27–7.30 (m, 4H), 6.91–6.97 (m, 2H), 3.75 (s, 2H), 3.38 (t, 2H), 3.25 (t, 2H), 1.46–1.65 (m, 4H), 0.93 (t, 3H), 0.85 (t, 3H); ^{13}C NMR ($CDCl_3$, 75.46 MHz) δ 171.2, 136.2, 134.9, 133.5, 130.9, 130.0, 128.8, 128.7, 125.5, 122.4, 117.4, 112.4, 106.6, 50.1, 48.2, 29.8, 22.4, 21.1, 11.5, 11.4; mass spectrum (15 eV) m/z 406 (M$^+$, ^{37}Cl, ^{37}Cl), 404 (M$^+$, ^{37}Cl, ^{35}Cl), 402 (M$^+$, ^{35}Cl, ^{35}Cl), 374, 370, 239, 204, 91; exact mass calcd for $C_{23}H_{24}Cl_2N_2O$ 402.1266, found 402.1266. Anal. ($C_{22}H_{24}Cl_2N_2O$) C, H, N.

2p: 85%, mp 184–185 °C; IR (KBr) 3286, 1628, 1581, 1483, 1432 cm^{-1}; ^{1}H NMR (CD_3COCD_3, 300 MHz) δ 10.11 (s, 1H), 7.49 (d, 2H), 7.40 (s, 1H), 7.28 (d, 2H), 7.25 (d, 1H), 6.84 (d, 1H), 3.85 (s, 2H), 3.21 (t, 2H), 3.12 (t, 2H), 2.85 (s, 3H), 2.80 (s, 3H), 1.31–1.48 (m, 4H), 0.75 (t, 3H), 0.63 (t, 3H); ^{13}C NMR ($CDCl_3$, 75.46 MHz) δ 171.0, 137.5, 135.6, 134.3, 130.0, 129.4, 129.3, 128.9, 128.1, 123.7, 119.2, 110.4, 106.0, 49.7, 47.8, 31.4, 22.0, 21.6, 21.2, 20.8, 11.4, 10.9; mass spectrum (15 eV) m/z 362 (M$^+$), 261, 234, 219, 204; exact mass calcd for $C_{24}H_{30}N_2O$ 362.2358, found 362.2358.

General Procedure for the Synthesis of Pyrrolidine and Piperidine Analogues 2q and 2r. A mixture of 15 mmol of the ethyl 2-phenylindole-3-acetate (prepared as described below) in 50 mL of aqueous 3 N NaOH was heated at reflux for 3 h. The cooled reaction mixture was made acidic with 6 N HCl. Ether workup gave the crude acid.

To a solution of 1.0 mmol of 2-phenylindole-3-acetic acid and 5.5 mmol of triethylamine in 30 mL of THF was added 1.0 mmol of ethyl chloroformate at 40 °C. The mixture was stirred for 10 min at −20 °C, and 1.0 mmol of pyrrolidine (or piperidine) was added. The temperature was allowed to rise to room temperature and stirring was continued for 20 min. Workup in the usual fashion afforded the expected products.

2q: 95%, mp 159–160 °C; IR (KBr) 3250, 1622, 1558, 1489 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.12 (s, 1H), 7.69 (d, 1H), 7.59 (d, 2H), 7.48 (dd, 2H), 7.33–7.39 (m, 2H), 7.11–7.22 (m, 2H), 3.90 (s, 2H), 3.46 (m, 2H), 3.24 (m, 2H), 1.79 (m, 2H), 1.76 (m, 2H); ^{13}C NMR ($CDCl_3$–DMSO-d_6, 75.46 MHz) δ 169.9, 136.0, 135.9, 132.9, 129.1, 128.8, 128.4, 127.9, 122.4, 119.9, 119.5, 111.0, 106.3, 46.7, 46.0, 32.2, 26.2, 24.3; mass spectrum (15 eV) m/z 304 (M$^+$), 206, 179, 55; exact mass calcd for $C_{20}H_{20}N_2O$ 304.1576, found 304.1576.

2r: 51%, mp 151–152 °C; IR (KBr) 3225, 2930, 1631, 1462 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.14 (s, 1H), 7.72 (d, 1H), 7.54 (d, 2H), 7.43 (dd, 2H), 7.35–7.41 (m, 2H), 7.11–7.22 (m, 2H), 3.92 (s, 2H), 3.51 (m, 2H), 3.17 (m, 2H), 1.40–1.48 (m, 4H), 1.14 (m, 2H); ^{13}C NMR ($CDCl_3$, DMSO-d_6, 75.46 MHz) δ 168.3, 135.3, 134.6, 132.0, 127.9, 127.6, 127.4, 126.6, 120.7, 118.2, 118.1, 110.2, 104.9, 45.8, 41.9, 29.9, 25.0, 24.6, 23.4; mass spectrum (15 eV) m/z 318 (M$^+$), 206, 203, 179, 69, 55; exact mass calcd for $C_{21}H_{22}N_2O$ 318.1732, found 318.1732.

2s: 72%, mp 127–128 °C, IR (KBr) 3203, 2958, 1622, 1503, 1460 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.16 (s, 1H), 7.63 (d, 1H), 7.53 (dd, 2H), 7.32 (d, 1H), 7.09–7.20 (m, 4H), 3.84 (s, 2H), 3.31 (t, 2H), 3.16 (t, 2H), 1.36–1.48 (m, 4H), 1.20–1.25 (m, 2H), 1.05–1.13 (m, 2H), 0.87 (t, 3H), 0.82 (t, 3H); ^{13}C NMR ($CDCl_3$, 75.46 MHz) δ 170.8, 162.5 (d, J = 246.8 Hz), 136.1, 134.7, 130.0 (d, J = 7.4 Hz), 129.0, 122.4, 119.9, 119.3, 115.7 (d, J = 21.1 Hz), 111.0, 107.0, 49.1, 46.1, 31.2, 30.8, 29.9, 20.3, 20.0, 13.9, 13.8; mass spectrum (15 eV) m/z 380 (M$^+$), 337, 251, 224, 156, 128, 100, 57. Anal. ($C_{24}H_{29}FN_2O$) C, H, N.

2t: 81%, mp 112–113 °C; IR (KBr) 3210, 3054, 2963, 1624, 1508, 1469 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.23 (s, 1H), 7.65 (d, 1H), 7.51 (dd, 2H), 7.29 (d, 1H), 7.08–7.19 (m, 4H), 3.84 (s, 2H), 3.29 (t, 2H), 3.13 (t, 2H), 1.35–1.49 (m, 4H), 1.16–1.28 (m, 6H), 0.96–1.03 (m, 2H), 0.83 (t, 6H); ^{13}C NMR ($CDCl_3$, 75.46 MHz) δ 170.7, 162.6 (d, J = 246.8 Hz), 136.1, 134.6, 130.1 (d, J = 7.8 Hz), 129.0, 122.5, 120.0, 119.5, 115.4 (d, J = 21.8 Hz), 110.9, 107.2, 48.2, 46.2, 30.9, 29.2, 29.0, 28.8, 27.5, 22.5, 22.4, 14.1, 14.0; mass spectrum (15 eV) m/z 408 (M$^+$), 375, 351, 280, 251, 224, 196, 140, 114, 71. Anal. ($C_{26}H_{33}N_2OF$) C, H, N.

2u: 60%, mp 170–171 °C; IR (KBr) 3383, 3281, 3053, 1657, 1585, 1525, 1508, 1230 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.34 (s, 1H), 7.61 (d, 1H), 7.46–7.56 (m, 5H), 7.17–7.33 (m, 5H), 7.09 (dd, 1H), 3.94 (s, 2H); ^{13}C NMR ($CDCl_3$, 75.46 MHz) δ 169.8, 162.9 (d, J = 250.9 Hz), 138.8, 136.1, 136.0, 130.2, 129.8 (d, J = 8.1 Hz), 128.5, 127.9, 127.5, 123.5, 122.9, 122.5, 121.1, 108.6, 116.5 (d, J = 23.0 Hz), 111.4, 104.9, 34.1; mass spectrum (15 eV) m/z 424 (M$^+$, ^{81}Br), 422 (M$^+$, ^{79}Br), 343, 278, 224, 204, 129, 90, 77.

2v: 2-Phenylindole-3-acetic acid was added to a mixture of 13.5 mmol of n-hexylamine, 13.5 mmol of phenyl N-phenylphosphoramidochloridate, and 27 mmol of triethylamine in 75 mL of dry methylene chloride. After stirring at ambient temperature for 1.5 h, the solvent was removed under reduced pressure, and the residue was chromatographed (silica gel, 1:3 ethyl acetate/n-hexane) to afford 4.15 g of **2v** as a white solid (83% yield); mp 113–114 °C (recrystallization from 1:10 ethyl acetate/n-hexane); IR (KBr) 3265, 3074, 1653, 1481 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz), δ 8.29 (s, 1H), 7.37–7.59 (m, 7H), 7.16–7.29 (m, 2H), 5.74 (br s, 1H), 3.84 (s, 2H), 3.18 (t, 2H), 1.61–1.97 (m, 6H), 0.82 (t, 3H); ^{13}C NMR ($CDCl_3$, 75.46 MHz) δ 171.7, 136.7, 136.2, 132.1, 129.1, 128.8, 128.1, 127.7, 122.8, 120.3, 118.6, 111.3, 105.1, 39.6, 33.1, 31.4, 29.4, 26.4, 22.5, 13.9; mass spectrum (15 eV) m/z 334 (M$^+$), 206, 193, 178, 152, 128, 102, 77, 55, exact mass calcd for $C_{22}H_{26}N_2O$ 334.2045, found 334.2045.

2w: 64%, mp 128–129 °C; IR (KBr) 3398, 3254, 3086, 1655, 1506, 1458, 1236 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.35 (s, 1H), 7.50–7.57 (m, 3H), 7.48 (d, 1H), 7.27 (dd, 1H), 7.15–7.21 (m, 3H), 5.78 (br s, 1H), 3.79 (s, 2H), 3.18 (dt, 2H), 1.33 (m, 2H), 1.15 (m, 6H), 0.82 (t, 3H); ^{13}C NMR ($CDCl_3$, 75.46 MHz) δ 171.3, 162.7 (d, J = 247.1 Hz), 136.0, 135.6, 129.6 (d, J = 7.7 Hz), 128.8, 128.2, 123.1, 120.6, 118.7, 116.2 (d, J = 21.6 Hz), 111.2, 105.5, 39.7, 33.0, 31.4, 29.5, 26.4, 22.5, 13.9; mass spectrum (15 eV) m/z 352 (M$^+$), 314, 251, 224, 196, 102, 77. Anal. ($C_{22}H_{25}FN_2O$) C, H, N.

2x: 80%, mp 146–147 °C; IR (KBr) 3215, 3048, 2965, 1627, 1509, 1463 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.12 (s, 1H), 7.63 (m, 1H), 7.53 (dd, 2H), 7.33 (d, 1H), 7.12–7.20 (m, 4H), 3.86 and 3.84 (s, s, 2H, cis and trans), 3.34 and 3.14 (t, t, 2H, cis and trans), 2.89 and 2.86 (s, s, 3H, cis and trans), 1.39 (m, 2H), 1.02–1.24 (m, 6H), 0.85 (t, 3H); mass spectrum (15 eV) m/z 366 (M$^+$), 295, 282, 224, 222, 128, 85, 77.

2y: 79%, mp 117–118 °C; IR (KBr) 3251, 3052, 1629, 1461 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.13 (s, 1H), 7.71 (d, J = 7.6 Hz, 1H), 7.56 (d, 2H), 7.45 (dd, 2H), 7.37 (d, 1H), 7.35 (d, 1H), 7.09–7.21 (m, 2H), 3.90 (s, 2H), 3.28 (t, 2H), 3.08 (t, 2H), 0.99–1.44 (m, 16H), 0.83 (t, 6H); ^{13}C NMR ($CDCl_3$, 75.46 MHz) δ 170.8, 138.1, 135.4, 132.9, 129.1, 128.4, 127.9, 122.4, 119.9, 119.8, 110.8, 107.1, 48.2, 46.2, 31.7, 31.5, 31.4, 29.0, 27.7, 26.7, 26.4, 22.6, 22.5, 14.1; mass spectrum (15 eV) m/z 418 (M$^+$), 347, 307, 233, 206, 154, 103, 85.

2z: mp 97–98 °C; IR (KBr) 3198, 2953, 1620, 1501, 1458, 1224 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.14 (s, 1H), 7.65 (d, J = 7.7 Hz, 1H), 7.54 (m, 2H), 7.32 (d, J = 7.6 Hz, 1H), 7.08–7.20 (m, 4H), 3.84 (s, 2H), 3.29 (t, 2H), 3.17 (t, 2H), 1.37–1.45 (m, 4H), 1.09–1.22 (m, 10H), 1.03 (m, 2H), 0.85 (t, 6H); ^{13}C NMR ($CDCl_3$, 75.46 MHz) δ 170.6, 162.6 (d, J = 246.6 Hz), 136.0, 134.6, 130.1 (d, J = 7.9 Hz), 129.1, 129.0, 122.6, 120.1, 119.6, 116.1 (d, J = 21.6 Hz), 110.8, 107.3, 48.3, 46.3, 31.7, 31.6, 30.9, 29.1, 27.8, 26.8, 26.5, 22.7, 22.6, 14.1, 14.0; mass spectrum (15 eV) m/z 436 (M$^+$) 407, 294, 251, 224, 196, 147, 128, 85. Anal. ($C_{28}H_{37}N_2OF$) C, H, N.

2aa: 78%, mp 92–93 °C; IR (KBr) 3234, 1627, 1510, 1462 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.38 (s, 1H), 7.59 (d, J = 1.8 Hz, 1H), 7.34 - 7.48 (m, 5H), 7.15 (d, J = 8.4 Hz, 1H), 7.06 (dd, J =

8.4, 1.8 Hz, 1H), 3.81 (s, 2H), 3.33 (t, 2H), 3.14 (t, 2H), 1.42–1.50 (m, 4H), 1.03–1.25 (m, 12H), 0.85 (two t, 6H); ^{13}C NMR ($CDCl_3$, 75.46 MHz) δ 170.6, 137.1, 134.5, 132.4, 130.3, 128.9, 128.2, 128.0, 125.5, 122.5, 118.8, 112.0, 106.8, 48.3, 46.3, 31.7, 31.6, 30.7, 29.1, 27.8, 26.8, 26.5, 22.7, 22.6, 14.1, 14.0; mass spectrum (15 eV) *m/z* 454 (M^+, ^{37}Cl), 452 (M^+, ^{35}Cl), 267, 240, 204, 154, 114, 85.

2bb: 76%, mp 98–99 °C; IR (KBr) 3204, 1623, 1506, 1461 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.71 (s, 1H), 7.44 (s, 1H), 7.35 (dd, 2H), 6.97–7.04 (m, 4H), 3.73 (s, 2H), 3.37 (t, 2H), 3.23 (t, 2H), 1.51 (m, 4H), 1.17–1.29 (m, 12H), 0.87 (two t, 6H); ^{13}C NMR ($CDCl_3$, 75.46 MHz) δ 170.9, 162.4 (d, J = 246.1 Hz), 136.4, 134.7, 130.0, 129.6 (d, J = 7.4 Hz) 128.6, 125.3, 122.3, 117.9, 115.6 (d, J = 21.4 Hz), 112.3, 106.4, 48.4, 46.5, 31.8, 31.6, 30.1, 29.2, 27.9, 26.8, 26.6, 22.7, 22.6, 14.1, 14.0; mass spectrum (15 eV) *m/z* 472 (M^+, ^{37}Cl), 470 (M^+, ^{35}Cl), 260, 258, 154, 85.

2cc: 72%, mp 78–79 °C; IR (KBr) 3189, 2963, 1624, 1503, 1468 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.94 (s, 1H), 7.40 (d, J = 1.8 Hz, 1H), 7.25 (s, 4H), 6.93 (dd, J = 7.6, 1.8 Hz, 1H), 6.87 (d, J = 7.6 Hz, 1H), 3.72 (s, 2H), 3.41 (t, 2H), 3.27 (t, 2H), 1.57 (m, 4H), 1.18–1.32 (m, 12H), 0.88 (t, 6H); ^{13}C NMR ($CDCl_3$, 75.46 MHz) δ 171.2, 136.2, 135.0, 133.5, 129.9, 128.7, 128.6, 125.1, 122.3, 117.3, 112.5, 106.5, 48.5, 46.6, 31.8, 31.6, 29.9, 29.2, 27.9, 26.9, 26.7, 22.8, 22.7, 14.2, 14.0; mass spectrum (15 eV) *m/z*, 490 (M^+, ^{37}Cl, ^{37}Cl), 488 (M^+, ^{37}Cl, ^{35}Cl), 486 (M^+, ^{35}Cl, ^{35}Cl), 415, 380, 353, 276, 274, 239, 212, 154, 128, 85.

2dd: 74%, mp 79–80 °C; IR (KBr) 3258, 3055, 1630, 1468 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.10 (s, 1H), 7.71 (d, 1H), 7.56 (d, 2H), 7.44 (m, 2H), 7.37 (dd, 2H), 7.11–7.19 (m, 2H), 3.90 (s, 2H), 3.28 (t, 2H), 3.08 (t, 2H), 0.91–1.44 (m, 24H), 0.86 (t, 6H); ^{13}C NMR ($CDCl_3$, 75.46 MHz) δ 170.7, 136.1, 135.4, 132.9, 129.2, 129.0, 128.4, 128.0, 122.5, 120.0, 119.9, 110.8, 107.3, 49.2, 48.2, 31.9, 31.8, 31.3, 29.5, 29.3, 29.2, 29.1, 27.8, 27.1, 26.8, 22.7, 14.1; mass spectrum (15 eV) *m/z* 474 (M^+), 268, 233, 206, 178, 128, 57. Anal. ($C_{32}H_{46}N_2O$) C, H, N.

2ee: 77%, mp 113–114 °C; IR (KBr) 3379, 3304, 3068, 2920, 1643, 1533, 1458 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.48 (s, 1H), 7.39–3.57 (m, 7H), 7.25 (dd, 1H), 7.18 (dd, 1H), 5.80 (br s, 1H), 3.84 (s, 2H), 3.16 (dt, 2H), 1.15–1.34 (m, 16H), 0.88 (t, 3H); ^{13}C NMR ($CDCl_3$, 75.46 MHz) δ 171.4, 136.6, 136.0, 132.0, 129.2, 128.9, 128.3, 127.7, 123.0, 120,5, 118.8, 111.2, 105.6, 39.6, 33.1, 31.9, 29.5, 29.4, 29.3, 29.2, 26.8, 22.7. 14.2, mass spectrum (15 eV) *m/z* 390 (M^+), 349, 291, 258, 206, 178, 128, 57.

2ff: 65%, mp 117–118 °C; IR (KBr) 3379, 3303, 3059, 2921, 1642, 1531 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.49 (s, 1H), 7.39–7.58 (m, 7H), 7.24 (dd, 1H), 7.18 (dd, 1H), 5.80 (br s, 1H), 3.84 (s, 2H), 3.16 (dt, 2H), 1.15–1.28 (m, 32H), 0.88 (t, 3H); ^{13}C NMR ($CDCl_3$, 75.46 MHz) δ 171.3, 136.6, 136.0, 132.0, 129.2, 128.9, 128.4, 127.8, 123.1, 120.6, 118.9, 111.1, 105.7, 39.6, 33.1, 32.0, 29.8, 29.6, 29.4, 29.3, 26.8, 22.7, 14.2; mass spectrum (15 eV) *m/z* 502 (M^+), 206, 178, 57.

3: By a similar procedure, compound **3** was prepared from 4-(4-chlorobenzoyl)butanoic acid in 76% yield: mp 173–174 °C; IR (KBr) 3281, 1628, 1483, 1465 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.10 (s, 1H), 7.59 (s, 1H), 7.46 (d, 2H), 7.43 (d, 2H), 7.27 (dd, 1H), 7.15 (dd, 1H), 3.17–3.28 (m, 4H), 3.05 (t, 2H), 2.61(m, 2H), 1.39–1.57 (m, 4H), 0.86 (t, 3H), 0.79 (t, 3H); ^{13}C NMR ($CDCl_3$, 75.46 MHz) δ 172.0, 134.8, 134.4, 133.9, 131.1, 129.9, 129.1, 125.4, 122.7, 118.4, 112.2, 112.1, 49.7, 47.8, 33.8, 22.2, 21.0, 20.6, 11.5, 11.2; mass spectrum (15 eV), *m/z* 420 (M^+, ^{37}Cl, ^{37}Cl), 418 (M^+, ^{37}Cl, ^{35}Cl), 416 (M^+, ^{35}Cl, ^{35}Cl), 274, 239, 204, 142, 114, 72, 58; exact mass calcd for $C_{23}H_{26}Cl_2N_2O$ 416.1422, found 416.1422. Anal. ($C_{23}H_{26}Cl_2N_2O$) C, H, N.

Methyl (*E*)-2-(4-Chlorophenyl)indole-3-propenoate. Freshly distilled phosphorus oxychloride (27.1 mmol) was added in a dropwise manner under N_2 to stirred, freshly distilled DMF (16 mL) at 0 °C. The resulting solution was allowed to stir for 30 min, and 2-(4-chlorophenyl)indole (24.2 mmol) was added gradually. During the addition the temperature of the reaction mixture was kept at 10 °C by cooling with ice water. The mixture was heated at 40 °C with stirring for 2 h, cooled to room temperature, and poured onto 500 g of ice. NaOH (2 N) was added dropwise to adjust the pH to 6, and the suspension was rapidly boiled for about 2 min. The resulting solution was allowed to cool to room temperature, and the precipitate was collected by filtration. The crude aldehyde (4.96 g, 80% yield) was used in the next step without further purification.

A solution of 15.7 mmol of the above aldehyde and 15.7 mmol of methyl (triphenylphosphoranylidene)acetate in 200 mL of benzene was heated at reflux for 10 h. The cooled solution was evaporated to dryness, and the solid was chromatographed (silica gel, 1:2 ethyl acetate/*n*-hexane) to afford 4.78 g (98%) of the title compound: IR (KBr) 3372, 1710, 1582, 1465 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.41 (s, 1H), 7.97 (d, 1H), 7.91 (d, J = 16.0 Hz, 1H), 7.39–7.50 (m, 5H) 7.28–7.34 (m, 2H), 6.59 (d, J = 16.0 Hz, 1H), 3.79 (s, 3H); ^{13}C NMR ($CDCl_3$, 75.46 MHz) δ 168.7, 140.8, 138.1, 139.4, 135.5, 130.4, 129.8, 129.5, 126.5, 123.8, 122.1, 121.0, 114.7, 111.5, 110.4, 51.6; mass spectrum (15 eV) *m/z* 313 (M^+, ^{37}Cl), 311 (M^+, ^{35}Cl), 280, 151, 117, 109, 95; exact mass calcd for $C_{18}H_{14}ClNO_2$ 311.0713; found 311.0713.

***N,N*-Di-*n*-propyl-2-(4-chlorophenyl)indole-3-propenamide (4).** To a solution of 15.6 mmol of trimethylaluminum in 80 mL of methylene chloride was added dropwise 15.6 mmol of dipropylamine at room temperature. The solution was stirred for 15 min, 7.07 mmol of the above ester was added gradually, and the resultant suspension was heated at reflux for 72 h. The cooled solution was treated by adding dropwise 50 mL of H_2O. Ether workup gave a yellow solid, which was purified by column chromatography (silica gel, 1:2 ethyl acetate/hexane) to afford 1.78 g of 4 (66% yield): mp 212–213 °C (recrystallization from 1:5 ethyl acetate/*n*-hexane); IR (KBr) 3381, 3050, 1621, 1583, 1496 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.93 (s, 1H), 7.90 (d, J = 16.0 Hz, 1H), 7.82 (d, 1H), 7.45 (d, 1H), 7.38 (d, 2H), 7.29 (d, 2H), 7.23–7.27 (m, 2H), 6.90 (d, J = 16.0 Hz, 1H), 3.33–3.42 (m, 4H), 1.62–1.72 (m, 4H), 0.97 (t, 3H), 0.83 (t, 3H); ^{13}C NMR ($CDCl_3$, 75.46 MHz) δ 167.8, 140.3, 136.7, 135.7, 134.6, 130.3, 129.0, 126.8, 123.2, 121.4, 120.3, 113.9, 112.0, 110.2, 106.3, 50.2, 48.9, 23.2, 21.4, 11.0; mass spectrum (15 eV) *m/z* 382 (M^+, ^{37}Cl), 380 (M^+, ^{35}Cl), 280, 217, 190, 108, 69, 58; exact mass calcd for $C_{23}H_{25}ClN_2O$ 380.1655, found 380.1655.

Ethyl 2-Phenylindole-3-acetate. Phenylhydrazine (80 mmol), 3-benzoylpropionic acid (80 mmol), and 12 mL of concentrated H_2SO_4 in 100 mL of ethanol were heated at reflux for 24 h. The cooled reaction mixture was poured onto 500 g of ice, and the resulting mixture was extracted with ether (2 × 300 mL). Drying and evaporation gave a red oil, which was purified by column chromatography (silica gel, 1:5 ethyl acetate/*n*-hexane) to afford 15.2 g (73%) of the title compound as a pale yellow solid: mp 61–62 °C; IR (KBr) 3370, 1718, 1585, 1458 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.17 (s, 1H), 7.67–7.71 (m, 3H), 7.47 (dd, 2H), 7.40 (dd, 2H), 7.20 (m, 2H), 4.12 (q, 2H), 3.98 (s, 2H), 1.07 (t, 3H); mass spectrum (15 eV) *m/z* 279 (M^+), 206, 179, 128, 102, 77, 58, 43; exact mass calcd for $C_{18}H_{17}NO_2$ 279.1259, found 279.1259.

Conversion of Ethyl 2-Phenylindole-3-acetate to Imidazoline 5. Ethylenediamine (18 mmol) was added dropwise to a stirred solution of trimethylaluminum (2.0 M in toluene, 8 mmol) in 28 mL of toluene at 10 °C. The solution was allowed to warm to room temperature, and the above ester (5.66 mmol) was added gradually. The reaction mixture was heated at reflux for 5.5 h. After cooling, the solution was treated dropwise with 10 mL of H_2O, diluted with 30 mL of methylene chloride and 30 mL of methanol, and refluxed on a steam bath for 15 min. After drying over Na_2SO_4, the solution was concentrated, and the residue was passed through a short column of neutral alumina (1:1 ethyl acetate/*n*-hexane was used as eluent) to remove any remaining salt. After removal of the solvent, the white solid was recrystallized from 1:10 ethyl acetate/*n*-hexane to afford 0.88 g (57% yield) of **5** as fine crystals: mp 179–180 °C; IR (KBr), 3404, 3134, 3060, 1616, 1458 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.40 (s, 1H), 7.58–7.63 (m, 3H), 7.46 (m, 2H), 7.39 (m, 2H), 7.21 (dd, 1H), 7.10 (dd, 1H), 3.91 (s, 2H), 3.56 (br s, 4H); ^{13}C NMR ($CDCl_3$, 75.46 MHz) 167.7, 136.4, 136.1, 132.4, 129.0, 128.9, 128.2, 127.9, 122.5, 119.9, 118.8, 112.2, 106.3, 49.9, 25.6; mass spectrum (15 eV) *m/z* 275 (M^+), 260, 206, 193, 178, 138, 109, 102, 77, 58, 43; exact mass calcd for $C_{16}H_{17}N_3$ 275.1422, found 275.1422. Anal. ($C_{16}H_{17}N_3$) C, H, N.

***N,N*-Di-*n*-Propyl-1-methyl-2-(4-chlorophenyl)indole-3-acetamide (6).** A mixture of 24.4 mmol of potassium hydroxide and 20 mL of dry DMSO was stirred at room temperature for 15 min, and then 6.1 mmol of *N,N*-dipropyl-2-(4-chlorophenyl)indole-3-acetamide (**2f**) was added slowly with ice water cooling. After stirring for 1 h at room temperature, 12.2 mmol of methyl iodide was added. After 1.5 h, the mixture was treated with 150

mL of H_2O, and the resulting mixture was extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with saturated brine and dried over Na_2SO_4. Pure **6** was obtained after removal of solvent (2.20 g, 94% yield): mp 103.5–104.5 °C (recrystallization from *n*-hexane); IR (KBr) 1641, 1466, 1427 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.66 (d, 1H), 7.46 (d, 2H), 7.35 (d, 2H), 7.29 (d, 1H), 7.20 (m, 1H), 7.13 (dd, 1H), 3.70 (s, 2H), 3.60 (s, 3H), 3.51 (t, 2H), 3.08 (t, 2H), 1.48 (m, 4H), 0.84 (t, 3H), 0.72 (t, 3H); ^{13}C NMR ($CDCl_3$, 75.46 MHz) δ 170.9, 137.5, 137.3, 134.4, 122.0, 130.1, 128.8, 127.8, 122.2, 119.8, 119.6, 109.4, 107.8, 49.8, 47.9, 31.1, 30.6, 23.0, 22.3, 11.4, 11.1; mass spectrum (15 eV) *m/z* 384 (M^+, ^{37}Cl), 382 (M^+, ^{35}Cl), 359, 343, 315, 269, 254, 241, 167, 71, 55; exact mass calcd for $C_{23}H_{27}ClN_2O$ 382.1812, found 382.1812. Anal. ($C_{23}H_{27}ClN_2O$) C, H, N.

Preparation of Lactam 7. Reaction of hydrogen azide with 1,4-naphthoquinone: To a solution of 23.7 g of 1,4-naphthoquinone in 150 mL of 95.9% H_2SO_4, 15.6 g of NaN_3 was added with stirring and cooling at 0 °C. After stirring for 3 days at 0–10 °C, the reaction mixture was poured onto 1 kg of ice. The precipitate was collected by filtration, washed repeatedly with water to neutrality and chromotographed (silica gel, 1:1 ethyl acetate/*n*-hexane) to afford 2.3 g of **15**.

A solution of 6.9 mmol of **15** in 150 mL of chloroform was catalytically hydrogenated at room temperature and 1 atm pressure in the presence of 0.5 g of 10% Pd/C. After all starting material had disappeared (checked by GC-MS), the catalyst was removed by filtration. After removal of the solvent at reduced pressure, the saturated lactam **16** was subjected to the standard Fischer indole synthesis to afford 1.21 g of **7** (7.5% yield from 1,4-naphthoquinone): mp 307–308 °C (recrystallization from isopropyl alcohol); IR (KBr) 3185, 1621, 1584, 1485 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.28 (s, 1H), 7.69 (d, 1H), 7.63 (dd, 1H), 7.56 (s, 1H), 7.22–7.45 (m, 4H), 7.11 (d, 1H), 3.68 (s, 2H); ^{13}C NMR (C_4D_8O, 75.46 MHz) δ 172.5, 139.1, 137.2, 133.6, 128.6, 128.3, 127.4, 124.4, 124.2, 123.0, 122.9, 120.0, 118.8, 111.8, 109.4, 32.4; mass spectrum (15 eV) *m/z* 248 (M^+), 219, 204, 190, 165, 124, 110, 96, 84, 77, 69, 55, 43; exact mass calcd for $C_{16}H_{12}N_2O$ 248.0950, found 248.0950. Anal. ($C_{16}H_{12}N_2O$) C, H, N.

***N,N*-Di-*n*-propyl-1-(2-bromobenzyl)indole-3-acetamide (17a).** By a procedure identical to that employed in the preparation of **2**, *N,N*-dipropylindole-3-acetamide was obtained from indole-3-acetic acid in 48% yield. The amide was then reacted with *o*-bromobenzyl bromide in the presence of KOH/DMSO to afford **17a** in 94% yield: IR (Nujol) 1641, 1588, 1489 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.64 (d, 1H), 7.58 (dd, 1H), 7.06–7.25 (m, 6H), 6.58 (br, 1H), 5.34 (s, 2H), 3.82 (s, 2H), 3.33 (t, 2H), 3.22 (t, 2H), 1.55 (m, 4H), 0.85 (t, 6H); mass spectrum (15 eV) *m/z* 428 (M^+, ^{81}Br), 426 (M^+, ^{79}Br), 384, 300, 298, 218, 171, 169, 129, 102, 90; exact mass calcd for $C_{22}H_{27}BrN_2O$ 426.1307, found 426.1307.

17b was prepared in a similar fashion in 80% yield: IR (neat) 2963, 1633, 1576, 1480 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.60 (d, 1H), 7.53 (dd, 1H), 7.27 (d, 1H), 7.17–7.25 (m, 2H), 7.06–7.14 (m, 4H), 4.15 (t, 2H), 3.79 (s, 2H), 3.31 (t, 2H), 3.21 (t, 2H), 2.73 (t, 2H), 2.14 (m, 2H), 1.48–1.59 (m, 4H), 0.85 (t, 6H); mass spectrum (15 eV) *m/z* 456 (M^+, ^{81}Br), 454 (M^+, ^{79}Br), 328, 326, 129, 90, 58.

17c: 81%; IR (neat) 3052, 1638, 1522, 1483 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.64 (d, 1H), 7.58 (m, 1H), 7.06–7.24 (m, 6H), 6.57 (m, 1H), 5.34 (s, 2H), 3.81 (s, 2H), 3.32 (t, 2H), 3.24 (t, 2H), 1.51 (m, 4H), 1.23–1.29 (m, 12H), 0.85 (two t, 6H); mass spectrum (15 eV) *m/z* 512 (M^+, ^{81}Br), 510 (M^+, ^{79}Br), 441, 368, 300, 298, 270, 218, 171, 169, 129, 85.

Palladium Catalyzed Intramolecular Ring Closure of 17. A mixture of 8.8 mmol of **17a**, 0.44 mmol of tetrakis(triphenylphosphine)palladium, and 8.8 mmol of potassium acetate in 120 mL of dry DMA was heated at reflux under N_2 for 6 h. The cooled reaction mixture was passed through a short column of alumina to remove inorganic substances, and the eluent was evaporated at 80 °C under reduced pressure to afford a yellow solid. The solid was chromatographed (silica gel, 1:3 ethyl acetate/*n*-hexane) to afford 2.30 g (74%) of **8a** as white solid: mp 118–119 °C (recrystallization from 1:5 ethyl acetate/*n*-hexane); IR (KBr) 1635, 1468, 1425 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.96 (d, 1H), 7.71 (d, 1H), 7.38–7.47 (m, 2H), 7.26–7.34 (m, 2H), 7.14 (dd, 1H), 7.09 (dd, 1H), 5.06 (s, 2H), 4.07 (s, 2H), 3.19–3.28 (m, 4H), 1.34–1.61 (m, 4H), 0.79 (t, 3H), 0.70 (t, 3H); ^{13}C NMR ($CDCl_3$, 75.46 MHz) δ 170.9, 141.8, 141.1, 133.6, 133.1, 132.2, 128.3, 126.9, 123.5, 122.0, 121.7, 119.9, 119.5, 109.2, 100.3, 49.9, 48.3, 47.9, 31.8, 22.2, 20.9, 11.4, 11.1; mass spectrum (15 eV) *m/z* 346 (M^+), 218, 159, 128, 109, 99, 84, 69, 55; exact mass calcd for $C_{23}H_{26}N_2O$ 346.2045, found 346.2045. Anal. ($C_{23}H_{26}N_2O$) C, H, N.

8b was prepared in a similar fashion in 81% yield: mp 118–119 °C; IR (KBr) 3011, 2953, 1633, 1450, 1427 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.79 (d, 1H), 7.51 (m, 1H), 7.34–7.48 (m, 4H), 7.21 (dd, 1H), 7.08 (dd, 1H), 4.11–4.21 (m, 2H), 3.89 (s, 2H), 3.01 (m, 2H), 2.92 (m, 2H), 2.63 (t, 2H), 2.25 (m, 2H), 1.36–1.58 (m, 4H), 0.81 (t, 3H), 0.58 (t, 3H); ^{13}C NMR ($CDCl_3$, 75.46 MHz) δ 171.0, 139.1, 138.2, 135.7, 132.1, 129.6, 129.4, 128.6, 127.8, 126.7, 121.9, 120.1, 119.2, 108.4, 106.0, 49.6, 47.9, 40.5, 31.2, 31.0, 30.8, 22.3, 21.0; mass spectrum (15 eV) *m/z* 374 (M^+), 246, 217, 128, 115, 86. Anal. ($C_{25}H_{30}N_2O$) C, H, N.

8c: 89%; mp 73–74 °C; IR (KBr) 3015, 2961, 1635, 1452, 1430 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.96 (d, J = 7.6 Hz, 1H), 7.69 (d, J = 7.7 Hz, 1H), 7.38–7.47 (m, 2H), 7.29–7.33 (m, 2H), 7.19 (dd, 1H), 7.10 (dd, 1H), 5.05 (s, 2H), 4.07 (s, 2H), 3.28 (t, 2H), 3.22 (t, 2H), 1.45 (m, 2H), 1.32 (m, 2H), 0.98–1.17 (m, 12H), 0.81 (t, 6H); ^{13}C NMR ($CDCl_3$, 75.46 MHz) δ 170.8, 141.8, 141.2, 133.6, 133.1, 132.2, 128.3, 126.8, 123.4, 122.1, 121.8, 119.9, 119.4, 109.2, 106.4, 48.4, 48.3, 46.1, 32.0, 31.7, 28.0, 27.7, 26.8, 26.5, 22.6, 14.0; mass spectrum (15 eV) *m/z* 430 (M^+), 345, 288, 219, 218, 144, 114, 55.

2-Phenyl-4-nitroindole-3-acetonitrile (19). To a suspension of 11.5 mmol of 2-phenyl-4-nitroindole (**18**) in 200 mL of dry methylene chloride was added gradually *N,N*-dimethylmethyleneammonium chloride (3.23 g, 34.5 mmol) at −10 °C. The resulting mixture was stirred overnight at room temperature, and 100 mL of 5% NaOH was added. The organic layer was separated, and the aqueous layer was extracted with 400 mL of ether. The combined organic layers were washed with saturated brine and dried over $MgSO_4$. Concentration by rotary evaporation and subsequent drying *in vacuo* yielded the crude gramine as a dark red oil.

The crude gramine was dissolved in 200 mL of absolute methanol. With cooling provided by an ice–water bath, 17.25 mmol of dimethyl sulfate and 69 mmol of sodium cyanide were added sequentially. The mixture was stirred for 18 h at room temperature. Workup by extraction with ether gave a dark red oil, which was purified by column chromatography (silica gel, 1:1 ethyl acetate/*n*-hexane) to afford 1.83 g of **19** (57% yield) as a yellow solid: IR (KBr) 3450, 3325, 2249, 1637, 1518, 1458, 1344, 1305 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.71 (s, 1H), 8.05 (d, 1H), 7.72 (d, 1H), 7.56 (m, 5H), 7.31 (dd, 1H), 4.04 (s, 2H); mass spectrum (15 eV) *m/z* 277 (M^+), 260, 230, 229, 220, 176, 87.

Ethyl 2-Phenyl-4-nitroindole-3-acetate (20). Absolute ethanol (40 mL) was saturated with dry hydrogen chloride. To this stirring solution were introduced sequentially 2.35 mmol of 2-phenyl-4-nitroindole-3-acetonitrile (**19**) and water (142 mg, 235 mmol). The resulting red solution was heated at reflux for 20 h. The cooled solution was diluted with 300 mL of ether and 100 mL of water. The organic layer was separated, washed with saturated brine, and dried over anhydrous $MgSO_4$. The solvent was removed, and the residue was chromatographed (silica gel, 1:1 ethyl acetate/*n*-hexane) to afford **20** (662 mg, 87%) as a yellow solid: ^1H NMR ($CDCl_3$, 300 MHz) δ 8.68 (s, 1H), 7.89 (d, 1H), 7.64 (d, 1H), 7.45–7.54 (m, 5H), 7.23 (dd, 1H), 4.14 (q, 2H), 3.99 (s, 2H), 1.25 (t, 3H); mass spectrum (15 eV) *m/z* 324 (M^+), 294, 251, 204, 165, 115, 102, 75.

Ethyl 2-Phenyl-4-aminoindole-3-acetate (21). A solution of ethyl 2-phenyl-4-nitroindole-3-acetate (**20**) (660 mg, 2.03 mmol) in 30 mL of absolute ethanol was hydrogenated over 0.2 g of Pd/C at room temperature and 1 atm pressure. After the red color of the solution had disappeared, the catalyst was filtered off, and the filtrate was evaporated to yield **21** as a colorless oil (570 mg, 96%): ^1H NMR ($CDCl_3$, 300 MHz) δ 8.05 (s, 1H), 7.68 (d, 2H), 7.48 (dd, 2H), 7.40 (m, 1H), 7.00 (d, 1H), 6.82 (d, 1H), 6.38 (d, 1H), 4.82 (br s, 2H), 4.24 (q, 2H), 3.93 (s, 2H), 1.31 (t, 3H); mass spectrum (15 eV) *m/z* 294 (M^+), 248, 221, 191, 110, 89, 77.

Lactam 9. A solution of ethyl 2-phenyl-4-aminoindole-3-acetate (**21**) (142 mg, 0.48 mmol) in 25 mL of toluene was heated at reflux for 72 h. The cooled solution was concentrated to yield

a solid residue which was chromatographed (silica gel, 1:1 ethyl acetate/*n*-hexane) to afford 83.1 mg of **9** (69% yield): mp 325 °C dec; IR (KBr) 3375, 3282, 3054, 1653, 1558 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.30 (s, 1H), 7.65 (s, 1H), 7.54 (d, J = 7.4 Hz, 2H), 7.46 (dd, 2H), 7.33 (m, 1H), 7.05 (m, 1H), 7.00 (d, J = 8.2 Hz, 1H), 6.38 (d, J = 7.2 Hz, 1H), 4.23 (s, 2H); mass spectrum (15 eV) m/z 248 (M^+), 219, 204, 165, 124, 109, 89, 63.

***N,N*-Di-*n*-propyl-1-(4-bromobenzyl)indole-3-acetamide (10).** Following the procedure provided above for the preparation of **2**, indole-3-acetic acid was converted into *N,N*-di-*n*-propylindole-3-acetamide in 48% yield. This amide (0.22 mmol) was dissolved in 3 mL of DMSO, and 0.1 g of potassium hydroxide was added. The resulting mixture was stirred for 1 h before 4-bromobenzyl bromide (0.22 mmol) was added. Stirring was continued for another 3 h, and then 10 mL of H_2O was added. Ether workup gave a yellow oil which was chromatographed to afford 91 mg (95%) of **10**: mp 92–93 °C; IR (KBr) 2967, 1632, 1468, 1443 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.60 (d, J = 7.9 Hz, 1H), 7.39 (d, J = 7.7 Hz, 2H), 7.12–7.19 (m, 3H), 7.06 (s, 1H), 6.96 (d, J = 7.8 Hz, 2H), 5.22 (s, 2H), 3.80 (s, 2H), 3.30 (t, 2H), 3.21 (t, 2H), 1.51–1.56 (m, 4H), 0.85 (t, 6H); ^{13}C NMR ($CDCl_3$, 75.46 MHz) δ 171.1, 136.6, 136.4, 131.9, 128.6, 128.1, 126.5, 122.1, 121.5, 119.5, 119.0, 109.6, 109.4, 49.5, 47.7, 30.9, 22.4, 20.9, 11.5, 11.3; mass spectrum (15 eV) m/z 428 (M^+, ^{81}Br), 426 (M^+, ^{79}Br), 300, 219, 171, 129, 90, 86.

Preparation of Amine 11. To a suspension of 35 mg (0.86 mmol) of lithium aluminum hydride in 5 mL of anhydrous ether under argon was added dropwise with stirring a solution of 250 mg (0.57 mmol) of amide **2z** in 5 mL of ether. The mixture was stirred and heated under reflux overnight, then quenched with water at 0 °C, and extracted with ether. After removal of the solvent, the crude product was purified by flash chromatography on ammonia-deactivated silica gel using 4:1 hexane/ethyl acetate as the eluent. Amine **11** was obtained as a white solid: 161 mg (67% yield); mp 82–84 °C; ^1H NMR ($CDCl_3$) δ 8.01 (s, 1H), 7.62 (d, 1H, J = 7.4 Hz), 7.57–7.52 (m, 2H), 7.37 (d, 1H, J = 7.4 Hz), 7.24–7.12 (m, 4H), 3.03–2.97 (m, 2H), 2.77–2.72 (m, 2H), 2.50 (t, 4H, J = 7.8 Hz), 1.44–1.42 (m, 4H), 1.34–1.25 (m, 12H), 0.89 (t, 6H, J = 6.9 Hz), ^{13}C NMR ($CDCl_3$) δ 162.2 (d, J = 240 Hz), 136.0, 133.8, 129.8 (d, J = 8.3 Hz), 129.4, 129.2, 122.4, 119.8, 119.0, 116.4, (d, J= 21 Hz), 111.5, 110.9, 54.3, 31.9, 27.3, 27.0, 22.7, 22.2, 14.1; mass spectrum (15 eV) m/z 422 (M^+), 421, 351, 238, 224, 198 (100%).

Preparation of the Thioamide 12. A mixture of **2z** (0.45 mmol) and Lawesson's reagent (0.28 mmol) in toluene was heated at reflux for 6 h. The cooled solution was concentrated, and the residual oil was chromatographed on silica gel with 1:10 ethyl acetate/hexane as eluent to afford 98 mg (95%) of the desired product as a colorless oil: IR (neat) 3050, 2960, 1530, 1482 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.09 (s, 1H), 7.85 (d, 1H), 7.56 (m, 2H), 7.32 (d, 1H), 7.13 (m, 4H), 4.37 (s, 2H), 3.90 (t, 2H), 3.33 (t, 2H), 1.57 (m, 4H), 0.95–1.26 (m, 12H), 0.84 (t, 6H); ^{13}C NMR ($CDCl_3$, 75.46 Hz) δ 187.3, 161.7 (d, J = 246.8 Hz), 135.8, 134.4, 129.9 (d, J = 7.8 Hz), 129.0, 128.9, 122.4, 120.0, 119.3, 116.1, (d, J = 21.4 Hz), 110.8, 107.3, 46.3, 30.1, 29.9, 29.1, 28.1, 26.9, 26.2, 22.3, 22.1, 13.9, 13.8 Hz; mass spectrum (15 eV) m/z 452 (M^+), 267, 224, 196, 144, 85.

Mitochondrial Preparation and [^{3}H]4′-Chlorodiazepam and [^{3}H]PK 11195 Binding. For binding studies with primary cultures of glial cells, cerebella were dissected from 8-day-old rat pups (Sprague–Dawley), and dissociated cells were prepared by enzymatic treatment with trypsin (0.25 mg/mL) according to a previously described method.[29] The cells were suspended in Eagle's minimum essential medium supplemented with 2 mM glutamine, 0.2% gentamicin, and 10% calf serum and were plated at a density of 1.5×10^6 cells/mL. Cultures were maintained in a humidified atmosphere of 5% CO_2/95% air at 37 °C.

The culture medium was changed on days 4, 7, 10, and 13. Such a culture is composed predominantly (95%) of astroglial cells as documented by staining with antibodies to glial fibrillary acid protein.[29] For binding studies the cells were harvested on day 14 *in vitro*, at which time the dishes were washed twice with phosphate-buffered saline. Buffer [50 mM Hepes (pH 7.4)] was added to the dishes to lyse the cells, and the protein concentration of the lysate was measured by the method of Bradford. An aliquot (150 μL) of the suspension, containing 4 μg of protein, was used immediately for the binding assay.

For binding studies, mitochondria were prepared from olfactory bulbs and adrenal cortical cells of Sprague–Dawley rats (200–250 g) killed by decapitation. Olfactory bulbs and adrenal glands were removed and homogenized gently in buffer A [10 mM Hepes (pH 7.4) and 0.32 M sucrose] with a Teflon-coated pestle. The homogenate was centrifuged (770 g) for 10 min at 4 °C, and the resulting supernatant was centrifuged at 5000 g for 10 min. The pellet from the second centrifugation was washed twice with buffer A to a final concentration of 1 mg of protein per mL. The binding assay was carried out with this partially purified mitochondrial preparation after centrifugation at 12 000 g and resuspension in 50 mM Hepes (pH 7.4). An aliquot of 150 μL containing 6–10 μg of protein was used in each assay tube.

A 50-μL aliquot of 0–50 nM solutions of [^{3}H]4′-chlorodiazepam or [^{3}H]PK 11195 (New England Nuclear, Boston, MA) was incubated with a 150-μL aliquot of tissue suspension in the presence of a 50-μL aliquot of various concentrations of the drugs to be tested dissolved in 50 mM Hepes (pH 7.4) containing 0.1% ethanol.

Nonspecific binding was defined as binding in the presence of 10 μM 4′-chlorodiazepam or PK 11195. Assays were terminated after 90 min at 4 °C by rapid filtration through a PHD sample harvester, Model 2000 (Cambridge Technology, Watertown, MA).

The cultured cerebellar glial cells express a large number of [^{3}H]4′-chlorodiazepam binding sites (20–30 pmol/mg protein) of high affinity (K_d = 2 to 3 nM) that are located mainly on mitochondria outer membranes.[30] Competition experiments were performed in triplicate with various concentrations of competing ligand. The IC_{50} values for each drug were calculated from isotherms generated by computer-assisted nonlinear regression analyses according to a least-squares curve-fitting program (Fig. P Scientific Fig Processor, Software Corporation). The K_i values for each drug were calculated from the IC_{50} according to Bylund and Yamamura.[31]

Elevated Plus Maze Test. The apparatus used was that described by File and Pellow.[34] The number of entries, the time spent in each arm, and path length were recorded with an HVS image system (Ormond Crescent, Hampton, England). The test was performed between 2 and 5 p.m. after the animals were allowed to adapt to the new environment for 1 h. Rats randomized for drug treatment were placed in the center of the maze, and the test was performed for 5 min. Drugs were administered per os 45 min before the test. For all behavioral tests, the statistical comparison between groups was performed by analysis of variance with Dunnett's test; the criteria for significance were $P < 0.5$ and $P < .01$.

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References

(1) Baulieu, E.-E.; Robel, P. J. Neurosteroids: a new brain function? *J. Steroid Biochem. Mol. Biol.* **1990**,*37*, 395–403.
(2) Jung-Testas, I.; Hu, Z.; Baulieu, E. E. Neurosteroids: biosynthesis of pregnenolone and progesterone in primary cultures of rat glial cells. *Endocrinol.* **1989**, *125*, 2083–2091.
(3) Puia, G.; Santi, M. R.; Vincini, S. Neurosteroids acts on recombinant human $GABA_A$ receptors. *Neuron* **1990**, *4*, 759–765.
(4) Costa, E.; Guidotti, A. Diazepam binding inhibitor (DBI): a peptide with multiple biological actions. *Life Sci.* **1991**, *49*, 325–344.
(5) Massotti, M.; Slobodyansky, E.; Konkel, D.; Costa, E.; Guidotti, A. Regulation of diazepam binding inhibitor in rat adrenal gland by adrenocorticotropin. *Endocrinol.* **1991**, *129*, 591–596.
(6) Sprengel, R.; Werner, P.; Seeburg, P. H. Molecular cloning and expression of cDNA encoding a peripheral-type benzodiazepine receptor. *J. Biol. Chem.* **1989**, *264*, 20415–20421. McEnery, M. W.; Snowman, A. M.; Trifletti, R. R.; Snyder, S. H. Isolation of the mitochondrial benzodiazepine receptor: association with the voltage dependent anion channel and the adenine nucleotide carrier. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 3170–3174.
(7) Papadopoulos, V.; Guarneri, P.; Krueger, K. E.; Guidotti, A.; Costa, E. Pregnenolone biosynthesis in C6 glioma cell mitochondria: regulation by a mitochondrial diazepam binding inhibitor receptor. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 5113–5117.
(8) Guarneri, P.; Papadopoulos, V.; Pan, B.; Costa, E. Regulation of pregnenolone synthesis in C6-2B glioma cells by 4′-chlorodiazepam. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 5118–5122.

(9) Langer, S. Z.; Arbilla, S.; Scatton, B.; Niddam, R.; Dubois, A. Receptors involved in the mechanism of action of zolpidem. In *Imidazopyridines in sleep disorders*; Sauvanet, J. P., Langer, S. Z., Morselli, P. L., Eds.; Raven Press: New York, 1988; pp 55–70.
(10) Auta, J.; Romeo, E.; Kozikowski, A. P.; Ma, D. Costa, E.; Guidotti, A. Participation of mitochondrial DBI receptors in the anticonflict, antineophobic, and anticonvulsant action of 2-aryl-3-indoleacetamide and imidazopyridine derivatives. *J. Pharmacol. Expt. Ther.* **1993**, *265*, 649–656.
(11) Majewska, M. D.; Harrison, N. L.; Schwartz, R. D.; Barker, J. L.; Paul, S. M. Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. *Science* **1986**, *232*, 1004–1007.
(12) Lambert, J. J.; Peters, J. A.; Cottrell, G. A. Actions of synthetic and endogenous steroids on the $GABA_A$ receptor. *Trends Pharmacol. Sci.* **1987**, *8*, 224–227.
(13) Mienville J.-M.; Vicini, S. Pregnenolone sulfate antagonizes $GABA_A$ receptor-mediated currents via a reduction of channel opening frequency. *Brain Res.* **1989**, *489*, 190–194.
(14) Puia, G.; Santi, M. R.; Vicini, S. Neurosteroids act on recombinant human $GABA_A$ receptors. *Neuron* **1990**, *4*, 759–765.
(15) Wu, F.; Gibbs, T. T.; Farb, D. H. Pregnenolone sulfate: a positive allosteric modulator at the N-methyl-D-asparate receptor. *Mol. Pharmacol.* **1991**, *40*, 333–336.
(16) Puia, G.; Santi, M. R.; Vicini, S.; Pritchett, D. B.; Seeburg, P. H.; Costa, E. Differences in the negative allosteric modulation of γ-aminobutyric acid receptors elicited by 4′-chlorodiazepam and by a β-carboxylate ester: A study with natural and reconstituted receptors. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 7275–7279.
(17) Benavides, J. J.; Guilloux, F.; Allam, D. E.; Uzan, A.; Mizoule, J.; Renault, C.; Dubroeucq, C.; Guérémy, C.; Le Fur, G. Opposite effects of an agonist, RO5–4864, and an antagonist, PK 11195, of the peripheral-type benzodiazepine binding sites on audiogenic seizures in DBA/2J mice. *Life Sci.* **1984**, *34*, 2613–2620.
(18) Pellow, S.; File, S. E. Behavorial actions of RO5–4864: a peripheral-type benzodiazepine? *Life Sci.* **1984**, *35*, 229–240.
(19) Romeo, E.; Auta, J.; Kozikowski, A.; 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 (mDRC). *J. Pharmacol. Exp. Ther.* **1992**, *262*, 971–978.
(20) Hughes, J.; Boden, P.; Costall, B.; Domeney, A.; Kelly, E.; Horwell, D. C.; Hunter, J. C.; Pinnock, R. D.; Woodruff, G. N. Development of a class of selective cholecystokinin type B receptor antagonists having potent anxiolytic activity. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 6728–6732.
(21) Carlin, R. B.; Fisher, E. E. Studies on the Fischer indole synthesis. *J. Am. Chem. Soc.* **1948**, *70*, 3421.
(22) Stetter, H.; Kuhlmann, H. The catalyzed nucleophilic addition of aldehydes to electrophilic double bonds. *Org. Reactions* **1991**, *40*, 407–496.
(23) Shriner, R. L.; Ashley, W. C.; Welch, E. 2-Phenylindole. *Organic Syntheses*; Wiley: New York, 1955; Collect. Vol. 3, pp 725–727.
(24) Glennon, R.; Chaurasia, C.; Titeler, M. Binding of indolylalkylamines at 5-HT_2 serotonin receptors: examination of a hydrophobic binding region. *J. Med. Chem.* **1990**, *33*, 2777–2784.
(25) Neef, G.; Eder, U.; Sauer, G. One-step conversions of esters to 2-imidazolines, benzimidazoles, and benzothiazoles by aluminum organic reagents. *J. Org. Chem.* **1981**, *46*, 2824–2826.
(26) Rossi, S.; Micheli, M.; Gianotti, M.; Salvatori, A. Benzocondensed heptaatomic heterocycles and their derivatives. VII. Synthesis of benzazepines of known anxiolytic drugs. *Gazz. Chim. Ital.* **1981**, *111*, 365–370.
(27) Kozikowski, A. P.; Ma. D. Palladium catalyzed synthesis of annelated indoles. *Tetrahedron Lett.* **1991**, *32*, 3317–3320. For related methodology, see: Grigg, R.; Sridharan, V.; Stevenson, P.; Sukirthalingam, S.; Worakun, T. The synthesis of fused ring nitrogen heterocycles via regiospecific intramolecular Heck reactions. *Tetrahedron* **1990**, *46*, 4003–4018.
(28) Bergman, J.; Sand, P. Synthesis of indoles via ring closure of 2-alkylnitroaniline derivatives. *Tetrahedron* **1990**, *46*, 6085–6112.
(29) Nicoletti, F.; Wroblewski, J. T.; Novelli, A.; Alho, H.; Guidotti, A.; Costa, E. The activation of inositol phospholipid mechanism as a signal transducing system for excitatory amino acids in primary cultures of cerebellar granule cells. *J. Neurosci.* **1986**, *6*, 1905–1911.
(30) Papadopoulos, V.; Berkovich, A.; Krueger, K. E.; Costa, E.; Guidotti, A. Diazepam binding inhibitor and its processing products stimulate mitochondrial steroid biosynthesis via an interaction with mitochondrial benzodiazepine receptors. *Endocrin.* **1991**, *129*, 1481–1487.
(31) Bylund, D. B.; Yamamura, H. I. Methods for receptor binding. In *Methods in neurotransmitter receptor analysis*; Yamamura, H. I., Enna, S. J., Kuhar, M. J., Eds.; Raven Press: New York, 1990; pp 1–35.
(32) The SS1 program developed by Dr. Yuan-Ping Pang at the Mayo Clinic Jacksonville scans all the energy-minimized conformations which are initially generated by rotating all possible rotatable bonds of a molecule at user defined increments. SYBYL (V5.41) program: Tripos Associates, Inc., 1699 S. Hanley Road, Suite 303, St. Louis, MO 63144.
(33) Flood, J. F.; Morley, J. E.; Roberts, E. Memory-enhancing effects in male mice of pregnenolone and steroids metabolically derived from it. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 1567–1571.
(34) File, S. E.; Pellow, S. The effects of triazolobenzodiazepines in two animal tests of anxiety and in the holeboard. *Br. J. Pharmacol.* **1985**, *86*, 729–735.