Novel 2-Phenylimidazo[1,2-*a*]pyridine Derivatives as Potent and Selective Ligands for Peripheral Benzodiazepine Receptors: Synthesis, Binding Affinity, and in Vivo Studies

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Received March 11, 1999

The substituent effects at positions 6 and 8 (compounds **17**−**31**) as well as at the amide nitrogen (compounds **32**−**40**) of a series of 2-phenylimidazo[1,2-*a*]pyridineacetamides were evaluated at both central (CBR) and peripheral (PBR) benzodiazepine receptors. The structure−activity relationship studies detailed herein indicate the key structural features required for high affinity and selectivity for PBR. Substitution on the imidazopyridine nucleus at position 8 with lipophilic substituents and the presence of one chlorine atom at the *para* position of the phenyl ring at C(2) are crucial features for high binding affinity and selectivity toward PBR. A small subset of active ligands (i.e., **17**, **20**, **26**, **34**, and **35**) were evaluated in vitro in *Xenopus* oocytes expressing cloned human $GABA_A$ receptors for their effects at CBR and in vivo for their ability to stimulate the synthesis of neurosteroids such as pregnenolone, progesterone, allopregnanolone, and allotetrahydrodeoxycorticosterone (THDOC). Compounds **17**, **20**, **26**, and **34** markedly increased the levels of neuroactive steroids in plasma and cerebral cortex, unlike compound **35**.

Introduction

The peripheral benzodiazepine receptor (PBR) is a multimeric protein complex located on the outer mitochondrial membrane of astroglial cells and is expressed in both central and peripheral tissues.[1] Even though the pharmacological role of PBR in the central nervous system has not been fully clarified yet, there is growing experimental evidence suggesting that high-affinity PBR ligands stimulate the synthesis of neurosteroids in glial cells.[2,3] In fact, PBR mediates the delivery of cholesterol to the inner mitochondrial membrane where it is oxidized by cytochrome P450 scc (side-chain cleavage) to pregnenolone, the parent molecule of endogenous steroids. Some neurosteroids [pregnenolone sulfate, 3α-hydroxy-5α-pregnan-20-one (3α-OH-DHP), 3α,21-dihydroxy-5α-pregnan-20-one (THDOC), and dehydroepiandrosterone] are known to modulate GABAergic and glutaminergic transmissions.[4−8] On the other hand, it has been proved that PBR expression is selectively increased in brain tumors, and this has led to the evaluation of PBR ligands as diagnostic imaging agents[9,10] or as receptor-mediated drug carriers to selectively target anticancer drugs to brain tumors.[11]

In recent years, considerable effort has been focused toward the identification of new PBR ligands with increased affinity and selectivity over central benzodiazepine receptors (CBR). PBR-selective ligands known to date belong to structurally unrelated classes of compounds such as benzodiazepines ([^{3}H]Ro-54864),[12] isoquinolines ([^{3}H]PK 11195),[13] imidazopyridines,[14] 2-aryl-3-indoleacetamides,[15a,b] benzofuranacetamides,[15c] and some benzothiazepines.[16]

Following our search for new selective benzodiazepine receptor ligands, we have previously described the synthesis of analogues of the imidazopyridines Alpidem (**1**) and Zolpidem (**2**) (Chart 1).[17] Our structure−activity relationship studies revealed considerable substituent effects at C(6) and C(6)−C(8) on the 2-phenylimidazo[1,2-*a*]pyridine heterocyclic ring system. The main conclusions from our previous studies can be summarized as follows: among compounds 6-monosubstituted at the pyridine nucleus, a high selectivity for PBR versus CBR has been observed for the 6-methoxy (**4**) and 6-nitro (**5**) (Chart 1) congeners [IC_{50}(CBR)/IC_{50}(PBR) = 84.2 and 232, respectively], whereas the 6,8-disubstituted compounds **6**−**11** (Chart 1) displayed an even higher selectivity for PBR [IC_{50}(CBR)/IC_{50}(PBR) > 1000].[17] In view of this high affinity and selectivity, these molecules might stimulate the production of neurosteroids and might represent useful tools for elucidating the physiological and pharmacological role of PBRs. To gain further insight into the structure−affinity relationships (SAR) and structure−selectivity relationships (SSR), several new ligands bearing substituents at the C(8) position have now been synthesized and evaluated in vitro for their binding affinity at CBR and PBR. In addition, the effects on binding and selectivity modifying the alkyl groups attached to the amido functionality have been explored. In this paper binding data as well as the potencies of some 2-phenylimidazo[1,2-*a*]pyridine derivatives in stimulating the synthesis of neurosteroids are reported and discussed.

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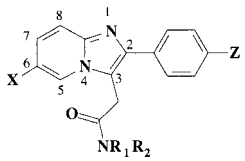
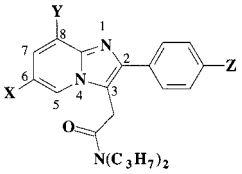
10.1021/jm991035g CCC: $18.00
Published on Web 09/01/1999

Chart 1

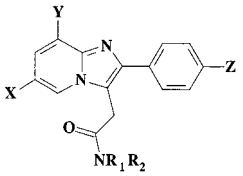


1 Alpidem X = Z = Cl; $R_1 = R_2 = C_3H_7$
2 Zolpidem X = Z CH_3; $R_1 = R_2 = CH_3$



3 X = Y = Z = H
4 X = OCH_3; Y = Z = H
5 X = NO_2; Y = Z = H
6 X = Y = Cl; Z = H
7 X = Y = Z = Cl
8 X = Y = Br; Z = Cl
9 X = CF_3 Y = Z = Cl
10 X = Br; Y = CH_3; Z = H
11 X = CH_3; Y = Br; Z = H

Table 1. Structure and Physical Properties of Compounds **17**−**40**

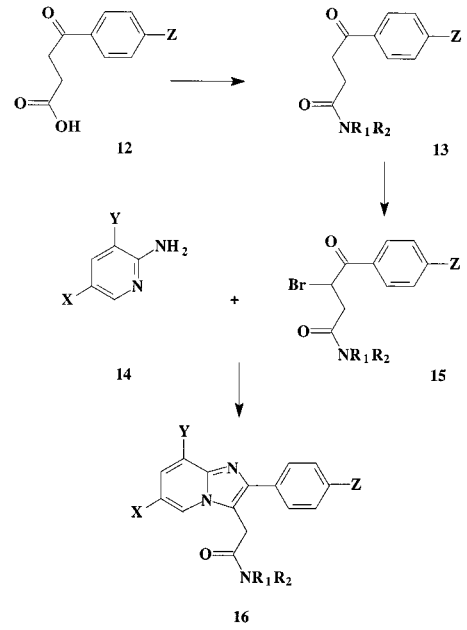


compd	X	Y	Z	R_1	R_2	mp (°C)	yield (%)
17	$COOCH_3$	H	H	C_3H_7	C_3H_7	oil	35
18	$CONH_2$	H	H	C_3H_7	C_3H_7	oil	40
19	H	CH_3	H	C_3H_7	C_3H_7	oil	20
20	H	CH_3	Cl	C_3H_7	C_3H_7	141−143	46
21	H	NO_2	H	C_3H_7	C_3H_7	oil	20
22	H	NO_2	Cl	C_3H_7	C_3H_7	204−206	18
23	H	OCH_3	H	C_3H_7	C_3H_7	118−120	16
24	H	OCH_3	Cl	C_3H_7	C_3H_7	130−132	18
25	H	Cl	H	C_3H_7	C_3H_7	97−99	25
26	H	Cl	Cl	C_3H_7	C_3H_7	175−177	31
27	H	NH_2	H	C_3H_7	C_3H_7	111−112	45
28	H	NH_2	Cl	C_3H_7	C_3H_7	95−97	22
29	H	$NHCH_3$	Cl	C_3H_7	C_3H_7	100−102	37
30	H	$NHCOCH_3$	H	C_3H_7	C_3H_7	135−137	40
31	H	$NHCOCH_3$	Cl	C_3H_7	C_3H_7	65−67	42
32	H	H	H	C_2H_5	C_2H_5	oil	30
33	H	H	Cl	C_3H_7	C_3H_7	132−133	30
34	H	H	H	C_4H_9	C_4H_9	107−109	34
35	H	H	Cl	C_4H_9	C_4H_9	107−109	37
36	H	H	H	C_6H_{13}	C_6H_{13}	oil	15
37	H	H	Cl	C_6H_{13}	C_6H_{13}	76−78	39
38	H	H	H	C_3H_7	H	157−159	20
39	H	H	Cl	C_3H_7	H	200−202	15
40	Cl	Cl	H	C_4H_9	H	167−169	35

Chemistry

A number of acetamidoimidazo[1,2-*a*]pyridine compounds (**17**−**26**, **32**−**40**) (Table 1) were synthesized using a previously reported method[17] with a modification (DMF in place of *n*-BuOH as solvent) that improved the overall yield (Scheme 1). As shown in Scheme 1, acetamidoimidazo[1,2-*a*]pyridine compounds **16** were prepared by condensation of suitably substituted 2-aminopyridines **14** with the appropriate bromoketoamides **15**. Compounds **15**, in turn, were prepared by reaction of 3-benzoylpropionic acids **12** with the appropriate dialkylamines in the presence of ethyl 1,2-dihydro-2-ethoxy-1-quinolinecarboxylate (EEDQ) as dehydrating agent. Next, treatment of the resulting amides **13** with bromine in carbon tetrachloride gave the desired compounds **15**. Catalytic hydrogenation of **21** and **22** in the presence of 10% Pd/C in ethanol yielded **27** and **28**,

Scheme 1



respectively. Reduction of the nitro group in compounds **21** and **22** was also accomplished using Sn/HCl, but no significant difference in terms of yield between the two procedures was observed. Treatment of compounds **27** and **28** with acetic anhydride afforded **30** and **31**, respectively. Compound **28** was allowed to react with dimethyl sulfate to give the methylamino derivative **29**. Physical data for new compounds are reported in Table 1.

Results and Discussion

Affinities of Imidazopyridine Derivatives for Peripheral and Central Benzodiazepine Receptors. The affinities of the tested compounds for CBR and PBR were evaluated by measuring their ability to displace [^{3}H]flunitrazepam and [^{3}H]PK 11195 from binding to membrane preparations from the cerebral cortex and ovary. Their affinities were compared with those of unlabeled PK 11195, a selective ligand for PBRs.[13] The measured binding affinities for CBR and PBR as well as their ratios, as a measure of in vitro selectivity, are shown in Table 2, together with similar data from our previous studies (compounds **3**, **5**−**10**).[17] A linear regression analysis on pIC_{50} values of the whole set of compounds listed in Table 2 showed a good correlation between data from ovary membranes and brain cerebral cortex cells ($n = 32$, $r^2 = 0.959$). This suggests that there are no significant differences in the PBR structure in the two tissues examined, and therefore we limited our SAR and SSR analyses to biological data coming from cortex membranes.

Structure−**Affinity and Structure**−**Selectivity Relationships.** Chemical variations were designed taking into consideration the pharmacophore model proposed by Bourguignon and Wermuth for PBR ligands.[18] Briefly, these authors suggested that Alpidem, which is not a selective PBR ligand, should interact with PBR and CBR in two different conformations arising from the planar (PBR) or out-of-plane (CBR) positions of the carbonyl group (Figure 1). Hydrogen-bonding (HB) acceptor capability of the imidazole nitrogen (δ1) was also hypothesized to play a role in enhancing binding

Table 2. Affinities of Compounds **3**, **5**−**10**, and **17**−**40** for CBR and PBR from Different Tissues[a]

compd	pIC_{50} CBR	pIC_{50} PBR cortex	ΔpIC_{50} PBR cor − CBR	pIC_{50} PBR ovary	ΔpIC_{50} PBR ov − CBR	CLogP[b]
3	6.28	7.00	0.72	7.02	0.74	3.94
5	5.47	8.12	2.65	8.11	2.64	3.75
6	2.40	8.52	6.12	8.37	5.97	5.37
7	2.40	9.00	6.60	8.58	6.18	6.09
8	5.00	9.10	4.10	8.80	3.80	6.39
9	5.00	8.74	3.74	8.55	3.55	6.29
10	4.27	8.79	4.52	8.58	4.31	6.02
17	4.92	7.78	2.86	7.57	2.65	3.96
18	5.58	6.34	0.76	6.52	0.94	2.85
19	4.85	7.70	2.85	7.38	2.53	4.44
20	2.40	8.85	6.45	8.55	6.15	5.15
21	4.80	7.07	2.27	6.92	2.12	3.75
22	5.00	8.24	3.24	8.06	3.06	4.46
23	5.27	7.33	2.06	7.31	2.04	4.16
24	4.19	7.78	3.59	7.68	3.49	4.87
25	4.59	7.64	3.05	7.80	3.21	4.66
26	2.40	8.37	5.97	8.13	5.73	5.37
27	5.27	8.37	3.10	8.24	2.97	3.39
28	5.07	8.20	3.13	8.34	3.27	4.11
29	4.00	8.43	4.43	8.10	4.10	4.90
30	4.96	6.59	1.63	6.35	1.39	3.59
31	4.09	8.00	3.91	7.48	3.39	4.29
32	5.68	6.36	0.68	6.18	0.50	2.89
33	7.19	8.00	0.81	7.48	0.29	4.65
34	6.30	7.77	1.47	7.64	1.34	4.99
35	7.04	8.23	1.19	7.98	0.94	5.71
36	3.93	7.72	3.79	7.88	3.95	7.11
37	3.83	6.74	2.91	6.75	2.92	7.83
38	6.09	5.70	−0.39	5.70	−0.39	2.60
39	6.93	6.23	−0.70	5.79	−1.14	3.32
40	4.00	6.53	2.53	6.41	2.41	4.57
PK 11195	4.67	8.85	4.18	8.74	4.07	4.61

[a] pIC_{50} values are the negative logarithms of the concentrations necessary for 50% inhibition (IC_{50}); data are means of 3 separate experiments performed in duplicate which differed by less than 10%. [b] Estimated by CLogP program. [20]

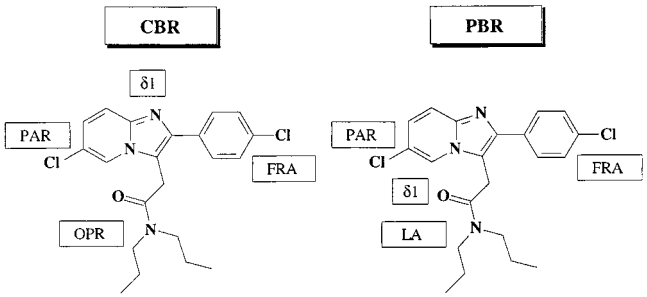


Figure 1. Comparison between the schematic representation of the interaction models of Alpidem with CBR and PBR: FRA, freely rotating aromatic ring region; δ1, electron-rich zone; OPR, out-of-plane region; PAR, planar aromatic region; LA, lipophilic area.

affinity for CBR.[19] The importance of the imidazole nitrogen as well as the amide lipophilic side chain(s) for the binding to the receptor was also clearly demonstrated.[15a,19]

We have previously reported high PBR/CBR selectivity for the (electron-withdrawing) 6-nitro (**5**) and (electron-donating) 6-methoxy (**4**) derivatives.[17] On this basis two new congeners bearing substituents with similar electron-withdrawing properties but different lipophilic characteristics, namely compounds **17** (6-$COOCH_3$) and **18** (6-$CONH_2$), were synthesized.

Interestingly, only the methoxycarbonyl derivative retained good affinity while improving the selectivity [IC_{50}(CBR)/IC_{50}(PBR) = 756 and 448 for cortex and ovary, respectively] (Table 2). These results suggest a lipophilic interaction from the 6-position to the PBR. Although many of the 6-substituted compounds possess good affinity at the PBR, it was observed that their selectivity over the CBR was still modest and lower than that of the reference compound PK 11195. Indeed, in our previous study[17] more important effects on selectivity resulted from the double substitution at C(6) and C(8) positions of the pyridine nucleus, leading to compounds more than 1000-fold selective for PBR versus CBR. However, in that paper the substituent effects of monosubstitution at position 8 were not investigated. Therefore we designed, synthesized, and tested a relatively large series of compounds containing 8-substituents with varying electronic, lipophilic, and steric properties.

At first glance, examination of the data reported in Table 2 for parent (**3**) and 8-monosubstituted compounds **19**, **21**, **23**, **25**, **27**, and **30** revealed no clear SAR and SSR. In fact, with the exception of the $NHCOCH_3$ substituent, any kind of substituent appears to significantly improve affinity and selectivity, with the best results being obtained for the hydrophilic electron-donating substituent NH_2 (compound **27**) and the lipophilic electron-withdrawing substituent Cl (compound **25**). However, deeper analysis of the SAR indicates that, with the exception of the 8-NH_2 compound (**27**), increased lipophilicity leads to better PBR binding affinity. Indeed, by dropping compound **27** from the analysis, a good linear correlation ($n = 6$, $r^2 = 0.89$, $s = 0.16$) was found between pIC_{50} of 8-substituted ligands and their octanol/water partition coefficients estimated by the CLogP software.[20]

Introduction of a chlorine atom at the *para* position of the C(2)-phenyl ring in the 8-substituted compounds, as well as in the parent compound **3**, strongly enhanced affinity and selectivity toward PBR (compare **3**, **20**, **22**, **24**, **26**, and **31** with **33**, **19**, **21**, **23**, **25**, and **30**, respectively). From a quantitative viewpoint, analysis of the binding affinities of the entire set of 8-substituted derivatives (compounds **3**, **19**−**31**) indicated a linear relationship with log *P*. Once again, compound **27** was a strong outlier, and excluding it from regression analysis gave a good linear correlation ($n = 13$, $r^2 = 0.66$, $s = 0.39$). No easy explanation of the anomalous behavior of the 8-NH_2 compound can be found unless a different binding mode is evoked. This hypothesis is supported by the observation that only in the case of the 8-NH_2 derivative the introduction of a chlorine atom to the *para* position of the 2-phenyl ring (compound **28**) results in no enhancement of binding affinity.

The relevant influence of lipophilicity on binding affinity and selectivity was also demonstrated for compounds **3** and **32**−**40** in which the number and branching of alkyl substituents on the amide nitrogen were varied. In this case, the relationship between pIC_{50} and log *P* was parabolic ($n = 10$, $r^2 = 0.75$, $s = 0.48$). Finally, to derive a general model, all the compounds **3**−**40** listed in Table 2 were taken into account, and as expected, a parabolic equation was derived ($n = 31$, $r^2 = 0.62$, $s = 0.58$). The unsatisfactory statistics of this equation could result from the fact that the descendent part of the parabola is described by two data points only,

Table 3. Modulatory Action of Compounds **17**, **20**, **26**, **34**, and **35** at Human $\alpha1\beta2\gamma2$ $GABA_A$ Receptors Expressed in *X. laevis* Oocytes[a]

compd	10 μM	50 μM	100 μM
17	17.1 ± 7.9	11.4 ± 7.3	11.6 ± 12.7
20	−9.6 ± 4.5	−38.7 ± 11.2*	−44.3 ± 12.7*
26	4.5 ± 10.7	−6.1 ± 4.8	−15.2 ± 5.2
34	17.4 ± 5.2	44.6 ± 12.3*	58.2 ± 23.8*
35	23.2 ± 7.4*	35.0 ± 13.2*	23.9 ± 8.9*
PK 11195	−16.0 ± 7.0	4.3 ± 7.5	9.1 ± 8.0

[a] Values are expressed as percent change induced by the different drugs from the control response obtained with GABA EC_{20} (concentration of GABA, usually ranging from 2 to 10 μM, which produced a Cl^- current whose amplitude was 20 ± 2% of the maximal response to GABA 10 mM). Data are means (from 6−14 different oocytes) ± SEM. *$p < 0.05$ vs control response (Student's *t*-test).

representing the most lipophilic compounds **36** and **37**. In fact, their elimination from the equation afforded a linear relationship with improved statistics.

As far as the SSR are concerned, a clear linear influence of lipophilicity on selectivity (expressed as ΔpIC_{50} in Table 2) was demonstrated for the 8-substituted compounds (**3**, **19**−**31**) and the *N*-alkyl-substituted compounds (**32**−**40**), with a significantly stronger effect on the former.

These findings suggest that the lipophilicity of the substituent at the 8-position is mainly responsible for the modulation of affinity. The existence of some outliers, however, indicates that other factors should be accounted for to fully explain the SSR. The synthesis of properly designed compounds could help in clarifying such a point.

Functional Studies. Compounds **17**, **20**, **26**, **34**, and **35**, as representative examples of the subsets here examined, were evaluated both for their effects on human recombinant $GABA_A$ receptors, to determine their capacity to interact at CBR, and for their effects on steroidogenesis in rats.

In Vitro Assay: Electrophysiology in *Xenopus* Oocytes. As summarized in Table 3, Cl^- currents elicited by GABA at $GABA_A$ receptors were enhanced by compounds **34** and **35**, with maximal effects observed at 100 and 50 μM, respectively. The facilitatory effects exerted by compounds **34** and **35** were reversed by the co-application of the selective benzodiazepine receptor antagonist flumazenil (10 μM) (data not shown). Compounds **17** and **26**, like PK 11195, failed to produce statistical significant changes in the amplitude of GABA-elicited currents, whereas compound **20** caused a decrease of GABA-elicited currents with maximal effect produced at 100 μM. However, this inhibitory effect was not mediated by benzodiazepine recognition sites since the receptor antagonist flumazenil did not block it (data not shown).

In Vivo Assay: Effect of Compounds on Steroidogenesis. Intraperitoneal administration of the compounds (25 mg/kg) resulted in significant increases in the plasma concentrations of neuroactive steroids measured after 30 min (Table 4). The effects of **20** were the most marked, with increases in pregnenolone, progesterone, allopregnanolone, and allotetrahydrodeoxycorticosterone (THDOC) of 126%, 103%, 85%, and 83%, respectively, while **35** did not significantly affect the amount of neuroactive steroids in plasma. With the exception of **35**, all the compounds markedly increased the levels of neuroactive steroids in brain (Table 5). Again, **20** was overall the most efficacious, with increases in pregnenolone, progesterone, allopregnanolone, and THDOC of 87%, 101%, 73%, and 50%, respectively. The effects of the others were less marked but significant, while **35** failed to affect the neuroactive steroid content in the cerebral cortex.

Table 4. Effect of ip Administration of Compounds **17**, **20**, **26**, **34**, and **35** (25 mg/kg) on Plasma Concentration of Neuroactive Steroid in Rats after 30 min[a]

	PRE (ng/mL)	PRO (ng/mL)	AP (ng/mL)	THDOC (ng/mL)	CTS (ng/mL)
vehicle	4.1 ± 0.3	6.3 ± 0.4	2.6 ± 0.5	3.5 ± 0.4	210 ± 20
17	7.3 ± 0.5[c]	13.7 ± 1.0[c]	4.9 ± 0.4[c]	5.3 ± 0.3[b]	290 ± 15
20	9.3 ± 1.0[c]	12.8 ± 0.4[c]	4.8 ± 0.3[c]	6.4 ± 0.6[c]	394 ± 30[c]
26	6.5 ± 1.0[c]	10.4 ± 0.7[c]	5.2 ± 0.3[c]	5.1 ± 0.2[b]	292 ± 26
34	7.7 ± 0.2[c]	11.3 ± 0.5[c]	4.4 ± 0.4[c]	4.8 ± 0.4[b]	327 ± 16[b]
35	5.2 ± 0.2	7.3 ± 0.5	3.3 ± 0.4	3.5 ± 0.4	260 ± 28

[a] PRE, pregnenolone; PRO, progesterone; AP, allopregnanolone; THDOC, allotetrahydrodeoxycorticosterone; CTS, corticosterone. Data are means ± SEM of 8−10 rats. [b] p 0.05. [c] p 0.01 vs vehicle-treated animals.

Table 5. Effect of ip Administration of Compounds **17**, **20**, **26**, **34**, and **35** (25 mg/kg) on the Concentration of Neuroactive Steroid in Cerebral Cortex of Rats after 30 min[a]

	PRE (ng/g of prot)	PRO (ng/g of prot)	AP (ng/g of prot)	THDOC (ng/g of prot)
vehicle	123 ± 10	52 ± 4	5.6 ± 0.4	5.0 ± 0.3
17	184 ± 17[c]	97 ± 4[c]	9.6 ± 0.5[c]	7.4 ± 0.4[c]
20	230 ± 17[c]	105 ± 10[c]	9.7 ± 0.5[c]	8.4 ± 0.3[c]
26	182 ± 9[c]	78 ± 5[c]	8.2 ± 0.2[c]	6.8 ± 0.5[b]
34	204 ± 11[c]	83 ± 6[b]	7.5 ± 0.4[c]	6.5 ± 0.5[b]
35	148 ± 9	55 ± 2	5.9 ± 0.2	5.6 ± 0.5

[a] PRE, pregnenolone; PRO, progesterone; AP, allopregnanolone; THDOC, allotetrahydrodeoxycorticosterone. Data are means ± SEM of 8−10 rats. [b] p 0.05. [c] $p < 0.01$ vs vehicle-treated animals.

Conclusions

We have shown that some 2-phenylimidazo[1,2-*a*]-pyridine derivatives investigated in the present study are potent and selective ligands for PBR and stimulate steroidogenesis in both the brain and periphery. In accordance with previous studies,[17,21] our binding data demonstrate that substitution at the 8-position of the imidazopyridine nucleus is a key factor for improving affinity and selectivity toward peripheral binding sites. In fact, members of the 8- and 6,8-disubstituted imidazopyridines show PBR selectivity > 10^3−10^5 and are among the most selective ligands identified so far. Substitution of the 8-position with lipophilic groups and the *para* position of the phenyl ring at C(2) with a chlorine atom are important for high affinity and selectivity. Another important structural feature concerns the number and length of alkyl substituents on the amide. Again, the higher the lipophilic character of the alkyl substituents, the better the affinity and selectivity. For affinity at PBR, this holds true to a log *P* value of ∼6, as beyond this value affinity decreases. However, as anticipated in our previous discussion, other properties, besides lipophilicity, should influence both affinity and selectivity. To clarify this important point, a deeper study of the SAR and SSR based upon 3D-QSAR and molecular modeling techniques is now in progress. Results from this study may be helpful to

support and/or integrate the pharmacophore model proposed by Bourguignon and Wermuth for PBR ligands.[18]

On the basis of the fact that several known ligands for the PBR possess some affinity also for the CBR, we evaluated the selectivity of these compounds by determining their effects at $GABA_A$ receptors. This was accomplished by expressing cloned human $GABA_A$ receptors in *Xenopus* oocytes and by observing the ability of the different drugs to alter GABA-evoked Cl^- currents. Our results indicate that compounds **34** and **35**, in good accordance with their ability to bind to cortical [^{3}H]flunitrazepam binding sites with a higher affinity than the other compounds, modulate in a positive and flumazenil-sensitive manner the responses to GABA. On the other hand, again in good agreement with binding data, compounds **17**, **20**, and **26**, which show almost no affinity for [^{3}H]flunitrazepam binding sites, did not modulate the action of GABA through the CBR. The ability of compound **20** to inhibit GABA-induced Cl^- currents was flumazenil-insensitive indicating that the effect is probably caused by a channel-blocking action exerted at high concentrations of this drug.

In the in vivo studies, compounds **17**, **20**, **26**, and **34** each exhibited potent effects on the peripheral and central synthesis of neuroactive steroids with **20** and **26** being the most potent. Consistent with previous studies[21,22] PK 11195 also induced significant increases in both the cortical and plasma concentrations of neuroactive steroids. Interestingly, compound **35**, even though it possesses high affinity (5−10 nM) for the PBR, did not show intrinsic activity. In fact, this compound failed to affect the neuroactive steroid content in both plasma and cerebral cortex. Since currently no ligands with antagonistic properties on steroidogenesis have been described, the availability of a such molecule together with additional selective ligands with different intrinsic activities may help to increase our understanding of the pharmacology and physiology of the PBR.

Experimental Section

Chemistry. Melting points were determined in open capillary tubes with a Büchi apparatus and are uncorrected. IR spectra were obtained on a Perkin-Elmer 283 spectrophotometer (KBr pellets for solid or Nujol for liquid). ^{1}H NMR spectra were determined on a Varian 390 or Bruker 300 MHz instrument. Chemical shifts are given in δ values downfield from Me_4Si as internal standard. Mass spectra were recorded on a Hewlett-Packard 5995c GC−MS low-resolution spectrometer. All compounds showed appropriate IR, ^{1}H NMR, and mass spectra. Elemental analyses were carried out with a Carlo Erba model 1106 analyzer, and results were within ±0.40% of the theoretical values. Silica gel 60 (Merck 70−230 mesh) was used for column chromatography. All the following reactions were performed under a nitrogen atmosphere. The starting 2-aminopyridine compounds are commercially available except for 2-amino-3-methoxypyridine prepared as follows, respectively. The 2-amino-3-chloropyridine was prepared following a literature procedure.[23,24] The preparation of *N,N*-di-*n*-propyl-3-bromo-3-benzoylpropionamide and *N,N*-di-*n*-propyl-3-bromo-3-(4-chlorobenzoyl)propionamide has previously been reported.[17]

2-Amino-3-methoxypyridine. To a solution of 2-amino-3-hydroxypyridine (1 g, 9.1 mmol) in methanol (30 mL) was added a freshly prepared ethereal solution of diazomethane (9.5 mmol) portionwise, and the resulting mixture was stirred at room temperature overnight. Evaporation of the solvent gave a residue which was purified by column chromatography [light petroleum ether/ethyl acetate 2/8 (v/v) as eluent] yielding the title compound in 45% yield: IR (KBr) 3840, 3470, 3240, 1060 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.70 (s, 3H, OCH_3), 4.56 (s, 2H, NH_2 disappeared on change with D_2O), 6.3−7.6 (m, 3H, Ar); MS *m/z* 124 (67, M^+), 81 (base). Anal. ($C_6H_8N_2O$) C, H, N.

General Procedure for Preparation of (2-Phenylimidazo[1,2-*a*]pyridin-3-yl)acetamides 17−26 and 32−40. To a solution of the suitably substituted 2-aminopyridine **3** (11 mmol) in DMF (50 mL) was added the appropriate 3-bromo-3-benzoylpropionamide **15** (11 mmol). The mixture was refluxed under stirring and under a nitrogen atmosphere for 7−20 h. The progress of reaction was monitored by TLC. The solvent was evaporated under reduced pressure; the residue was purified by silica gel column chromatography [light petroleum ether/ethyl acetate 8/2 (v/v) as eluent] to give the amides **17**−**26** and **32**−**40**. Physical data are summarized in Table 1.

***N,N*-Di-*n*-propyl-(2-phenyl-6-methoxycarbonylimidazo-[1,2-*a*]pyridin-3-yl)acetamide (17):** IR (KBr) 1720, 1620 cm^{-1} ; ^{1}H NMR ($CDCl_3$) δ 0.63 (t, J = 6 Hz, 3H, CH_3), 0.83 (t, J = 6 Hz, 3H, CH_3), 1.3−1.6 (m, 4H, CH_2), 3.00 (t, J = 6 Hz, 2H, CH_2−N), 3.20(t, J = 6 Hz, 2H, CH_2−N), 3.90 (s, 3H, OCH_3), 4.03 (s, 2H, CH_2CO), 7.3−7.7 (m, 7H, Ar), 8.90 (s, 1H, Ar); MS *m/z* 393 (M^+, 21), 265 (base). Anal. ($C_{23}H_{27}N_3O_3$) C, H, N.

***N,N*-Di-*n*-propyl-(2-phenyl-6-carboxamidoimidazo[1,2-*a*]pyridin-3-yl)acetamide (18):** IR (KBr) 3345, 3185, 1675, 1635 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.80 (t, J = 9 Hz, 3H, CH_3), 0.90 (t, J = 6 Hz, 3H, CH_3), 1.3−1.7 (m, 4H, CH_2), 3.20 (t, J = 9 Hz, 2H, CH_2−N), 3.30 (t, J = 6 Hz, 2H, CH_2−N), 3.60 (br s, 2H, NH_2), 4.10 (s, 2H, CH_2CO), 7.3−7.7 (m, 7H, Ar), 8.80 (s, 1H, Ar); MS *m/z* 378 (M^+, 23), 250 (base). Anal. ($C_{22}H_{26}N_4O_2$) C, H, N.

***N,N*-Di-*n*-propyl-(2-phenyl-8-methylimidazo[1,2-*a*]pyridin-3-yl)acetamide (19):** IR (KBr) 1630 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.63 (t, J = 9 Hz, 3H, CH_3), 0.90 (t, J = 9 Hz, 3H, CH_3), 1.3−1.7 (m, 4H, CH_2), 2.73 (s, 3H, CH_3), 3.06 (t, J = 9 Hz, 2H, CH_2−N), 3.26 (t, J = 9 Hz, 2H, CH_2−N), 4.03 (s, 2H, CH_2CO), 6.7−8.1 (m, 7H, Ar), 8.26 (d, J = 9 Hz, 1H, Ar); MS *m/z* 349 (M^+, 23), 221 (base). Anal. ($C_{22}H_{27}N_3O$) C, H, N.

***N,N*-Di-*n*-propyl-[2-(4-chlorophenyl)-8-methylimidazo-[1,2-*a*]pyridin-3-yl]acetamide (20):** IR (KBr) 1625 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.67 (t, J = 2.5 Hz, 3H, CH_3), 0.80 (t, J = 2.5 Hz, 3H, CH_3), 1.4−1.6 (m, 4H, CH_2), 2.62 (s, 3H, CH_3), 3.04 (t, J = 2.5 Hz, 2H, CH_2−N), 3.24 (t, J = 0.5 Hz, 2H, CH_2−N), 4.05 (s, 2H, CH_2CO), 6.7−7.6 (m, 6H, Ar), 8.11 (d, J = 2.5 Hz, 1H, Ar); MS *m/z* 383 (M^+, 22), 255 (base). Anal. ($C_{22}H_{26}ClN_3O$) C, H, N.

***N,N*-Di-*n*-propyl-(2-phenyl-8-nitroimidazo[1,2-*a*]pyridin-3-yl)acetamide (21):** IR (KBr) 1625 cm^{-1} ; ^{1}H NMR ($CDCl_3$) δ 0.50 (t, J = 9 Hz, 3H, CH_3), 0.66 (t, J = 9 Hz, 3H, CH_3), 1.1−1.5 (m, 4H, CH_2), 2.90 (t, J = 9 Hz, 2H, CH_2N), 3.10 (t, J = 9 Hz, 2H, CH_2−N), 4.03 (s, 2H, CH_2CO), 7.2−7.6 (m, 7H, Ar), 8.60 (d, J = 9 Hz, 1H, Ar); MS *m/z* 380 (M^+, 55), 252 (base). Anal. ($C_{21}H_{24}N_4O_3$) C, H, N.

***N,N*-Di-*n*-propyl-[2-(4-chlorophenyl)-8-nitroimidazo-[1,2-*a*]pyridin-3-yl]acetamide (22):** IR (KBr) 1620 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.72 (t, J = 2.5 Hz, 3H, CH_3), 0.82 (t, J = 2.5 Hz, 3H, CH_3), 1.4−1.6 (m, 4H, CH_2), 3.07 (t, J = 2.5 Hz, 2H, CH_2N), 3.25 (t, J = 0.5 Hz, 2H, CH_2−N), 4.12 (s, 2H, CH_2CO), 6.9−7.7 (m, 5H, Ar), 8.20 (dd, J = 2 and 5 Hz, 1H, Ar), 8.62 (dd, J = 2 and 3 Hz, 1H, Ar); MS *m/z* 414 (M^+, 14), 256 (base). Anal. ($C_{21}H_{23}ClN_4O_3$) C, H, N.

***N,N*-Di-*n*-propyl-(2-phenyl-8-methoxyimidazo[1,2-*a*]-pyridin-3-yl)acetamide (23):** IR (KBr) 1630 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.57 (t, J = 2.5 Hz, 3H, CH_3), 0.78 (t, J = 2.5 Hz, 3H, CH_3), 1.3−1.4 (m, 2H, CH_2), 1.4−1.5 (m, 2H, CH_2), 2.97 (t, J = 2.5 Hz, 2H, CH_2−N), 3.22 (t, J = 0.5 Hz, 2H, CH_2−N), 4.00 (s, 3H, CH_3O), 4.10 (s, 2H, CH_2CO), 6.47 (d, J = 2.5 Hz, 1H, Ar), 6.71 (t, J = 2.5 Hz, 1H, Ar), 7.3−7.4 (m, 3H, Ar), 7.6−7.7 (m, 2H, Ar), 7.9−8.0 (m, 1H, Ar); MS *m/z* 365 (M^+, 30), 237 (base). Anal. ($C_{22}H_{27}N_3O_2$) C, H, N.

***N,N*-Di-*n*-propyl-[2-(4-chlorophenyl)-8-methoxyimidazo-[1,2-*a*]pyridin-3-yl]acetamide (24):** IR (KBr) 1630 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.66 (t, $J = 2.5$ Hz, 3H, CH_3), 0.80 (t, $J = 2.5$ Hz, 3H, CH_3), 1.3−1.6 (m, 4H, CH_2), 3.03 (t, $J = 2.5$ Hz, 2H, CH_2−N), 3.24(t, $J = 0.5$ Hz, 2H, CH_2−N), 4.00 (s, 3H, OCH_3), 4.10 (s, 2H, CH_2CO), 6.4−6.5 (m, 1H, Ar), 6.7−6.8 (m, 1H, Ar), 7.3−7.4 (m, 2H, Ar), 7.6−7.7 (m, 2H, Ar), 7.85 (dd, $J = 2$ and 5 Hz, 1H, Ar); MS *m/z* 399 (M^+, 13), 271 (base). Anal. ($C_{22}H_{26}ClN_3O_2$) C, H, N.

***N,N*-Di-*n*-propyl-(2-phenyl-8-chloroimidazo[1,2-*a*]pyridin-3-yl)acetamide (25):** IR (KBr) 1620 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.53 (t, $J = 9$ Hz, 3H, CH_3), 0.73 (t, $J = 9$ Hz, 3H, CH_3), 1.1−1.6 (m, 4H, CH_2), 2.93 (t, $J = 9$ Hz, 2H, CH_2−N), 3.23 (t, $J = 9$ Hz, 2H, CH_2−N), 4.07 (s, 2H, CH_2CO), 6.5−7.8 (m, 7H, Ar), 8.27 (d, $J = 8$ Hz, 1H, Ar); MS *m/z* 369 (M^+, 23), 241 (base). Anal. ($C_{21}H_{24}ClN_3O$) C, H, N.

***N,N*-Di-*n*-propyl-[2-(4-chlorophenyl)-8-chloroimidazo-[1,2-*a*]pyridin-3-yl]acetamide (26):** IR (KBr) 1630 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.73 (t, $J = 9$ Hz, 3H, CH_3), 0.86 (t, $J = 9$ Hz, 3H, CH_3), 1.3−1.6 (m, 4H, CH_2), 3.10 (t, $J = 9$ Hz, 2H, CH_2−N), 3.30 (t, $J = 9$ Hz, 2H, CH_2−N), 4.10 (s, 2H, CH_2CO), 6.8−7.7 (m, 6H, Ar), 8.20 (d, $J = 9$ Hz, 1H, Ar); MS *m/z* 403 (M^+, 22), 275 (base). Anal. ($C_{21}H_{23}Cl_2N_3O$) C, H, N.

***N,N*-Diethyl-(2-phenylimidazo[1,2-*a*]pyridin-3-yl)acetamide (32):** IR (Nujol) 1650 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.90 (t, $J = 6$ Hz, 3H, CH_3), 1.10 (t, $J = 6$ Hz, 3H, CH_3), 3.13 (q, $J = 9$ Hz, 2H, CH_2), 3.33 (q, $J = 9$ Hz, 2H, CH_2), 4.13 (s, 2H, CH_2CO), 6.7−7.7 (m, 8H, Ar), 8.30 (d, $J = 9$ Hz, 1H, Ar); MS *m/z* 307 (M^+, 22), 207 (base). Anal. ($C_{19}H_{21}N_3O$) C, H, N.

***N,N*-Di-*n*-propyl-[2-(4-chlorophenyl)imidazo[1,2-*a*]pyridin-3-yl]acetamide (33):** IR (KBr) 1620 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.68 (t, $J = 2.5$ Hz, 3H, CH_3), 0.81 (t, $J = 2.5$ Hz, 3H, CH_3), 1.4−1.5 (m, 4H, CH_2), 3.06 (t, $J = 0.5$ Hz, 2H, CH_2−N), 3.25 (t, $J = 0.5$ Hz, 2H, CH_2−N), 4.08 (s, 2H, CH_2CO), 6.8−7.6 (m, 7H, Ar), 8.23 (dd, $J = 2$ and 3 Hz, 1H, Ar); MS *m/z* 369 (M^+, 26), 241 (base). Anal. ($C_{21}H_{24}ClN_3O$) C, H, N.

***N,N*-Di-*n*-butyl-(2-phenylimidazo[1,2-*a*]pyridin-3-yl)-acetamide (34):** IR (KBr) 1620 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.83 (t, $J = 4.5$ Hz, 3H, CH_3), 0.90 (t, $J = 4.5$ Hz, 3H, CH_3), 1.1−1.6 (m, 8H, CH_2), 3.10 (t, $J = 6$ Hz, 2H, CH_2−N), 3.33 (t, $J = 6$ Hz, 2H, CH_2−N), 4.16 (s, 2H, CH_2CO), 6.8−7.8 (m, 8H, Ar), 8.40 (d, $J = 9$ Hz, 1H, Ar); MS *m/z* 363 (M^+, 22), 207 (base). Anal. ($C_{23}H_{29}N_3O$) C, H, N.

***N,N*-Di-*n*-butyl-[2-(4-chlorophenyl)imidazo[1,2-*a*]pyridin-3-yl]acetamide (35):** IR (KBr) 1620 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.70 (t, $J = 3$ Hz, 6H, CH_3), 0.9−1.4 (m, 8H, CH_2), 3.10 (t, $J = 9$ Hz, 2H, CH_2−N), 3.30 (t, $J = 9$ Hz, 2H, CH_2−N), 4.10 (s, 2H, CH_2CO), 6.7−7.8 (m, 7H, Ar), 8.20 (d, $J = 6$ Hz, 1H, Ar); MS *m/z* 397 (M^+, 24), 241 (base). Anal. ($C_{23}H_{28}ClN_3O$) C, H, N.

***N,N*-Di-*n*-hexyl-(2-phenylimidazo[1,2-*a*]pyridin-3-yl)-acetamide (36):** IR (neat) 1630 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.86 (t, $J = 6$ Hz, 6H, CH_3), 1.0−1.5 (m, 16H, CH_2), 3.03 (t, $J = 6$ Hz, 2H, CH_2−N), 3.26 (t, $J = 6$ Hz, 2H, CH_2−N), 4.13 (s, 2H, CH_2CO), 6.7−7.8 (m, 8H, Ar), 8.33 (d, $J = 8$ Hz, 1H, Ar); MS *m/z* 419 (M^+, 15), 207 (base). Anal. ($C_{27}H_{37}N_3O$) C, H, N.

***N,N*-Di-*n*-hexyl-[2-(4-chlorophenyl)imidazo[1,2-*a*]pyridin-3-yl]acetamide (37):** IR (KBr) 1630 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.90 (t, $J = 3$ Hz, 6H, CH_3), 1.0−1.7 (m, 16H, CH_2), 3.10 (t, $J = 6$ Hz, 2H, CH_2−N), 3.30 (t, $J = 6$ Hz, 2H, CH_2−N), 4.10 (s, 2H, CH_2CO), 6.8−7.8 (m, 7H, Ar), 8.20 (d, $J = 8$ Hz, 1H, Ar); MS *m/z* 453 (M^+, 20), 241 (base). Anal. ($C_{27}H_{36}ClN_3O$) C, H, N.

***N-n*-Propyl-(2-phenylimidazo[1,2-*a*]pyridin-3-yl)acetamide (38):** IR (KBr) 3225, 1630 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.76 (t, $J = 2.5$ Hz, 3H, CH_3), 1.3−1.5 (m, 2H, CH_2), 3.1−3.2 (m, 2H, CH_2−NH), 3.99 (s, 2H, CH_2CO), 5.77 (br s, 1H, NH), 6.8−7.7 (m, 8H, Ar), 8.03 (dd, $J = 0.3$ and 0.6 Hz, 1H, Ar); MS *m/z* 293 (M^+, 23), 207 (base). Anal. ($C_{18}H_{19}N_3O$) C, H, N.

***N-n*-Propyl-[2-(4-chlorophenyl)imidazo[1,2-*a*]pyridin-3-yl]acetamide (39):** IR (KBr) 3230, 1635 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.78 (t, $J = 2.5$ Hz, 3H, CH_3), 1.3−1.5 (m, 2H, CH_2), 3.1−3.2 (m, 2H, CH_2−NH), 3.98 (s, 2H, CH_2CO), 5.84 (br s, 1H, NH), 6.8−7.7 (m, 7H, Ar), 8.05 (d, $J = 2.3$ Hz, 1H, Ar); MS *m/z* 327 (M^+, 24), 241 (base). Anal. ($C_{18}H_{18}ClN_3O$) C, H, N.

***N-n*-Butyl-(2-phenyl-6,8-dichloroimidazo[1,2-*a*]pyridin-3-yl)acetamide (40):** IR (KBr) 3270, 1640 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.84 (t, $J = 2.5$ Hz, 3H, CH_3), 1.1−1.3 (m, 2H, CH_2), 1.3−1.4 (m, 2H, CH_2), 3.21 (q, $J = 4.5$ Hz, 2H, CH_2−NH), 3.87 (s, 2H, CH_2CO), 5.93 (br s, 1H, NH), 7.3−7.7 (m, 6H, Ar), 8.08 (d, $J = 0.5$ Hz, 1H, Ar); MS *m/z* 375 (M^+, 11), 275 (base). Anal. ($C_{19}H_{19}Cl_2N_3O$) C, H, N.

General Procedure for Preparation of (8-Aminoimidazo[1,2-*a*]pyridin-3-yl)acetamides 27 and 28. A mixture of (8-nitroimidazo[1,2-*a*]pyridin-3-yl)acetamide **21** or **22** (7.9 mmol) and 10% palladium on carbon in ethanol (30 mL) was shaken on a hydrogenation apparatus at 24 atm of hydrogen. The progress of reaction was monitored by TLC. Then the catalyst was filtered, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography [light petroleum ether/ethyl acetate 8/2 (v/v) as eluent] to give the amide **27** or **28**, respectively.

Alternatively, the title compounds were prepared according to the following procedure. A mixture of (8-nitroimidazo[1,2-*a*]pyridin-3-yl)acetamide **21** or **22** (4.8 mmol) in concentrated HCl (10 mL) and granular tin (0.40 g, 3.37 mmol) was heated at 100 °C for 2 h. After cooling at room temperature, the mixture was made alkaline with NaOH and the organic phase extracted with $CHCl_3$ (3 × 20 mL), washed, and dried (Na_2SO_4). Evaporation of the solvent gave a residue which was purified by silica gel column chromatography [light petroleum ether/ethyl acetate 8/2 (v/v) as eluent] to give the amide **27** or **28**, respectively. Physical data are summarized in Table 1.

***N,N*-Di-*n*-propyl-(2-phenyl-8-aminoimidazo[1,2-*a*]pyridin-3-yl)acetamide (27):** IR (KBr) 1620 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.59 (t, $J = 2.5$ Hz, 3H, CH_3), 0.79 (t, $J = 2.5$ Hz, 3H, CH_3), 1.4−1.5 (m, 4H, CH_2), 3.00 (t, $J = 2.5$ Hz, 2H, CH_2−N), 3.23 (t, $J = 2.5$ Hz, 2H, CH_2−N), 4.05 (s, 2H, CH_2CO), 4.59 (s, 2H, NH_2), 6.3−7.8 (m, 8H, Ar); MS *m/z* 350 (M^+, 25), 222 (base). Anal. ($C_{21}H_{26}N_4O$) C, H, N.

***N,N*-Di-*n*-propyl-[2-(4-chlorophenyl)-8-aminoimidazo-[1,2-*a*]pyridin-3-yl]acetamide (28):** IR (KBr) 1630 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.69 (t, $J = 2.5$ Hz, 3H, CH_3), 0.81 (t, $J = 2.5$ Hz, 3H, CH_3), 1.3−1.6 (m, 4H, CH_2), 3.06 (t, $J = 2.5$ Hz, 2H, CH_2−N), 3.25 (t, $J = 0.5$ Hz, 2H, CH_2−N), 4.02 (s, 2H, CH_2CO), 4.50 (s, 2H, NH_2), 6.3−7.9 (m, 7H, Ar); MS *m/z* 384 (M^+, 23), 256 (base). Anal. ($C_{21}H_{25}ClN_4O$) C, H, N.

Preparation of *N,N*-Di-*n*-propyl-[2-(4-chlorophenyl)-8-methylaminoimidazo[1,2-*a*]pyridin-3-yl]acetamide (29). To a mixture of (8-aminoimidazo[1,2-*a*]pyridin-3-yl)acetamide **28** (0.30 g, 0.78 mmol) and $NaHCO_3$ (0.24 g, 2.90 mmol) in water/methanol (8/2, 50 mL) was dropwise added dimethyl sulfate (0.33 g, 2.6 mmol) maintaining the reaction temperature at 18−20 °C with a water bath. The reaction mixture was stirred at room temperature for 1 h, heated at 60 °C for 15 min, and then diluted with water. To the stirred and ice-cooled mixture was added ethanolamine (2 mL); the stirring was prolonged overnight, and then the mixture was extracted with $CHCl_3$ (3 × 50 mL) to give an oil which solidified on standing and was identified as compound **29**: IR (KBr) 3410, 1625 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.68 (t, $J = 2.5$ Hz, 3H, CH_3), 0.81 (t, $J = 2.5$ Hz, 3H, CH_3), 1.3−1.5 (m, 4H, CH_2), 2.96 (d, $J = 1$ Hz, 3H, CH_3), 3.06 (t, $J = 2.6$ Hz, 2H, CH_2−N), 3.25 (t, $J = 2.5$ Hz, 2H, CH_2−N), 4.01 (s, 2H, CH_2CO), 5.24 (br s, 1H, NH), 6.09 (d, $J = 2.5$ Hz, 1H, Ar), 6.70 (t, $J = 2.4$ Hz, 1H, Ar), 7.3−7.6 (m, 5H, Ar); MS *m/z* 398 (M^+, 19), 270 (base). Anal. ($C_{22}H_{27}ClN_4O$) C, H, N.

General Procedure for Preparation of (8-Acetamidoimidazo[1,2-*a*]pyridin-3-yl)acetamides 30 and 31. A mixture of (8-aminoimidazo[1,2-*a*]pyridin-3-yl)acetamide **27** or **28** (0.86 mmol) in acetic anhydride (25 mL) was stirred at room temperature overnight. The solvent was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography [light petroleum ether/ethyl acetate 1/1 (v/v) as eluent] to give the amide **30** or **31**, respectively. Physical data are summarized in Table 1.

***N,N*-Di-*n*-propyl-(2-phenyl-8-acetamidoimidazo[1,2-*a*]-pyridin-3-yl)acetamide (30):** IR (KBr) 3400, 3300, 1620 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.65 (t, $J = 2.5$ Hz, 3H, CH_3), 0.81 (t, $J = 2.5$ Hz, 3H, CH_3), 1.4−1.6 (m, 4H, CH_2), 2.28 (s, 3H, CH_3-CO), 3.04 (t, $J = 2.5$ Hz, 2H, CH_2−N), 3.25 (t, $J = 0.5$ Hz, 2H, CH_2−N), 4.07 (s, 2H, CH_2CO), 6.81 (t, $J = 2.5$ Hz, 1H, Ar), 7.4−7.7 (m, 5H, Ar), 7.96 (dd, $J = 2.3$ and 0.3 Hz, 1H, Ar), 8.16 (d, $J = 2.5$ Hz, 1H, Ar), 8.72 (s, 1H, NH); MS *m*/*z* 392 (M^+, 15), 264 (base). Anal. ($C_{23}H_{28}N_4O_2$) C, H, N.

***N,N*-Di-*n*-propyl-[2-(4-chlorophenyl)-8-acetamidoimidazo[1,2-*a*]pyridin-3-yl]acetamide (31):** IR (KBr) 3300, 1630 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.72 (t, $J = 2.5$ Hz, 3H, CH_3), 0.83 (t, $J = 2.5$ Hz, 3H, CH_3), 1.4−1.6 (m, 4H, CH_2), 2.36 (s, 3H, CH_3CO), 3.09 (t, $J = 2.5$ Hz, 2H, CH_2−N), 3.29 (t, $J = 0.5$ Hz, 2H, CH_2−N), 4.02 (s, 2H, CH_2CO), 6.8−7.6 (m, 6H, Ar), 8.2−8.3 (m, 1H, Ar), 9.20 (s, 1H, NH); MS *m*/*z* 426 (M^+, 16), 298 (base). Anal. ($C_{23}H_{27}ClN_4O_2$) C, H, N.

Biological Methods. Materials. Adult male or female Sprague−Dawley CD rats (Charles River, Como, Italy), with body masses of 200−250 g at the beginning of the experiments, were maintained under an artificial 12-h light/dark cycle (light on from 08.00 to 20.00 h) at a constant temperature of 23 ± 2 °C and 65% humidity. Food and water were freely available, and the animals were acclimatized for >7 days before use. Experiments were performed between 0800 and 1400 h. Animal care and handling throughout the experimental procedure were performed in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). The experimental protocol were approved by the Animal Ethical Committee of the University of Cagliari.

In Vitro Receptor Binding Assays. 1. [^{3}H]Flunitrazepam Binding. Cerebral cortex was homogenized with a Polytron PT 10 in 50 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.4) and centrifuged twice at 20000*g* for 10 min. The pellet was reconstituted in 50 volumes of Tris-HCl buffer and was used for the binding assay. Aliquots of 400 μL of tissue homogenate (0.4−0.5 mg of protein) were incubated in the presence of [^{3}H]flunitrazepam at a final concentration of 0.5 nM, in a total incubation volume of 1000 μL. The drugs were added in 100-μL aliquots. After a 60-min incubation at 0 °C, the assay was determined by rapid filtration through glass-fiber filter strips (Whatman GF/B). The filters were rinsed with 2−4-mL portions of ice-cold Tris-HCl buffer as described above. Radioactivity bound to the filters was quantitated by liquid scintillation spectrometry. Nonspecific binding was determined as binding in the presence of 5 μM diazepam and represented about 10% of total binding.

2. [^{3}H]PK 11195 Binding. After sacrifice brain and ovary were rapidly removed, cerebral cortex was dissected, and both tissues were stored at −80 °C until assay. The tissues were thawed and homogenized in 50 volumes of Dulbecco's phosphate-buffered saline (PBS) pH 7.4 at 4 °C with a Polytron PT 10 (setting 5 for 20 s). The homogenate was centrifuged at 40000*g* for 30 min, and the pellet was resuspended in 50 volumes of PBS and recentrifuged. The new pellet was resuspended in 20 (cerebral cortex) or 150 (ovary) volumes of PBS and used for the assay. [^{3}H]PK 11195 binding was determined in a final volume of 1000 μL of tissue homogenate (0.15−0.20 mg protein for the cerebral cortex and 0.01−0.02 mg of protein for the ovary), 100 μL of [^{3}H]PK 11195 (s.a. 85.5 Ci/mmol; New England Nuclear) at final assay concentration of 1 nM, 5 μL of drug solution or solvent, and 795 μL of PBS buffer (pH 7.4 at 25 °C). Incubations (0 °C) were initiated by addition of membranes and were terminated 90 min later by rapid filtration through glass-fiber filter strips (Wathaman GF/B), which were rinsed with 2 × 4 mL of ice-cold PBS buffer using cell harvester filtration manifold (Brandel). Filter-bound radioactivity was quantified by liquid scintillation spectrometry. Nonspecific binding was defined as binding in the presence of 10 μM unlabeled PK 11195 (Sigma).

Functional in Vitro Studies: Electrophysiological Studies Using *Xenopus* Oocytes. Complementary DNAs encoding the human α1, β2, and γ2 $GABA_A$ receptor subunits were subcloned into the pCDM8 expression vector (Invitrogen, San Diego, CA). The cDNAs were purified with the Promega Wizard Plus Miniprep DNA Purification System (Madison, Wl) and then resuspended in sterile distilled water, divided into portions, and stored at −20 °C until used for injection. Stage V and VI oocytes were manually isolated from sections of *Xenopus laevis* ovary, placed in modified Barth's saline (MBS) containing 88 mM NaCl, 1 mM KCl, 10 mM Hepes-NaOH (pH 7.5), 0.82 mM $MgSO_4$, 2.4 mM $NaHCO_3$, 0.91 mM $CaCl_2$, and 0.33 mM $Ca(NO_3)_2$ and treated with 0.5 mg/mL collagenase type IA (Sigma) in collagenase buffer (83 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 5 mM Hepes-NaOH, pH 7.5) for 10 min at room temperature, to remove the follicular layer. A mixture of $GABA_A$ receptor α1, β2, and γ2 subunit cDNAs (1.5 ng/30 nL) was injected into the oocyte nucleus using a 10-μL glass micropipet (10−15-μm tip diameter). The injected oocytes were cultured at 19 °C in sterile MBS supplemented with streptomycin (10 μg/mL), penicillin (10 U/mL), gentamicin (50 μg/mL), 0.5 mM theophylline, and 2 mM sodium pyruvate. Electrophysiological recordings began approximately 24 h following cDNA injection. Oocytes were placed in a 100-μL rectangular chamber and continuously perfused with MBS solution at a flow rate of 2 mL/min at room temperature. The animal pole of oocytes was impaled with two glass electrodes (0.5−3 MΩ) filled with filtered 3 M KCl, and the voltage was clamped at −70 mV with an Axoclamp 2-B amplifier (Axon Instruments, Burlingame, CA). Currents were continuously recorded on a strip-chart recorder. Resting membrane potential usually varied between −30 and −50 mV. Drugs were perfused for 20 s (7−10 s was required to reach equilibrium in the recording chamber). Intervals of 5−10 min were allowed between drug applications.

Functional in Vivo Studies: Effect of Compounds 17, 20, 26, 34, and 35 on Plasma Concentration of Neuroactive Steroids. 1. Drug Administration. Compounds **17**, **20**, **26**, **34**, and **35** were dissolved by adding 3 drops of Tween 80/5 mL of saline and were administered intraperitoneally in a volume of 0.3 mL/100 g of body mass.

2. Extraction and Assay of Steroids. Male rats were killed at the indicated times either by guillotine (for measurement of plasma steroids) or by focused microwave irradiation (70 W c-2 for 4 s) to the head (for measurement of brain steroid). This latter procedure results in a virtually instantaneous inactivation of brain enzymes, thus minimizing postmortem steroid metabolism. Brains were rapidly (<1 min) removed from the skull, and the cerebral cortices were dissected and frozen at −20 °C until steroid extraction. Steroids were extracted and purified as previously described.[25] Briefly, steroids present in the cerebral cortical homogenates (400 mg of tissue protein in 4 mL of phosphates-buffered saline, pH 7.0 were extracted three times with ethyl acetate, and the combined organic phases were dried under vacuum. The residue was dissolved in 5 mL of *n*-hexane and applied to Seppak silica cartridges (Waters), and components were eluted with *n*-hexane and 2-propanol (7:3, v:v). Steroids were separated and further purified by high-performance liquid chromatography (HPLC) on a 5-μm Lichrosorb-diol column (250 × 4 mm) (Phenomenex) with a discontinuous gradient of 2-propanol (0−30%) in *n*-hexane. Progesterone, which coelutes with cholesterol, was further purified by washing the corresponding dried fractions twice with 200 μL of dimethyl sulfoxide and water (400 μL). Progesterone was then extracted from the aqueous phase twice with 1.5-mL volumes of *n*-hexane. The recovery (70−80%) of steroids through the extraction and purification procedure was monitored by adding a trace amount (6000−8000 cpm; s.a. ranging from 20 to 80 Ci/mmol) of tritiated standard to the brain homogenate. Steroids were quantified by radioimmunoassay as previously described[25,26] with specific antibodies to pregnenolone, progesterone, and corticosterone (ICN, Costa Mesa, CA).

Antibodies to THDOC and allopregnanolone were raised in rabbits and sheep and characterized as previously described.[26] Blood was collected from the trunk of rats killed by guillotine into heparinized tubes and centrifuged at 900*g* for 20 min at room temperature; the plasma was frozen until assays for

steroids. Steroids were extracted from plasma with 1.5 mL of ethyl acetate.

Statistics. Data are presented as means ± SEM and were analyzed by analysis of variance (ANOVA) followed by Newman−Keuls test.

Acknowledgment. This work was supported by a grant from Ministero dell'Università e della Ricerca Scientifica e Tecnologica (MURST). We thank Mr. Giovanni Dipinto for skillful technical assistance in recording mass spectra and determining microanalytical data.

Supporting Information Available: General procedure for preparation of *N*,*N*-di- or *N*-monoalkyl-3-benzoylpropionamides and *N*,*N*-di- or *N*-monoalkyl-3-bromo-3-benzoylpropionamides and their spectral data. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM991035G