Structure−Activity Relationships and Effects on Neuroactive Steroid Synthesis in a Series of 2-Phenylimidazo[1,2-*a*]pyridineacetamide Peripheral Benzodiazepine Receptors Ligands

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A series of 36 imidazopyridineacetamides (**2**−**37**) were designed and synthesized to evaluate the effects of structural changes on the amide nitrogen at both central (CBRs) and peripheral benzodiazepine receptors (PBRs). These changes include variations in the length and number of the alkyl groups as well as introduction of different aromatic, heteroaromatic, and conformationally constrained groups. The affinities of these compounds for CBRs and PBRs were determined, and the results indicate that bulkiness of the substituents, their branching, and length beyond an optimal value may cause hindrance to the ligand in its interaction with the receptor. The presence of aromatic or conformationally constrained substituents on the carboxamide nitrogen can be conducive to high affinity and selectivity. Furthermore, the ability of a subset of the most active ligands to stimulate synthesis of neuroactive steroids in plasma and brain was evaluated in vivo and in vitro. Compound **3** exhibited very marked effects on the peripheral and central synthesis of neuroactive steroids, while **36** (potent at subnanomolar level) showed a slight ability to affect neuroactive steroid content in the cerebral cortex.

Introduction

Benzodiazepines are used clinically as sedatives, hypnotics, anxiolytics, muscle relaxants, and anticonvulsants. In the brain, two major classes of benzodiazepine receptors have been identified: the "central-type" and the "peripheral-type" receptors. Central-type benzodiazepine receptors (CBRs) are present exclusively in the central nervous system (CNS) and mediate the classical effects of benzodiazepines. Peripheral-type benzodiazepine receptors (PBRs) are pharmacologically distinct from CBRs and are present in peripheral tissues and also in glial cells of the CNS. PBRs are composed of a multimeric protein complex located on the outer mitochondrial membrane of astroglial cells.[1] Although the pharmacological role of PBRs in the CNS has not yet been fully clarified, a wide range of pharmacological activities, including anticonvulsant, anxiolytic, immunomodulating, and cardiovascular, have been associated with their activation. In particular, there is growing experimental evidence suggesting that high-affinity PBR ligands stimulate neurosteroids synthesis 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,5α-THPROG), 3α,21-dihydroxy-5α-pregnan-20-one (3α,5α-THDOC), and dehydroepiandrosterone] are known to modulate GABAergic and glutamatergic transmissions.[4−8] Moreover, it has been proved that PBR expression selectively increases in brain tumors, and this led to evaluating appropriate PBR ligands as diagnostic imaging agents[9,10] or as receptor-mediated drug carriers to selectively target anticancer drugs to brain tumors.[11−13] The abundance of PBRs in cancers of colon, breast, ovary, and liver suggests a role of these receptors in tumorigenesis.[14−17] There is also experimental evidence that PBRs are involved in various neurological diseases, while PBR-specific ligands have also been shown to induce apoptosis and cell cycle arrest in cancer cells.[18,19]

The sequence of primary, secondary, and tertiary structure of PBRs in several animal species including human, bovine, rat, and murine has been clarified.[20] Furthermore, considerable efforts have been focused on the identification of new PBR ligands characterized by high affinity and selectivity. The known PBR-selective ligands belong to structurally different compounds such as benzodiazepines ([^{3}H] Ro-54864),[21] isoquinolines ([^{3}H] PK-11195),[22] 2-aryl-3-indoleacetamides (FGIN-1−27),[23] *N*-phenoxyphenyl-*N*-isopropoxybenzylacetamides (DAA1097),[24] and some benzothiazepines[25] (Chart 1).

We have recently shown that some 2-phenylimidazo-[1,2-*a*]pyridineacetamides are potent and selective ligands for PBRs and stimulate steroidogenesis in both the brain and periphery.[26−29] Among these ligands, *N*,*N*-di-*n*-propyl-[2-(4-chlorophenyl)-6,8-dichloroimidazo[1,2-*a*]-pyridin-3-yl)]acetamide (denoted as CB 34)[28] has been

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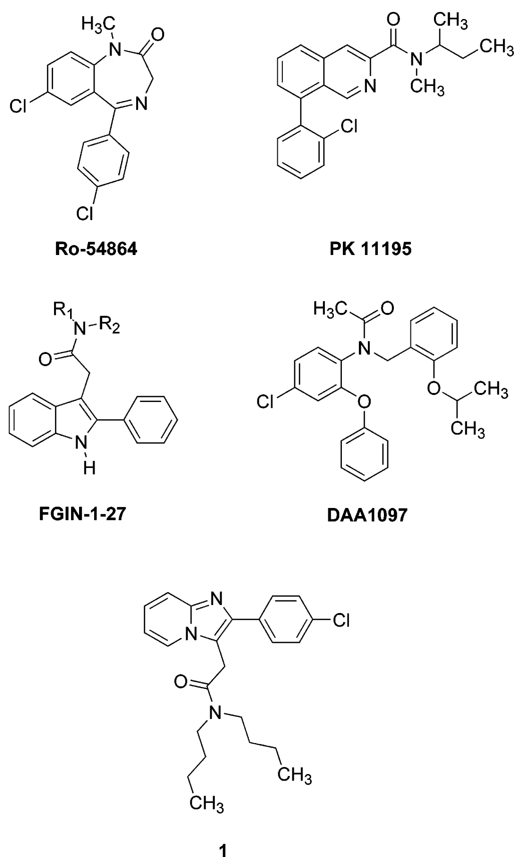
Chart 1



shown to be a particularly interesting compound because of its affinity, selectivity, and stimulation of the steroidogenesis.[26-29] Our 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.[26-29] Substitutions at the 8-position with lipophilic groups and at the para position of the phenyl ring at C(2) with a chlorine atom are crucial for high affinity and selectivity. With the exception of *N,N*-di-*n*-butyl-[2-(4-chlorophenyl)imidazo-[1,2-*a*]pyridin-3-yl)]acetamide **1** (Chart 1), all the *N,N*-dipropylimidazopyridineacetamides studied markedly increased the level of neuractive steroids in brain and therefore can be considered full or partial agonists on steroidogenesis.[27] Compound **1**, even though it possess high affinity for the PBR, showed a low receptor selectivity and failed to affect the neuractive steroid content in both plasma and cerebral cortex.[27] On the other hand, to the best of our knowledge, PBR ligands with high affinity and selectivity which do not stimulate neurosteroid synthesis (antagonists) have never been described. Therefore, the identification of PBR ligands endowed with antagonistic properties on steroidogenesis remains a worthwhile goal. Such molecules may be the proper pharmacological tool to increase our understanding of the physiology and pharmacology of PBRs.

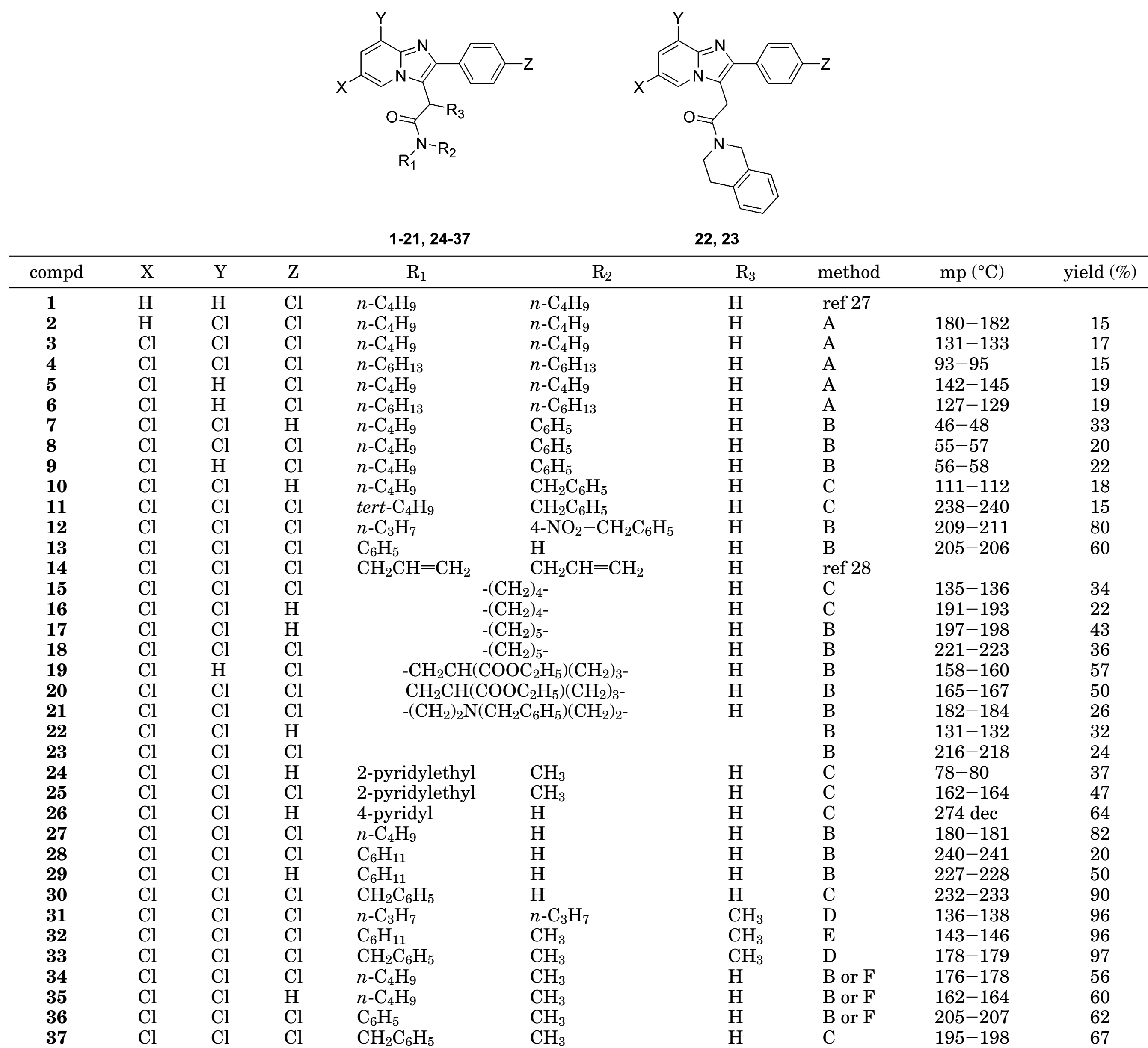
On the basis of information from molecular biology experiments, increasingly accurate PBR ligand−receptor interaction models have been developed.[30] These modeling studies lead to the suggestion that high PBR affinity requires (i) the presence of a suitably located and oriented carbonyl C(=O)N; (ii) the presence of lipophilic substituents attached to the C(=O)N fragment; (iii) the presence of an aromatic or heteroaromatic system with a pendant phenyl ring. Recently, a unique 3D interaction model of endogenous and synthetic PBR ligands has been described[31] which confirmed two lipophilic regions (L1 and L3), one polar group (H2), and a lipophilic region modulating the receptor binding (L4) in which H2 is expressed as essential elements of the PBR interaction. Modeling of the receptor's 3D structure is based on knowledge of the receptor protein's primary structure. Receptor-mapping and receptor-fitting approaches, indeed, led to the suggestion that the amino acid residues Ser 41, Trp 107, and Trp 161 of the receptor protein are key elements in the ligand−receptor interaction. In particular, the amide carbonyl group of the ligand should be involved in a hydrogen bonding acceptor interaction with Ser 41, while the hydrophobic groups attached to C(=O)N should interact with Trp 107 and Trp 161.[30]

The series of 2-phenylimidazo[1,2-*a*]pyridineacetamides **2**−**37** (Table 1) herein studied was designed taking into account the chemical structure of compound **1**. Thus, the present study probed variations in the length and number of the alkyl groups on the amide nitrogen, as well as introduction of different aromatic, heteroaromatic, and conformationally constrained groups. This was done with a 2-fold aim: first, in order to find molecules characterized by high affinity and selectivity which are also antagonist of neurosteroids synthesis, and second, to improve our definition of the structure−activity relationships in this specific region in the 2-phenylimidazopyridineacetamide series of PBR ligands.

Chemistry

The imidazopyridineacetamides **2**−**6** (Table 1) were synthesized using a previously reported method outlined in Scheme 1 (Method A). Briefly, compounds **2**−**6** were prepared by condensation in DMF at reflux of suitably substituted 2-aminopyridines **41** with the appropriate bromoketoamides **40** which, in turn, were prepared by reaction of 3-benzoylpropionic acids **38** 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 **39** with bromine in carbon tetrachloride gave the desired compounds **40**. As shown in Scheme 2, the imidazopyridineacetamides **7**−**9**, **12**, **13**, **17**−**23**, **27**−**29**, **34**−**36** were prepared by condensation of the imidazopyridine-acetic acids **42**[13] with the appropriate mono- or dialkylamine hydrochlorides in anhydrous THF and in the presence of carbonyldiimidazole (CDI) (Method B). The synthesis of compounds **10**, **11**, **15**, **16**, **24**−**26**, **30**, and **37** was accomplished by the procedure shown in Scheme 2 involving the condensation of the imidazopyridineacetic acids **42**[13] with the appropriate mono- or dialkylamines in anhydrous THF and in the presence of EEDQ (Method C). An attempt was made to prepare the tertiary amide *N*-benzyl-*N*-methyl-[2-(4-chlorophenyl)imidazo[1,2-*a*]pyridin-3-yl)]acetamide **37** (Table 1) by *N*-methylation of the corresponding secondary amide **30** by treatment with NaH in DMF and methyl iodide. Under these conditions however, the dimethyl-substituted compound **33** was obtained (Scheme 3, Method D). Similarly, the dimethyl-substituted compound **32** was obtained by treatment of the secondary amide **28**

Table 1. Structure and Physical Properties of Compounds **1**−**37**



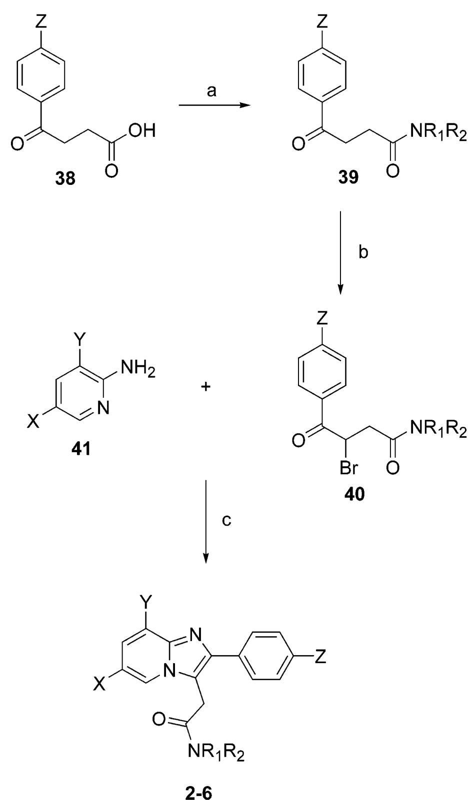
compd	X	Y	Z	R_1	R_2	R_3	method	mp (°C)	yield (%)
1	H	H	Cl	*n*-C_4H_9	*n*-C_4H_9	H	ref 27		
2	H	Cl	Cl	*n*-C_4H_9	*n*-C_4H_9	H	A	180−182	15
3	Cl	Cl	Cl	*n*-C_4H_9	*n*-C_4H_9	H	A	131−133	17
4	Cl	Cl	Cl	*n*-C_6H_{13}	*n*-C_6H_{13}	H	A	93−95	15
5	Cl	H	Cl	*n*-C_4H_9	*n*-C_4H_9	H	A	142−145	19
6	Cl	H	Cl	*n*-C_6H_{13}	*n*-C_6H_{13}	H	A	127−129	19
7	Cl	Cl	H	*n*-C_4H_9	C_6H_5	H	B	46−48	33
8	Cl	Cl	Cl	*n*-C_4H_9	C_6H_5	H	B	55−57	20
9	Cl	H	Cl	*n*-C_4H_9	C_6H_5	H	B	56−58	22
10	Cl	Cl	H	*n*-C_4H_9	$CH_2C_6H_5$	H	C	111−112	18
11	Cl	Cl	Cl	*tert*-C_4H_9	$CH_2C_6H_5$	H	C	238−240	15
12	Cl	Cl	Cl	*n*-C_3H_7	4-NO_2−$CH_2C_6H_5$	H	B	209−211	80
13	Cl	Cl	Cl	C_6H_5	H	H	B	205−206	60
14	Cl	Cl	Cl	$CH_2CH{=}CH_2$	$CH_2CH{=}CH_2$	H	ref 28		
15	Cl	Cl	Cl	-$(CH_2)_4$-		H	C	135−136	34
16	Cl	Cl	H	-$(CH_2)_4$-		H	C	191−193	22
17	Cl	Cl	H	-$(CH_2)_5$-		H	B	197−198	43
18	Cl	Cl	Cl	-$(CH_2)_5$-		H	B	221−223	36
19	Cl	H	Cl	-$CH_2CH(COOC_2H_5)(CH_2)_3$-		H	B	158−160	57
20	Cl	Cl	Cl	$CH_2CH(COOC_2H_5)(CH_2)_3$-		H	B	165−167	50
21	Cl	Cl	Cl	-$(CH_2)_2N(CH_2C_6H_5)(CH_2)_2$-		H	B	182−184	26
22	Cl	Cl	H				B	131−132	32
23	Cl	Cl	Cl				B	216−218	24
24	Cl	Cl	H	2-pyridylethyl	CH_3	H	C	78−80	37
25	Cl	Cl	Cl	2-pyridylethyl	CH_3	H	C	162−164	47
26	Cl	Cl	H	4-pyridyl	H	H	C	274 dec	64
27	Cl	Cl	Cl	*n*-C_4H_9	H	H	B	180−181	82
28	Cl	Cl	Cl	C_6H_{11}	H	H	B	240−241	20
29	Cl	Cl	H	C_6H_{11}	H	H	B	227−228	50
30	Cl	Cl	Cl	$CH_2C_6H_5$	H	H	C	232−233	90
31	Cl	Cl	Cl	*n*-C_3H_7	*n*-C_3H_7	CH_3	D	136−138	96
32	Cl	Cl	Cl	C_6H_{11}	CH_3	CH_3	E	143−146	96
33	Cl	Cl	Cl	$CH_2C_6H_5$	CH_3	CH_3	D	178−179	97
34	Cl	Cl	Cl	*n*-C_4H_9	CH_3	H	B or F	176−178	56
35	Cl	Cl	H	*n*-C_4H_9	CH_3	H	B or F	162−164	60
36	Cl	Cl	Cl	C_6H_5	CH_3	H	B or F	205−207	62
37	Cl	Cl	Cl	$CH_2C_6H_5$	CH_3	H	C	195−198	67

with potassium *tert*-butoxide in DMF and methyl iodide (Scheme 3, Method E). It is likely that this double-methylation reaction is due to the fact that the methylene group in the starting secondary amides is acidic enough with a dissociation constant comparable with that of a CONH moiety. Thus, the methyl-substituted derivative **31** was prepared starting from the tertiary amide *N,N*-di-*n*-propyl-[2-(4-chlorophenyl)-6,8-dichloroimidazo[1,2-*a*]pyridin-3-yl)]acetamide (i.e., CB 34)[26] by treatment with NaH in DMF and methyl iodide. The double-methylation reaction was prevented by using *n*-BuLi at −45 °C and quenching the resulting anion with methyl triflate. Under the latter experimental conditions, the desired monomethyl-substituted tertiary amides **34** and **35** were obtained starting from the imidazoacetamide **27** and *N*-*n*-butyl-(2-phenyl-6,8-dichloroimidazo[1,2-*a*]pyridin-3-yl)acetamide,[27] respectively (Scheme 3, Method F). Similarly, treatment of compound **13** with *n*-BuLi and methyl triflate gave the corresponding monomethyl tertiary amide **36**. All compounds were fully characterized by IR, ^{1}H NMR, mass spectra, and elemental analyses (Experimental Section). An interesting feature of the ^{1}H NMR spectra of these imidazoacetamides concerns the signals associated with the methylene linked at the carbonyl group and the methyl and methylene groups characterizing the alkyl chains of CO−N<R_1R_2. The CH_2CO gives rise to a singlet in the range 3.7−4.6 δ only when the R_1 and R_2 groups are of similar size. When these two groups are very different in size, (e. g., in the case of compounds **10**, **12**, **22**−**25**, **32**−**35**, and **37**) the signals of the CH_2-CO and those of the protons in the R_1 and R_2 groups are duplicated due to the partial double bond character of the amide bond. Physical data for new compounds are reported in Table 1.

Results and Discussion

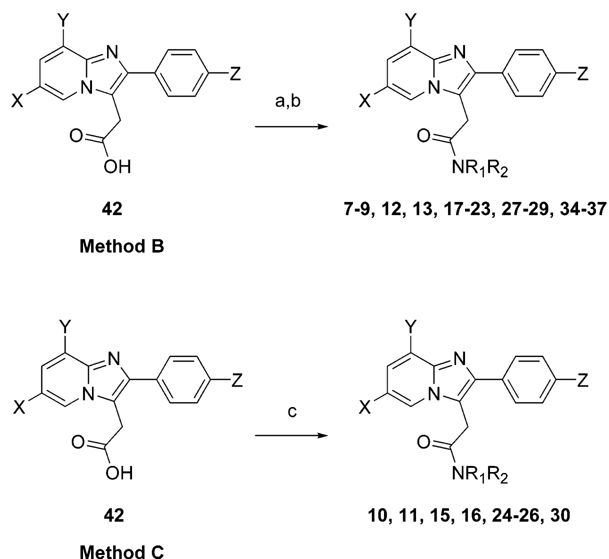
Affinities of Imidazopyridine Derivatives for Peripheral and Central Benzodiazepine Receptors. The affinities of the 36 tested compounds for CBR and PBR were evaluated by measuring their ability to displace [^{3}H]-flunitrazepam and [^{3}H]PK 11195 from

Scheme 1[a]



[a] Method A, Reagents: (a) R_1R_2NH, EEDQ,THF; (b)Br_2/CCl_4; (c) DMF, reflux.

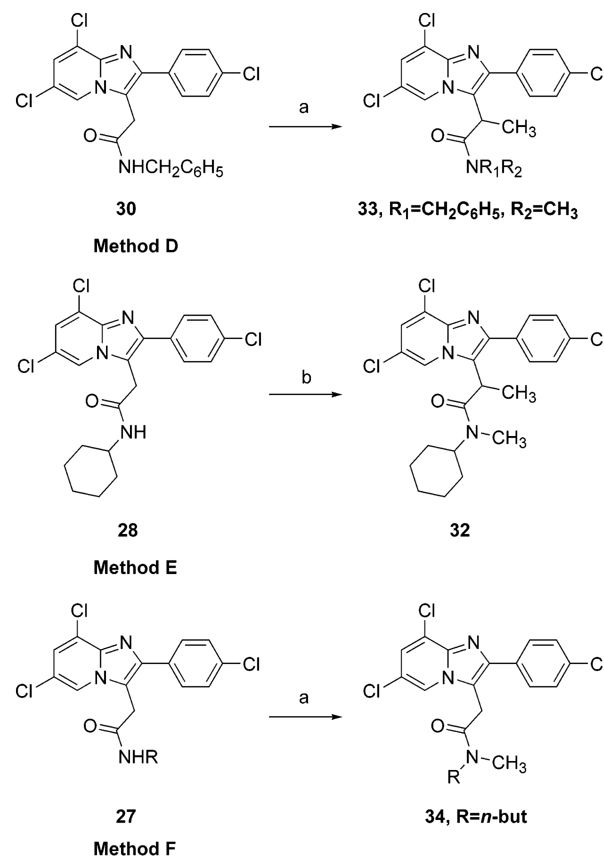
Scheme 2[a]



[a] Method B, Reagents (a): CDI, THF; (b)$^{+}H_2NR_1R_2\ Cl^{-}$. Method C, Reagents: (a) R_1R_2NH, EEDQ,THF.

binding to membrane preparations from the cerebral cortex and ovary. Their effects were compared with those of unlabeled PK 11195, a selective ligand for PBRs[22] and with that of CB34.[26,29] The measured binding affinities for CBR and PBR expressed as K_i are shown in Table 2. For the sake of comparison with previously reported data, the affinities as well as their ratios, as a measure of in vitro selectivity, are expressed also as pIC_{50}. A linear regression analysis on pIC_{50} values for the whole set of compounds listed in Table 2 showed a good correlation between the data from ovary membranes and from brain cerebral cortex cells ($n = 35$, $r^2 = 0.870$). 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 analysis to biological data from cortex membranes.

Scheme 3[a]



[a] Method D, Reagents: (a) NaH, room temperature, CH_3I, DMF. Method E, Reagents: (a) Potassium *tert*-butoxide, CH_3I, DMF. Method F, Reagents: (a) *n*-BuLi −45°C, methyl triflate, THF.

Structure−Affinity (SAR) and Structure−Selectivity Relationships (SSR). As mentioned above, even though compound **1** was characterized by high affinity for the PBR, it showed poor receptor selectivity and failed to affect neuroactive steroids content both in plasma and cerebral cortex. Therefore, our initial efforts were directed to enhancing the selectivity of compound **1** chosen as template. Thus, compounds **2**, **3**, **5** as 6- and/ or 8-Cl-substituted analogues of **1** were synthesized, taking into account that substitution at the 6- and/or 8-position leads to compounds with enhanced affinity and selectivity for PBR versus CBR. A pairwise comparison of binding data showed a marked improvement in selectivity only for the 8-Cl-substituted compounds **2** and **3** over template **1** (i.e., pIC_{50} PBR cortex 8.104, 8.284, and 8.230,[27] respectively; selectivity index ΔpIC_{50} 3.104, 3.284, and 1.190,[27] respectively), whereas compound **5**, although endowed with good affinity at the PBR, showed a modest selectivity (i.e., pIC_{50} PBR cortex 8.485; selectivity index ΔpIC_{50} 1.434). No significant improvement in selectivity was caused by increasing the chain length of the alkyl substituents on the carboxamide nitrogen as observed for compounds **4** and **6**. These findings are consistent with suggestions from QSAR analysis[32] on the 2-phenylimidazo[1,2-*a*]pyridine derivatives which suggested that a four carbon chain

Table 2. Affinities of Compounds **2**−**37** for CBR and PBR from Different Tissues[a]

compd	K_i (nM) CBR	Hill slope CBR	pIC_{50} CBR	K_i (nM) PBR cortex	Hill slope cortex	pIC_{50} PBR cortex	ΔpIC_{50} PBR cor-CBR	K_i (nM) PBR ovary	Hill slope ovary	pIC_{50} PBR ovary	ΔpIC_{50} PBR ov-CBR
1			7.040			8.230	1.190			7.980	0.940
2	6880	1.02	5.000	4.639	0.88	8.104	3.104	17.193	1	7.646	2.646
3	7750	1.04	5.000	2.679	0.92	8.284	3.284	13.179	1.08	7.731	2.731
4	7750	1.03	5.000	221.659	0.87	6.424	1.424	1892.631	0.88	5.584	0.584
5	68.90	1	7.051	1.685	0.93	8.485	1.434	4.769	0.97	8.172	1.121
6	2560	0.99	5.481	3.012	0.82	8.292	2.811	35.314	0.93	7.312	1.831
7	6880	0.98	5.000	6.779	0.85	7.939	2.939	3.499	0.98	8.337	3.337
8	6880	0.97	5.000	7.841	0.9	7.876	2.876	3.649	0.99	8.319	3.319
9	506	0.96	6.133	0.884	1.1	8.824	2.691	8.824	1	7.936	1.803
10	7010	1	5.000	14.143	1.02	7.616	2.616	64.667	0.86	7.047	2.047
11	7590	1.02	5.000	2101.357	0.85	5.464	0.464	12932.951	1.02	4.740	−0.260
12	6880	0.88	5.000	16.034	0.93	7.566	2.566	50.528	0.91	7.157	2.157
13	6880	0.87	5.000	11.731	0.94	7.701	2.701	18.273	1.012	7.599	2.599
14	7750	0.85	5.000	4.769	0.88	8.092	3.092	4.061	0.91	8.252	3.252
15	7010	0.93	5.000	125.649	0.9	6.668	1.668	607.744	0.66	6.074	1.025
16	6880	0.99	5.000	757.466	0.91	5.907	0.907	6646.968	0.89	5.029	0.029
17	7590	0.98	5.000	95.905	0.85	6.804	1.804	1033.925	1.03	5.837	0.837
18	7590	0.97	5.000	3.054	0.87	8.301	3.301	65.375	0.96	7.036	2.036
19	130	1	6.725	19.636	1.05	7.454	0.729	207.468	0.88	6.520	−0.205
20	6880	1.02	5.000	79.773	0.93	6.845	1.845	1737.374	0.9	5.597	0.597
21	6880	1	5.000	12705.88	0.95	4.682	−0.318	515896.848	0.93	3.139	−1.861
22	6880	1	5.000	23.640	0.83	7.412	2.412	164.149	0.93	6.636	1.636
23	6880	1	5.000	2.969	0.97	8.313	3.313	25.283	0.88	7.449	2.449
24	7750	0.99	5.000	1279.258	0.92	5.663	0.663	3667.789	0.95	5.296	0.296
25	7750	0.88	5.000	529.978	0.87	6.046	1.046	3685.193	1.12	5.294	0.294
26	7750	0.93	5.000	1237.991	1.025	5.677	0.677	2974.550	1.16	5.387	0.387
27	6880	0.99	5.000	169.951	1.01	6.409	1.409	170.426	1.2	6.538	1.538
28	7590	0.92	5.000	139.889	1.45	6.640	1.640	748.974	1.89	5.977	0.977
29	7010	1.01	5.000	771.429	1.1	5.878	0.878	941.535	0.81	5.884	0.884
30	7010	1.02	5.000	98.766	1.3	6.772	1.772			ND	ND
31	7010	1	5.000	701.299	0.95	5.920	0.920	1931.372	0.78	5.572	0.572
32	7010	1.03	5.000	2992.208	1.27	5.288	0.288	2212.535	0.87	5.513	0.513
33	7010	1	5.000	5774.026	1.037	5.005	0.005	8334.674	0.86	4.937	−0.063
34	6880	1	5.000	0.302	1.2	9.347	4.347	2.465	0.98	8.481	3.481
35	6880	0.99	5.000	2.157	0.98	8.456	3.456	1.718	0.97	8.638	3.638
36	6880	0.88	5.000	0.203	0.97	9.481	4.481	1.268	0.93	8.770	3.770
37	6880	0.87	5.000	1.379	0.93	8.623	3.623			ND	ND
PK 11195	7590	1.13	5.000	4.269	1.05	8.155	3.155	1.704	1.066	8.620	3.620

[a] pIC_{50} values are the negative logarithms of the molar concentrations necessary for 50% inhibition (IC_{50}); data are means of three separate experiments performed in duplicate which differed by less than 10%; ND, not determined.

is the optimum length for the alkyl substitution on the carboxamide nitrogen. Replacement of one *n*-butyl group in **3** with a phenyl one did not improve affinity and selectivity (compare **3** with **7**, **8**, and **9**). Introduction of a benzyl group (compound **10**) or a 4-NO_2-benzyl group (compound **12**) instead of one *n*-butyl group in the parent compound led to a good selectivity for PBR but a slight decrease in receptor binding. Interestingly, the low affinity of the *ter*-butyl-substituted imidazopyridine **11** (pIC_{50} 5.464) in comparison to the corresponding *n*-butyl derivative **10** (pIC_{50} 7.616) suggests that increasing the branching of the alkyl substituent on the carboxamide nitrogen may cause hindrance to the carboxamide site of the ligand in occupying the cavity within the receptor. It should be noted that all these aromatic compounds (i.e., **7**−**13**) were designed in order to take advantage of a potential hydrophobic interaction $\pi-\pi$ between their aromatic moieties and the amino acid residues Trp 107 and Trp 161 on the receptor protein.

We next examined the biological effects of conformationally constrained analogues. These compounds were designed taking into consideration that antagonists are often conformationally restricted analogues of agonists.[33] Thus, inspection of the data reported in Table 2 for the dipropenyl- (**14**), pyrrolidino- (**15** and **16**), and piperidino- (**17** and **18**) derivatives revealed that the best results in terms of affinity and selectivity were obtained for compounds **18** and **14**. These results prompted us to synthesize new congeners of **18** with a polar group such as the ethoxycarbonyl group on the piperidino moiety (i.e., **19** and **20**), the 4′-benzyl-piperazino analogue **21,** and the benzofused analogues **22** and **23**. Interestingly, only the latter two compounds retained a good affinity selectivity (i.e., pIC_{50} PBR cortex 7.412 and 8.313; selectivity index ΔpIC_{50} 2.412 and 3.313, respectively) (Table 2). These data suggest that the presence of polar substituents or ionizable groups on the amide nitrogen seems to be detrimental for high affinity and selectivity. This was confirmed by introducing a heteroaryl substituent such as the 2-pyridylethyl group; the resulting tertiary amides **24** and **25**, indeed, showed a marked decrease in affinity and selectivity toward PBR.

Monosubstitution on the carboxamide nitrogen led to a significant decrease in affinity and selectivity for PBR, as observed for the set of compounds **26**−**30**. Therefore, these data support the conclusion that a disubstitution on the carboxamide nitrogen with linear or conformationally restricted alkyl substituents of optimum length is of fundamental importance for interaction with the corresponding complementary site of the receptor. We next examined the effects on affinity and selectivity of disubstitution on the carboxamide nitrogen with alkyl groups of very different lengths and namely, one *n*-butyl group or phenyl group or benzyl group, each coupled with a methyl group. Another reason for designing these *N*-methyl congeners **34**−**37** was to exploit the presence

of a methyl group, to develop a potential positron emission tomography (PET) tracer that would provide selective imaging of PBR in vivo. Interestingly, compounds **34**–**37** proved to be among the most active compounds in this series. Particularly, compounds **34** and **36** are potent even at subnanomolar level. To characterize better the role of the carboxamide nitrogen in these compounds, the propanamides **31**–**33** were also evaluated. The observed loss of activity and selectivity highlights the importance of the presence of a carbonyl C(=O)N group suitably located and oriented for binding to PBR [compare **31** and **33** with *N*,*N*-di-*n*-propyl-[2-(4-chlorophenyl)-6,8-dichloroimidazo[1,2-*a*]pyridin-3-yl)]acetamide (CB 34)[26] and **37**, respectively]. It is likely that the presence of the additional methyl group in **31**–**33** leads to a conformational change with consequent unfavorable orientation of the carbonyl group for the interaction with the receptor.

Quantitative Structure–Affinity Relationship Studies. To gain further insight into the relationships between the binding affinity to PBRs and physicochemical properties, a QSAR study of the 2-phenylimidazopyridines **2**–**37** was carried out. The data set was divided into subsets A [characterized by compounds having open chain alkyl substituents (R_1 and R_2) on the carboxamide nitrogen, i.e., compounds **2**–**11**, **13**, **14**, **27**–**37**] and B which considers the whole data set). Each compound in subset A was described by 14 parameters. As a descriptor of molecular lipophilicity, the octanol/water partition coefficient value calculated using the CLOGP program[34] was used. Bulkiness was parametrized by molar volume (mv/1000), calculated with the ACDLab program,[35] molar refractivity (mr/1000)[34] and, for each alkyl substituent, STERIMOL descriptors (i.e., the length L, the minimum width B_1 and the maximum width B_5 of the alkyl substituents) developed by Verloop and co-workers.[36] The main advantage of using the STERIMOL parameters over other more common steric variables is that they allow one to define the dimensions of a given substituent along fixed directions in a three-dimensional space and, in this case, they should help to assess the optimal size of the alkyl groups. A number of indicator variables were also used to account for the presence or absence of a chlorine atom in the para position of the phenyl group (I_{z1}), the presence or absence of a chlorine atom both in the para position of the phenyl group and at the 8-position of the 2-phenylimidazo[1,2-*a*]pyridine system (I_{z2}), the presence of an acetamide or propanamide group (I_{CH3}) and next whether the carboxamide nitrogen is di- or monosubstituted (I) (Table 3). The variable N, denoting the number of carbon atoms of the alkyl substituents on the carboxamide nitrogen, was also included. In subset A, compounds **12**, **24**–**26** were not included because their STERIMOL parameters are not reported in standard compilations.

A selection of the pertinent variables was carried out by considering the intercorrelation among predictors (physicochemical parameters) and their influence (explained variance of the biological data) on the regression model. A cluster analysis based on correlation coefficients among variables (Figure 1a) showed which parameters contain comparable information, namely mr, mv, N, B_5R_2, LR_2, and and Clog P as well as I and

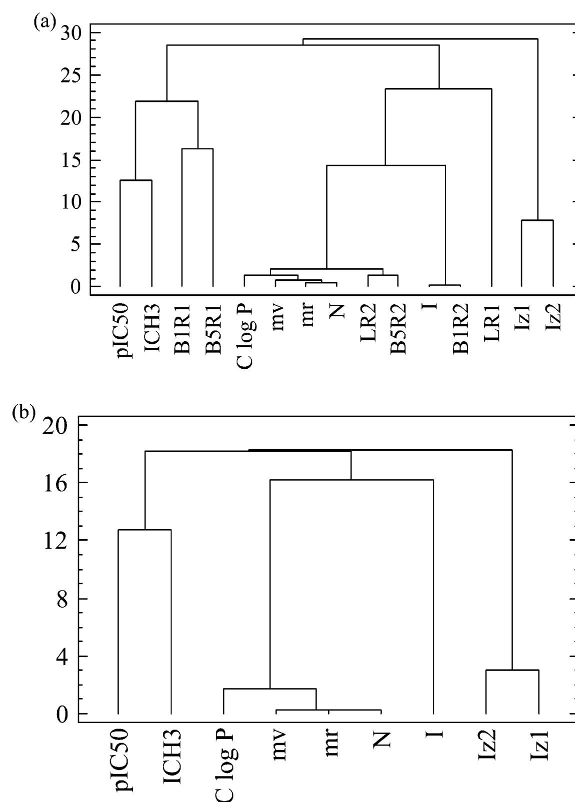


Figure 1. (a) Dendrogram of similarity among variables of subset A obtained using a hierarchical cluster analysis based on correlation coefficients. (b) Dendrogram of similarity among variables of subset B obtained using a hierarchical cluster analysis based on correlation coefficients.

B_1R_2. This cluster analysis also proved the presence of an acetamide group to be somehow related to pIC_{50}. Thus, we performed a stepwise multiparametric regression analysis (MRA) using only six predictors (mr, I_{CH3}, I, I_{z1}, LR_1, and B_1R_1), retaining only QSARs with cross-validated (leave-one-out procedure, loo) $q^2 > 0.5$. MRA carried out on subset A retained four variables (mr, I_{CH3}, I, and B_1R_1) to generate eq 1, explaining about 80% of the variance in the PBR binding data:

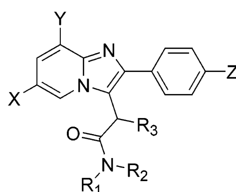
$$pIC_{50}\ (\text{PBR cortex}) = 12.39\ (\pm 4.0) - 491.43\ (\pm 342)\ mr + 2.66\ (\pm 0.8)\ I_{CH3} + 1.84\ (\pm 0.8)\ I - 1.64\ (\pm 1.16)\ B_1R_1 \quad (1)$$

$$n = 23,\ r^2 = 0.799,\ q^2 = 0.576$$

where n represents the number of data points, r^2 the squared correlation coefficient, q^2 the loo cross-validation coefficient (an assessment of the 'internal' predictive ability of the QSAR model).

Equation 1 relates PBR cortex affinity mainly to steric effects parametrized by molar refractivity and the minimum width B_1 of the R_1 alkyl substituent. Bulky substituents on the amide nitrogen are detrimental for PBR affinity. Furthermore, the significant contribution to the QSAR model of I_{CH3} and I suggests the importance for binding to PBR of effects not already accounted for by steric constants (mr and B_1R_1). The positive coefficients of I_{CH3} and I in eq 1 indicate that the presence of an acetamide group and disubstitution on carboxamide nitrogen favor the affinities for PBR. These find-

Table 3. Physicochemical Parameters Used in the QSAR Study of 2-Phenylimidazo[1,2-*a*]pyridineacetamides **2**−**37**



compd	Log P[a]	mv[b]	mr[b]	I_{z1}	I_{z2}	I_{CH3}	I	N	LR1	LR2	B_1R1	B_1R2	B_5R1	B_5R2
2	6.430	0.3575	0.01213	1	1	1	1	8	6.17	6.17	1.52	1.52	4.54	4.54
3	7.140	0.3668	0.01262	1	1	1	1	8	6.17	6.17	1.52	1.52	4.54	4.54
4	9.260	0.4310	0.01448	1	1	1	1	12	8.22	8.22	1.52	1.52	5.96	5.96
5	6.430	0.3575	0.01213	1	0	1	1	8	6.17	6.17	1.52	1.52	4.54	4.54
6	8.540	0.4217	0.01399	1	0	1	1	12	8.22	8.22	1.52	1.52	5.96	5.96
7	6.750	0.3620	0.01279	0	0	1	1	10	6.17	6.28	1.52	1.71	4.54	3.11
8	7.470	0.3712	0.01328	1	1	1	1	10	6.17	6.28	1.52	1.71	4.54	3.11
9	6.750	0.3620	0.01279	1	0	1	1	10	6.17	6.28	1.52	1.71	4.54	3.11
10	6.000	0.3620	0.01279	0	0	1	1	11	6.17	4.62	1.52	1.52	4.54	6.02
11	6.890	0.3909	0.01374	1	1	1	1	11	4.11	4.62	2.6	1.52	3.11	3.17
12	6.460	0.3764	0.01389	1	1	1	1	10	4.92	-	1.52	-	3.49	-
13	5.700	0.3018	0.01142	1	1	1	0	6	6.28	2.06	1.71	1	3.11	1
14	5.520	0.3347	0.01164	1	1	1	1	6	5.11	5.11	1.52	1.52	3.78	3.78
15	4.600	0.2758	0.01059	1	1	1	1	4	-	-	-	-	-	-
16	3.890	0.2665	0.01010	0	0	1	1	4	-	-	-	-	-	-
17	4.450	0.2826	0.01056	0	0	1	1	5	-	-	-	-	-	-
18	5.160	0.2918	0.01105	1	1	1	1	5	-	-	-	-	-	-
19	4.860	0.3336	0.01214	1	0	1	1	8	-	-	-	-	-	-
20	5.570	0.3428	0.01263	1	1	1	1	8	-	-	-	-	-	-
21	6.470	0.3704	0.01393	1	1	1	1	11	-	-	-	-	-	-
22	5.230	0.3183	0.01215	0	0	1	1	9	-	-	-	-	-	-
23	5.940	0.3275	0.01264	1	1	1	1	9	-	-	-	-	-	-
24	3.860	0.3345	0.01211	0	0	1	1	8	-	-	-	-	-	-
25	4.570	0.3438	0.01260	1	1	1	1	8	-	-	-	-	-	-
26	5.030	0.2904	0.01121	0	0	1	0	5	-	-	-	-	-	-
27	5.280	0.2973	0.01077	1	1	1	0	4	6.17	2.06	1.52	1	4.54	1
28	5.720	0.3018	0.01152	1	1	1	0	6	6.17	2.06	1.91	1	3.49	1
29	5.010	0.2925	0.01103	0	0	1	0	6	6.17	2.06	1.91	1	3.49	1
30	5.720	0.3179	0.01189	1	1	1	0	7	4.62	2.06	1.52	1	6.02	1
31	6.390	0.3499	0.01216	1	1	0	1	6	4.92	4.92	1.52	1.52	3.49	3.49
32	6.310	0.3383	0.01245	1	1	0	1	7	6.17	2.87	1.91	1.52	3.49	2.04
33	5.970	0.3543	0.01281	1	1	0	1	8	4.62	2.87	1.52	1.52	6.02	2.04
34	5.560	0.3186	0.01123	1	1	1	1	5	6.17	2.87	1.52	1.52	4.54	2.04
35	4.840	0.3090	0.01074	0	0	1	1	5	6.17	2.87	1.52	1.52	4.54	2.04
36	5.880	0.3231	0.01189	1	1	1	1	7	6.28	2.87	1.71	1.52	3.11	2.04
37	5.66	0.3391	0.01235	1	1	1	1	8	4.62	2.87	1.52	1.52	6.02	2.04

[a] Estimated by CLOGP program.[34] [b] Bulkiness was parametrized by molar volume (mv, cm^3) calculated by ACDLab program[35] and molar refractivity (mr) estimated by CLOGP software.[34] Both calculated molar volumes and molar refractivities were normalized multiplying them by a factor of 1×10^{-3} The other physicochemical descriptors reported in the Table are so defined: I_{z1} presence (value 1) or absence (value 0) of Cl at Z position; I_{z2} presence (value 1) or absence (value 0) of a Cl both at Z and Y positions; I_{CH3} presence of an acetamido (value 1) or presence of propanamide group (value 0); I whether the carboxamide nitrogen is bisubstituted (value 1) or monosubstituted (value 0); N the total number of carbon atoms of the alkylsubstituents on the carboxamide nitrogen; LR1 and LR2 are the L Verloop's parameters for R1 and R2, respectively; B_1R1 and B_1R2 are the B_1 Verloop's parameters for R1 and R2, respectively; B_5R1 and B_5R2 are the B_5 Verloop's parameters for R1 and R2, respectively. The Verloop's parameters were taken from standard compilations.

ings are consistent with previous QSAR analysis[32] which, as mentioned above, suggested that a four-carbon chain is the optimum length for the alkyl substitution on the carboxamide nitrogen. However, in the present study, it was found that the minimum width B_1 of the R_1 alkyl substituent, rather than its length and the number of carbon atoms, is a relevant factor for increasing PBR binding affinity.

We next explored the best quantitative structure−activity relationship of the whole data set (subset B). As molecular descriptors, parameters of lipophilicity (Clog P), bulk (mv, mr), indicator variables (I_{z1}, I_{z2}, I_{CH3}, I), and N were examined. Again, a cluster analysis based on correlation coefficients among variables (Figure 1b) showed which parameters contain comparable information. Moreover it was found that mv, mr, and Clog P are interdependent ($r^2 > 0.500$).

The partial least squares (PLS) method is particularly suited for the extraction of a few highly significant factors from large sets of correlated descriptors. Therefore, in this paper, correlation between the eight selected descriptors and pIC_{50} values was also established by PLS. The best computational model was obtained involving five predictors namely, mr (molar refractivity mr in quadratic term), Clog P, I_{CH3}, and I with a maximum of five PLS components explaining more than 75% of the variance in the PBR binding data. This model gave r^2 of 0.752 and q^2 of 0.648.

$$\mathrm{pIC}_{50}\ (\mathrm{PBR\ cortex}) = -65.06\ (\pm 27.04) + 11300\ (\pm 4368)\ mr - 507.46\ (\pm 181.14)\ mr^2 + 2.84\ (\pm 0.86)\ I_{\mathrm{CH3}} + 1.72\ (\pm 0.68)\ I + 1.08\ (\pm 0.34)\ \mathrm{Clog\ P} \quad (2)$$

$$n = 36,\ r^2 = 0.752,\ q^2 = 0.648$$

These relationships suggest that increases in molar refractivity after some optimum value is reached may

cause hindrance to the carboxamide site of the ligand in interacting with the receptor. Again, the positive coefficient of I_{CH3} and I in eq 2 suggests that the presence of an acetamide group and the double substitution on carboxamide nitrogen favor affinity for PBR. From eq 2 (which considers the whole data set) lipophilicity emerges as a physicochemical property which also correlates with binding affinity. Actually, the importance of the lipophilicity was clearly demonstrated by a significant decrease in receptor affinity of compounds **19**−**21** characterized by hydrophilic substituents on the amide nitrogen. As a consequence, these results induced us to the decision to stop the project of further introduction of hydrophilic substituents in this specific region.

With the same independent variables in eqs 1 and 2, a preliminary attempt was made to explore the features causing selectivity for PBR binding [expressed as ΔpIC_{50} (PBR cortex)] and statistically poorer relationships were derived (i.e., $n = 23$, $r^2 = 0.717$, $q^2 = 0.495$ and $n = 36$, $r^2 = 0.621$, $q^2 = 0.491$, respectively) suggesting that further studies with larger sample size are necessary to reach a conclusion concerning selectivity requirements in this series of ligands.

While the above QSARs highlighted the main physicochemical factors eliciting the binding of 2-phenylimidazo[1,2-*a*]pyridine derivatives to PBR and could have value for molecular design, we further explored the pharmacological profile of the representative compounds by measuring the effects on steroidogenesis in rats.

Functional Studies. Compounds **2**, **3**, **7**, **14**, **18**, **34**, **35**, and **36**, as potent and selective examples of the subsets examined here, were evaluated both for their effects on human recombinant $GABA_A$ receptors, to determine their capacity to interact with CBRs, and for their capability to stimulate the synthesis in plasma and brain of neuroactive steroids in rats.

In Vitro Assay: Electrophysiology in *Xenopus* Oocytes. As illustrated in Figure 2, Cl^- currents evoked by GABA at $GABA_A$ receptors were not significantly modified by compounds **2**, **3**, **14**, **18**, and **36** tested at concentrations of 3, 10, and 30 μM. Similarly, PK 11195 failed to produce statistical significant changes in the amplitude of GABA-evoked currents. By contrast, compound **35** markedly enhanced GABA-evoked currents (97 ± 6% and 145 ± 10%, at concentrations of 10 and 30 μM, respectively).

In Vivo Assay: Effect of Compounds on Steroidogenesis. As shown in Table 4, intraperitoneal administration of compounds **2**, **3**, **7**, **14**, **34**, and **35** (25 mg/kg) resulted in a significant increase in the concentration of neuroactive steroids both in plasma and in brain. The effects of compound **3** was most marked: progesterone (PROG): + 121% and +111% in brain and plasma, respectively; 3α,5α-THPROG: + 52% and + 96% in brain and plasma, respectively; 3α,5α-THDOC: + 52% and 131% in the brain and plasma, respectively. In contrast, compounds **18** and **36** showed little effects; in particular **36** was able to significantly enhance the cerebrocortical and plasmatic levels of PROG (+ 39%; $p < 0.05$; and + 55%; $p < 0.01$, respectively) and the plasma levels of 3α,5α-THPROG (+ 45%, $p < 0.01$) while failing to significantly modify brain 3α,5α-THPROG as well as brain and peripheral levels of 3α,5α-THDOC.

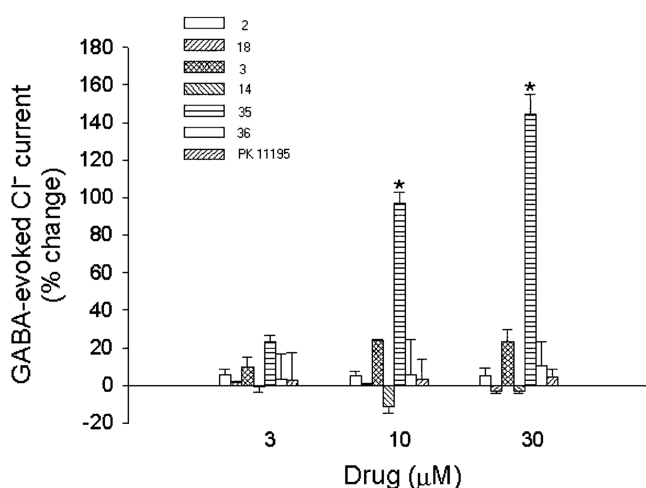


Figure 2. Modulatory action of compounds **2**, **3**, **14**, **18**, **35**, **36**, and PK 11195 at human α1β2γ2L $GABA_A$ receptors expressed in *Xenopus* oocytes. Values are expressed as percentage change induced by the different drugs from the control response obtained with GABA EC_{10} (concentration of GABA, usually ranging from 0.5 to 8 μM, which produced a Cl^- current the amplitude of which was 10 ± 3% of the maximal response to 1 mM GABA). Data are means (from four to six different oocytes) ± SEM. *$p < 0.01$ vs control response (ANOVA and Scheffè's test).

On the basis of these results, we examined whether **36**, which appears to act as a partial agonist at the PBR, could antagonize CB 34-induced steroidogenesis.[26,37] Prior treatment (−10 min) with **36**, at a dose of 25 mg/kg, significantly decreased the CB 34-induced increases in the brain (Figure 3) and plasma (data not shown) concentrations of 3α,5α-THPROG.

Conclusions

Consistent with previous studies,[27,32] the results of this work suggest that bulkiness of the substituents on the amide nitrogen of the imidazopyridine PBR ligands is a property mainly responsible for modulation of affinity. Second, increasing the branching of the alkyl substituent on the carboxamide nitrogen may cause hindrance to the ligand in interacting with the receptor region which should be of restricted size. Third, the presence of aromatic or conformationally constrained substituents on the carboxamide nitrogen may favor high affinity and selectivity, while the presence of polar substituents or ionizable groups in this region seems to be detrimental. A QSAR analysis of the measured binding data indicated that besides the important role played by disubstitution on the carboxamide nitrogen, the affinity of compounds **2**−**37** to PBRs proved to be linearly correlated with lipophilicity constant and molar refractivity through a parabolic relationship.

In in vivo studies, compounds **2**, **3**, **7**, **14**, and **34** each exhibited potent and selective effects on the peripheral and central synthesis of neuroactive steroids, with **3** being the most potent derivatives. The capability of compound **36** to reduce the stimulatory effect exerted by CB 34 on the cerebrocortical concentration of 3α,5α-THPROG is consistent with a partial agonist activity. We also show that there is no correlation between PBR affinity and selectivity and steroidogenic activity. The whole set of compounds examined **2**, **3**, **7**, **14**, **18**, **34**, **35**, and **36**, indeed, although endowed with high affinity

Table 4. Effect of ip Administration of Compounds **2**, **3**, **7**, **14**, **18**, **34**, **35**, and **36** (25 mg/kg) on Cerebrocortical and Plasmatic Levels of Neuroactive Steroids in Rats after 30 min[a,b]

	cerebral cortex (ng/g)			plasma (ng/mL)		
compounds	progesterone	3α,5α-THPROG	3α,5α-THDOC	progesterone	3α,5α-THPROG	3α,5α-THDOC
vehicle	5.2 ± 0.3	6.0 ± 0.6	2.3 ± 0.3	9.0 ± 1.0	14.0 ± 0.9	7.0 ± 0.9
2	10.3 ± 1.7*	8.8 ± 1.1*	2.9 ± 0.1	26.0 ± 3.7**	23.4 ± 4.0*	14.9 ± 0.8**
3	11.5 ± 1.1**	9.1 ± 1.2*	3.5 ± 0.5*	19.0 ± 1.8**	27.5 ± 3.7**	16.2 ± 2.1**
7	5.8 ± 1.0	16.3 ± 1.6**	2.6 ± 0.3	17.3 ± 2.0**	21.3 ± 1.9**	8.9 ± 1.8
14	11.5 ± 1.2**	8.6 ± 1.4	3.2± 0.1*	12.2 ± 1.0*	18.1 ± 0.2	9.0 ± 0.8
18	7.8 ± 0.6**	7.3 ± 0.6	2.9 ± 0.8	12.2 ± 1.0*	19.4 ± 2.0*	9.6 ± 0.5*
34	8.9 ± 1.1*	7.4 ± 1.8	3.9 ± 0.4*	14.9 ± 1.8*	16.2 ± 1.9	8.1 ± 1.2
35	10.6 ± 0.6**	9.2 ± 1.3*	4.1 ± 0.5**	13.6 ± 0.7**	16.5 ± 2.0	10.4 ± 1.2*
36	7.2 ± 0.7*	7.4 ± 0.6	2.8 ± 0.3	13.9 ± 0.1**	20.3 ± 0.2**	8.3 ± 0.6

[a] Data are expressed as nanograms of steroids per gram of tissue and are the means ± SEM of values from at least eight rats. [b] *$p < 0.05$; **$p < 0.01$ vs vehicle-treated animals.

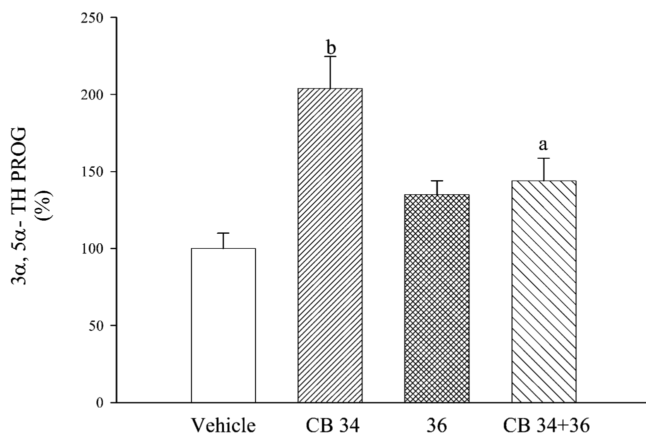


Figure 3. Effect of pretreatment with compound **36** on the CB 34-induced increases in the cerebrocortical concentration of 3α,5α-THPROG. Rats were injected with compound **36** (25 mg/kg ip) 10 min before CB 34 and were killed 30 min after CB 34. Data are means ± SD from eight rats and are expressed as a percentage of concentration of vehicle-treated animals. [a]$p < 0.05$; [b]$p < 0.01$ vs vehicle group.

and selectivity, did not produce similar effects on neurosteroid synthesis. This could suggest that PBR agonists and antagonists may occupy similar but not identical binding sites and that, as occurs for other receptor systems, the transition from agonist to antagonist activity is consequent to very small structural changes. Of interest in this regard is the consideration that a rank order of intrinsic activity can be envisaged. Thus, the *N,N*-di-*n*-butyl- and the *N*-butyl,*N*-methyl-substituted-imidazopyridines **3** and **35** exhibited high efficacy in increasing the peripheral and central synthesis of neuroactive steroids (full agonism). Compounds **2, 14**, and **34** showed a moderate increase in steroidogenic activity. The effects of congeners **7** and **18** was less marked but significant, while the *N*-phenyl,*N*-methyl derivative **36** (highly potent and selective) showed little efficacy in increasing steroids content in the cerebral cortex. This ligand is the first compound in this series able to produce a small increase in neurosteroid concentration and to reduce the CB 34-induced increase in the cerebrocortical levels of 3α,5α-THPROG.

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 IR Fourier transform spectrophotometer in KBr pellets. ^{1}H NMR spectra were determined on a Varian Mercury 300 MHz instrument. Chemical shifts are given in *d* values downfield from Me_4Si as a internal standard. Mass spectra were recorded on an Agilent 6890-5973 MSDGC-MS spectrometer. Elemental analyses were carried out with a Eurovector EuroEA 3000 C, H, N analyzer, and results were within ±0.40% of theoretical values. Silica gel 60 (Merck 70−230 mesh) was used for column chromatography. All the following reactions were performed under a nitrogen atmosphere.

Materials. The preparation of 2-phenylimidazo[1,2-*a*]pyridine-3-acetic acids (**42**) was accomplished following a reported procedure.[13] Ethyl 1,2-dihydro-2-ethoxy-1-quinolinecarboxylate (EEDQ), *N,N′*dicyclohexylcarbodiimide (DCC), 1,1′-carbonyldiimidazole (CDI), anhydrous tetrahydrofuran, and triethylamine (TEA) were purchased from Sigma-Aldrich (Italy). All the mono- or dialkylamines were commercially available; the amine hydrochlorides used in method B, when not commercially available, were prepared as follows: HCl gas was bubbled into a stirred solution of amine in anydrous CH_2Cl_2 for 15 min and then excess HCl gas was removed under a stream of nitrogen. The solvent was evaporated under reduced pressure to give the hydrochlorides as white solids which were used immediately.

General Procedure for Preparation of (2-Phenylimidazo[1,2-*a*]pyridin-3-yl)acetamides 2−6. Method A. To a solution of the suitably substituted 2-aminopyridine **41** (11 mmol) in DMF (50 mL) was added the appropriate 3-bromo-3-benzoyl propionamide[26,27] (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, and the residue was purified by silica gel column chromatography [light petroleum ether/ethyl acetate 8/2 (v/v) as eluent)] to give the amide **2**−**6**. Physical data of these compounds are summarized in Table 1.

***N,N*-Di-*n*-butyl-[2-(4-chlorophenyl)-8-chloroimidazo-[1,2-*a*]pyridin-3-yl]acetamide (2):** IR (KBr): 1630 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 0.8−0.9 (m, 6H, CH_3), 0.9−1.1 (m, 2H, CH_2), 1.1−1.3 (m, 2H, CH_2), 1.3−1.5 (m, 4H, CH_2), 3.07 (t, 2H, CH_2N), 3.28 (t, 2H, CH_2N), 4.05 (s, 2H, CH_2CO), 6.7−6.8 (m, 1H, Ar), 7.2−7.3 (m, 1H, Ar), 7.41 (d, J = 8.4 Hz, 2H, Ar), 7.62 (d, J = 8.4 Hz, 2H, Ar), 8.20 (d, J = 7.0 Hz, 1H, Ar); MS *m/z* 431 (M$^+$, 19), 275 (base); Anal. ($C_{23}H_{27}Cl_2N_3O$) C, H, N.

***N,N*-Di-*n*-butyl-[2-(4-chlorophenyl)-6,8-dichloroimidazo[1,2-*a*]pyridin-3-yl]acetamide (3):** IR (KBr): 1630 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 0.8−1.0 (m, 6H, CH_3), 1.0−1.6 (m, 8H, CH_2), 3.1−3.2 (m, 2H, CH_2N), 3.3−3.4 (m, 2H, CH_2N), 4.03 (s, 2H, CH_2CO), 7.30 (d, J = 1.7 Hz, 1H, Ar), 7.43 (d, J = 8.3 Hz, 2H, Ar), 7.60 (d, J = 8.3 Hz, 2H, Ar), 8.23 (d, J = 1.7 Hz, 1H, Ar); MS *m/z* 465 (M$^+$, 11), 309 (52); Anal. ($C_{23}H_{26}Cl_3N_3O$) C, H, N.

***N,N*-Di-*n*-hexyl-[2-(4-chlorophenyl)-6,8-dichloroimidazo[1,2-*a*]pyridin-3-yl]acetamide (4):** IR (KBr): 1645 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 0.7−0.9 (m, 6H, CH_3), 1.0−1.6 (m, 16H, CH_2), 3.0−3.1 (m, 2H, CH_2N), 3.3−3.4 (m, 2H, CH_2N), 4.03 (s, 2H, CH_2CO), 7.30 (d, J = 1.4 Hz, 1H, Ar), 7.43 (d, J = 8.3 Hz, 2H, Ar), 7.59 (d, J = 8.3 Hz, 2H, Ar), 8.26 (d, J = 1.4 Hz, 1H,

Ar); MS *m/z* 309 [M^+- $(C_6H_{13})_2NCO$, 53]; Anal. ($C_{27}H_{34}Cl_3N_3O$) C, H, N.

***N,N*-Di-*n*-butyl-[2-(4-chlorophenyl)-6-chloroimidazo[1,2-*a*]pyridin-3-yl]acetamide (5):** IR (KBr): 1630 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 0.8−1.0 (m, 6H, CH_3), 1.0−1.6 (m, 8H, CH_2), 3.1−3.2 (m, 2H, CH_2N), 3.3−3.4 (m, 2H, CH_2N), 4.02 (s, 2H, CH_2-CO), 7.14 (dd, J = 9.4 and 1.9 Hz, 1H, Ar), 7.40 (d, J = 8.5 Hz, 2H, Ar), 7.5−7.6 (m, 3H, Ar), 8.2−8.3 (m, 1H, Ar); MS *m/z* 431 (M^+, 15), 275 (base); Anal. ($C_{23}H_{27}Cl_2N_3O$) C, H, N.

***N,N*-Di-*n*-hexyl-[2-(4-chlorophenyl)-6-chloroimidazo[1,2-*a*]pyridin-3-yl]acetamide (6):** IR (KBr): 1640 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 0.8−0.9 (m, 6H, CH_3), 1.0−1.6 (m, 16H, CH_2), 3.0−3.2 (m, 2H, CH_2N), 3.3−3.4 (m, 2H, CH_2N), 4.05 (s, 2H, CH_2-CO), 7.16 (dd, J = 9.4 and 1.9 Hz, 1H, Ar), 7.43 (d, J = 8.5 Hz, 2H, Ar), 7.5−7.7 (m, 3H, Ar), 8.28 (d, J = 1.2 Hz, 1H, Ar); MS *m/z* 487 (M^+, 15), 275 (base); Anal. ($C_{27}H_{35}Cl_2N_3O$) C, H, N.

General Procedure for Preparation of (2-Phenylimidazo[1,2-*a*]pyridin-3-yl)acetamides 7−9, 12, 13, 17−23, 27−29, 34−36. Method B. A solution of the appropriate acid **42** (1 mmol) and CDI (1.3 mmol) in anhydrous THF (20 mL) was stirred at room temperature. Then, the suitable amine hydrochloride ($HNR_1R_2 \cdot HCl$) (1.5 mmol) was added and the mixture stirred at room temperature or under a gentle heating for 4−12 h. The reaction mixture was washed with water, extracted with $CHCl_3$ (3 × 30 mL), and dried over Na_2SO_4. Solvent was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography [light petroleum ether/ethyl acetate 8/2 (v/v) as eluent)] to give the required compounds **7**−**9**, **12**, **13**, **17**−**23**, **27**−**29**, **34**−**36**. Physical data of these compounds are summarized in Table 1.

***N*-*n*-Butyl-*N*-phenyl-(2-phenyl-6,8-dichloroimidazo[1,2-*a*]pyridin-3-yl)acetamide (7):** IR (KBr): 1630 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 0.86 (t, J = 7.3 Hz, 3H, CH_3), 1.2−1.4 (m, 2H, CH_2), 1.4−1.6 (m, 2H, CH_2), 3.60−3.70 (m, 2H, CH_2N), 3.77 (s, 2H, CH_2CO), 7.0−7.2 (m, 2H, Ar), 7.2−7.4 (m, 7H, Ar), 7.4−7.6 (m, 2H, Ar), 8.15 (d, J = 1.9 Hz, 1H, Ar); MS *m/z* 451 (M^+, 28), 275 (base); Anal. ($C_{25}H_{23}Cl_2N_3O$) C, H, N.

***N*-*n*-Butyl-*N*-phenyl-[2-(4-chlorophenyl)-6,8-dichloroimidazo[1,2-*a*]pyridin-3-yl]acetamide (8):** IR (KBr): 1640 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 0.88 (t, J = 7.3 Hz, 3H, CH_3), 1.2−1.4 (m, 2H, CH_2), 1.4−1.6 (m, 2H, CH_2), 3.6−3.7 (m, 2H, CH_2N), 3.79 (s, 2H, CH_2CO), 7.0−7.2 (m, 2H, Ar), 7.2−7.4 (m, 6H, Ar), 7.46 (d, J = 8.5 Hz, 2H, Ar), 8.19 (d, J = 1.9 Hz, 1H, Ar); MS *m/z* 485 (M^+, 29), 309 (base); Anal. ($C_{25}H_{22}Cl_3N_3O$) C, H, N.

***N*-*n*-Butyl-*N*-phenyl-[2-(4-chlorophenyl)-6-chloroimidazo[1,2-*a*]pyridin-3-yl]acetamide (9):** IR (KBr): 1640 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 0.84 (t, J = 7.4 Hz, 3H, CH_3), 1.2−1.4 (m, 2H, CH_2), 1.4−1.6 (m, 2H, CH_2), 3.67 (t, J = 7.6 Hz, 2H, CH_2N), 3.77 (s, 2H, CH_2CO), 7.0−7.2 (m, 3H, Ar), 7.2−7.4 (m, 5H, Ar), 7.4−7.6 (m, 3H, Ar), 8.1−8.2 (m, 1H, Ar); MS *m/z* 451 (M^+, 22), 275 (base); Anal. ($C_{25}H_{23}Cl_2N_3O$) C, H, N.

***N*-*n*-Butyl-*N*-4-nitrobenzyl-[2-(4-chlorophenyl)-6,8-dichloroimidazo[1,2-*a*]pyridin-3-yl]acetamide (12):** IR (KBr):1645 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 0.73 (t, 2.1H, CH_3), 0.84 (t, 0.9H, CH_3), 1.4−1.6 (m, 2H, CH_2), 3.12 (t, J = 6.5 Hz, 1,-4H, CH_2N), 3.34 (t, J = 6.5 Hz, 0,6H, CH_2N), 4.02 (s, 0,6H, CH_2CO), 4.16 (s, 1,4H, CH_2CO), 4.40 (s, 0,6H, CH_2−Ar), 4.64 (s, 1,4H, CH_2−Ar), 6.9−7.7 (m, 8H, Ar), 8.1−8.4 (m, 2H, Ar); MS *m/z* 530 (M^+, 8), 309 (base); Anal. ($C_{25}H_{21}Cl_3N_4O_3$) C, H, N.

***N*-Phenyl-[2-(4-chlorophenyl)-6,8-dichloroimidazo[1,2-*a*]pyridin-3-yl]acetamide (13):** IR (KBr):3265, 1650 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 4.08 (s, 2H, CH_2CO), 7.0−7.2 (m, 2H, Ar), 7.2−7.5 (m, 5H, Ar), 7.6−7.7 (m, 3H, Ar), 8.16 (d, J = 6.5 Hz, 1H, Ar); MS *m/z* 429 (M^+, 16), 309 (base); Anal. ($C_{21}H_{14}Cl_3N_3O$) C, H, N.

2-Phenyl-3-[(2-oxo-2-piperidin-1-ylethyl)]-6,8-dichloroimidazo[1,2-*a*]pyridine (17): IR (KBr):1627 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 1.3−1.4 (m, 2H, CH_2), 1.5−1.7 (m, 4H, CH_2), 3.2−3.3 (m, 2H, CH_2), 3.5−3.6 (m, 2H, CH_2), 4.09 (s, 2H, CH_2-CO), 7.34 (d, J = 1.5 Hz, 1H, Ar), 7.4−7.5 (m, 3H, Ar), 7.6−7.7 (m, 2H, Ar), 8.33 (d, J = 1.5 Hz, 1H, Ar); MS *m/z* 387 (M^+, 15), 275 (base); Anal. ($C_{20}H_{19}Cl_2N_3O$) C, H, N.

2-(4-Chlorophenyl)-3-[(2-oxo-2-piperidin-1-ylethyl)]-6,8-dichloroimidazo[1,2-*a*]pyridine (18): IR (KBr): 1640 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 1.3−1.4 (m, 2H, CH_2), 1.5−1.7 (m, 4H, CH_2), 3.3−3.4 (m, 2H, CH_2), 3.5−3.6 (m, 2H, CH_2), 4.06 (s, 2H, CH_2CO), 7.35 (d, J = 1.5 Hz, 1H, Ar), 7.42 (d, J = 8.4 Hz, 2H, Ar), 7.61 (d, J = 8.4 Hz, 2H, Ar), 8.25 (d, J = 1.5 Hz, 1H, Ar); MS *m/z* 421 (M^+, 18), 309 (base); Anal. ($C_{20}H_{18}Cl_3N_3O$) C, H, N.

Ethyl 1-[2-(4-chlorophenyl)-6-chloroimidazo[1,2-*a*]pyridin-3-yl]acetyl)piperidin-3-yl]acetate (19): IR (KBr): 1724, 1634 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 1.1−1.4 (m, 3H, CH_3), 1.5−1.8 (m, 2H, CH_2), 1.8−2.1 (m, 2H, CH_2), 2.4−2.6 (m, 1H, CH), 3.1−3.5 (m, 2H, CH_2), 3.7−3.9 (m, 1H, CH_2), 3.9−4.4 (m, 5H, CH_2CO, CH_2O and CHCO), 7.1−7.2 (m, 1H, Ar), 7.4−7.6 (m, 5H, Ar), 8.2−8.3 (m, 1H, Ar); MS *m/z* 459 (M^+, 17), 275 (base); Anal. ($C_{23}H_{23}N_3Cl_2O_3$) C, H, N.

Ethyl 1-[2-(4-chlorophenyl)-6,8-dichloroimidazo[1,2-*a*]-pyridin-3-yl]acetyl)piperidine-3-yl]acetate (20): IR (KBr): 1727, 1644 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 1.2−1.3 (m, 3H, CH_3), 1.5−1.8 (m, 2H, CH_2), 1.8−2.1 (m, 2H, CH_2), 2.4−2.6 (m, 1H, CH), 3.1−3.5 (m, 2H, CH_2), 3.7−3.9 (m, 1H, CH_2), 3.9−4.4 (m, 5H, CH_2CO, CH_2O and CHCO), 7.2−7.3 (m, 1H, Ar), 7.4−7.7 (m, 4H, Ar), 8.2−8.3 (m, 1H, Ar); MS *m/z* 493 (M^+, 21), 309 (base); Anal. ($C_{23}H_{22}N_3Cl_3O_3$) C, H, N.

2-(4-Chlorophenyl)-3-[(4-benzylpiperazin-1-yl)-2-oxoethyl]-6,8-dichloroimidazo[1,2-*a*]pyridine (21): IR (KBr): 1640 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 2.3−2.4 (m, 4H, CH_2N), 3.4−3.8 (m, 6H, CH_2N and CH_2−Ar), 4.0 (s, 2H, CH_2CO), 7.2−7.8 (m, 10H, Ar), 8.17 (s, 1H, Ar); MS *m/z* 512 (M^+, 5), 309 (30); Anal. ($C_{26}H_{23}Cl_3N_4O$) C, H, N.

2-Phenyl-3-[(6,8-dichloroimidazo[1,2-*a*]pyridin-3-yl)-acetyl]-1,2,3,4-tetrahydroisoquinoline (22): IR (KBr):1620 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 2.65 (t, J = 6.5 Hz, 1,1H, CH_2), 2.85 (t, J = 6.5 Hz, 0.9H, CH_2), 3.45 (t, J = 6.5 Hz, 1.1H, CH_2), 3.80 (t, J = 6.5 Hz, 0.9H, CH_2), 4.17 (s, 1.1H, CH_2CO), 4.20 (s, 0.9H, CH_2CO), 4.35 (s, 0.9H, CH_2), 4.71 (s, 1.1H, CH_2), 6.5−7.8 (m, 10H, Ar), 8.31 (d, J = 6.7 Hz, 0.55H, Ar), 8.38 (d, J = 1.8 Hz, 0.45H, Ar); MS *m/z* 435 (M^+, 7), 275 (base); Anal. ($C_{24}H_{19}Cl_2N_3O$) C, H, N.

2-(4-Chlorophenyl)-3-[(6,8-dichloroimidazo[1,2-*a*]pyridin-3-yl)acetyl]-1,2,3,4-tetrahydroisoquinoline (23): IR (KBr):1620 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 2.70 (t, J = 6.5 Hz, 1.1H, CH_2), 2.85 (t, J = 6.5 Hz, 0.9H, CH_2), 3.56 (t, J = 6.5 Hz, 1.1H, CH_2), 3.80 (t, J = 6.5 Hz, 0.9H, CH_2), 4.12 (s, 1.1H, CH_2CO), 4.14 (s, 0.9H, CH_2CO), 4.44 (s, 0.9H, CH_2), 4.72 (s, 1.1H, CH_2), 6.7−7.8 (m, 9H, Ar), 8.22 (d, J = 1.8 Hz, 0.55H, Ar), 8.26 (d, J = 1.8 Hz, 0.45H, Ar); MS *m/z* 469 (M^+, 4), 309 (60); Anal. ($C_{24}H_{18}Cl_3N_3O$) C, H, N.

***N*-*n*-Butyl-[2-(4-chlorophenyl)-6,8-dichloroimidazo[1,2-*a*]pyridin-3-yl]acetamide (27):** IR (KBr): 3295, 1643 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 0.88 (t, 3H, CH_3), 1.2−1.6 (m, 4H, CH_2), 3.25 (q, 2H, CH_2NH), 3.92 (s, 2H, CH_2CO), 7.3−7.4 (m, 1H, Ar), 7.43 (d, J = 8.4 Hz, 2H, Ar), 7.70 (d, J = 8.4 Hz, 2H, Ar), 8.1−8.2 (m, 1H, Ar); MS *m/z* 409 (M^+, 14), 309 (base); Anal. ($C_{19}H_{18}Cl_3N_3O$) C, H, N.

***N*-Cyclohexyl-[2-(4-chlorophenyl)-6,8-dichloroimidazo[1,2-*a*]pyridin-3-yl]acetamide (28):** IR (KBr): 3295, 1630 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 0.9−1.2 (m, 2H, CH_2), 1.2−1.4 (m, 2H, CH_2), 1.5−1.9 (m, 6H, CH_2), 3.7−3.8 (m, 1H, CH), 3.87 (s, 2H, CH_2CO), 5.52 (d, J = 7.0 Hz, 1H, NH), 7.33 (d, J = 1.6 Hz, 1H, Ar), 7.43 (d, J = 8.5 Hz, 2H, Ar), 7.65 (d, J = 8.5 Hz, 2H, Ar), 8.13 (d, J = 1.6 Hz, 1H, Ar); MS *m/z* 435 (M^+, 15), 309 (base); Anal. ($C_{21}H_{20}Cl_3N_3O$) C, H, N.

***N*-Cyclohexyl-(2-phenyl-6,8-dichloroimidazo[1,2-*a*]-pyridin-3-yl)acetamide (29):** IR (KBr): 3290, 1635 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 0.9−1.2 (m, 2H, CH_2), 1.2−1.5 (m, 2H, CH_2), 1.5−1.9 (m, 6H, CH_2), 3.8−3.9 (m, 1H, CH), 3.90 (s, 2H, CH_2-CO), 5.70 (br s, 1H, NH), 7.2−7.6 (m, 4H, Ar), 7.6−7.8 (m, 2H, Ar), 8.0−8.3 (m, 1H, Ar); MS *m/z* 401 (M^+, 15), 275 (base); Anal. ($C_{21}H_{21}N_3Cl_2O$) C, H, N.

***N*-*n*-Butyl,*N*-methyl-[2-(4-chlorophenyl)-6,8-dichloroimidazo[1,2-*a*]pyridin-3-yl]acetamide (34):** IR (KBr): 1640

cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 0.8−1.0 (m, 3H, CH_3), 1.1−1.6 (m, 4H, CH_2), 2.9−3.0 (m, 3H, CH_3N), 3.1−3.3 (m, 1H, CH_2N), 3.3−3.5 (m, 1H, CH_2N), 4.02 (s, 1H, CH_2CO), 4.05 (s, 1H, CH_2CO), 7.3−7.4 (m, 1H, Ar), 7.43 (d, J = 8.2 Hz, 2H, Ar), 7.5−7.7 (m, 2H, Ar), 8.1−8.2 (m, 0.5H, Ar), 8.2−8.3 (m, 0.5H, Ar); MS *m/z* 423 (M^+, 14), 309 (base); Anal. ($C_{20}H_{20}Cl_3N_3O$) C, H, N.

***N*-*n*-Butyl,*N*-methyl-(2-phenyl-6,8-dichloroimidazo[1,2-*a*]pyridin-3-yl)acetamide (35):** IR (KBr): 1642 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 0.8−1.0 (m, 3H, CH_3), 1.0−1.4 (m, 2H, CH_2), 1.4−1.6 (m, 2H, CH_2), 2.89 (s, 1.5H, CH_3N), 2.92 (s, 1.5H, CH_3N), 3.1−3.2 (m, 1H, CH_2N), 3.3−3.5 (m, 1H, CH_2N), 4.06 (s, 1H, CH_2CO), 4.09 (s, 1H, CH_2CO), 7.29 (d, J = 1.9 Hz, 1H, Ar), 7.4−7.5 (m, 3H, Ar), 7.6−7.7 (m, 2H, Ar), 8.23 (d, J = 1.9 Hz, 0.5H, Ar), 8.31 (d, J = 1.9 Hz, 0.5H, Ar); MS *m/z* 389 (M^+, 12), 275 (base); Anal. ($C_{20}H_{21}Cl_2N_3O$) C, H, N.

***N*-Methyl-*N*-phenyl-[2-(4-chlorophenyl)-6,8-dichloroimidazo[1,2-*a*]pyridin-3-yl]acetamide (36):** IR (KBr): 1650 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 3.30 (s, 3H, CH_3N), 3.85 (s, 2H, CH_2CO), 7.1−7.2 (m, 2H, Ar), 7.3−7.4 (m, 6H, Ar), 7.4−7.6 (m, 2H, Ar), 8.2−8.3 (m, 1H, Ar); MS *m/z* 443 (M^+, 18), 309 (base); Anal. ($C_{22}H_{16}Cl_3N_3O$) C, H, N.

General Procedure for Preparation of (2-phenylimidazo[1,2-*a*]pyridin-3-yl)acetamides 10, 11, 15, 16, 24−26, 30, and 37. Method C. To a stirred solution of the required imidazo[1,2-*a*]pyridine-3-acetic acid **42** (1 mmol) in anhydrous THF (20 mL) were added EEDQ (1.2 mmol) and the appropriate amine and then TEA (1.3 mmol) dropwise. Stirring was prolonged at room temperature for 6−12 h and then the mixture poured into 20 mL of water and extracted with $CHCl_3$ (3 × 30 mL). The organic layer was separated, washed with water and brine, and dried over Na_2SO_4. Solvent was evaporated under reduced pressure, and the resulting residue was purified by silica gel column chromatography [light petroleum ether/ethyl acetate 8/2 (v/v) as eluent)] to give the required compounds. **10**, **11**, **15**, **16**, **24**−**26**, **30**, and **37**. Physical data of these compounds are summarized in Table 1.

***N*-Benzyl-*N*-*n*-butyl-(2-phenyl-6,8-dichloroimidazo[1,2-*a*]pyridin-3-yl)acetamide (10):** IR (KBr):1645 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 0.79 (t, 1.35 H, CH_3), 0.91 (t, 1.65H, CH_3), 0.95−1.15 (m, 0.9 H, CH_2), 1.2−1.3 (m, 1.10 H, CH_2), 1.4−1.6 (m, 2H, CH_2), 3.08 (t, J = 6.5 Hz, 1.1H, CH_2), 3.43 (t, J = 6.5 Hz, 0.9H, CH_2), 4.01 (s, 0.9H, CH_2CO), 4.13 (s, 1.1H, CH_2CO), 4.41 (s, 0.9H, CH_2−Ar), 4.58 (s, 1.1H, CH_2−Ar), 6.9−9.0 (m, 12H, Ar); MS *m/z* 465 (M^+, 15), 275 (base); Anal. ($C_{26}H_{25}N_3Cl_2O$) C, H, N.

***N*-Benzyl-*N*-*tert*-butyl-[2-(4-chlorophenyl)-6,8-dichloroimidazo[1,2-*a*]pyridin-3-yl]acetamide (11):** IR (KBr): 1620 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 1.45 (s, 9H, $C(CH_3)_3$), 4.02 (s, 2H, CH_2CO), 4.56 (s, 2H, CH_2N), 7.05 (d, J = 6.0 Hz, 2H, Ar), 7.2−7.6 (m, 6H, Ar), 7.46 (d, J = 8.0 Hz, 2H, Ar), 8.19 (s, 1H, Ar); MS *m/z* 499 (M^+, 4), 309 (70); Anal. ($C_{26}H_{24}Cl_3N_3O$) C, H, N.

2-(4-Chlorophenyl)-6,8-dichloro-3-(2-oxo-2-pyrrolidin-1-ylethyl)imidazo[1,2-*a*]pyridine (15): IR (KBr):1625 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 1.8−2.0 (m, 4H, CH_2), 3.34 (t, J = 6.5 Hz, 2H, CH_2N), 3.49 (t, J = 6.5 Hz, 2H, CH_2N), 4.00 (s, 2H, CH_2CO), 7.33 (d, J = 1.8 Hz, 1H, Ar), 7.44 (d, J = 8.3 Hz, 2H, Ar), 7.62 (d, J = 8.3 Hz, 2H, Ar), 8.26 (d, J = 1.8 Hz, 1H, Ar); MS *m/z* 407 (M^+, 19), 309 (base); Anal. ($C_{19}H_{16}Cl_3N_3O$) C, H, N.

2-Phenyl-6,8-dichloro-3-(2-oxo-2-pyrrolidin-1-ylethyl)imidazo[1,2-*a*]pyridine (16): IR (KBr):1628 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 1.8−2.0 (m, 4H, CH_2), 3.29 (t, J = 6.5 Hz, 2H, CH_2N), 3.48 (t, J = 6.5 Hz, 2H, CH_2N), 4.03 (s, 2H, CH_2CO), 7.31 (d, J = 1.8 Hz, 1H, Ar), 7.4−7.5 (m, 3H, Ar), 7.66 (d, J = 6.7 Hz, 2H, Ar), 8.31 (d, J = 1.8 Hz, 1H, Ar); MS *m/z* 373 (M^+, 17), 275 (base); Anal. ($C_{19}H_{17}Cl_2N_3O$) C, H, N.

2-[6,8-Dichloro-2-phenylimidazo[1,2-*a*]pyridin-3-yl]-*N*-methyl-*N*-(2-pyridin-3-ylethyl)acetamide (24): IR (KBr): 1645 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 2.81 (s, 1.5H, CH_3), 2.94 (s, 1.5H, CH_3), 2.9−3.0 (m, 2H, CH_2), 3.7−3.8 (m, 2H, CH_2), 3.91 (s, 1H, CH_2CO), 3.96 (s, 1H, CH_2CO), 7.0−7.7 (m, 9H, Ar), 7.69 (s, 0.5H, Ar), 8.13 (s, 0.5H, Ar), 8.3−8.6 (m, 1H, Ar); MS *m/z* 438 (M^+, 26), 275 (42); Anal. ($C_{23}H_{20}Cl_2N_4O$) C, H, N.

2-[6,8-Dichloro-2-(4-chlorophenyl)imidazo[1,2-*a*]pyridin-3-yl]-*N*-methyl-*N*-(2-pyridin-3-ylethyl)acetamide (25): IR (KBr):1645 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 2.88 (s, 1.5H, CH_3), 2.98 (s, 1.5H, CH_3), 3.03 (t, 2H, CH_2), 3.7−3.8 (m, 2H, CH_2), 3.92 (s, 1H, CH_2CO), 3.95 (s, 1H, CH_2CO), 7.0−7.6 (m, 8H, Ar), 7.6−7.7 (m, 0.5H, Ar), 8.11 (d, J = 1.6 Hz, 0.5H, Ar), 8.4−8.6 (m, 1H, Ar); MS *m/z* 472 (M^+, 17), 309 (37); Anal. ($C_{23}H_{19}Cl_3N_4O$) C, H, N.

2-(6,8-dichloro-2-phenylimidazo[1,2-*a*]pyridin-3-yl)-N-pyridin-4-ylacetamide (26): IR (KBr): 3240, 1704 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 4.35 (s, 2H, CH_2CO), 7.3−7.6 (m, 5H, Ar), 7.6−7.8 (m, 3H, Ar), 8.42 (d, J = 6.3 Hz, 2H, Ar), 8.85 (d, J = 1.6 Hz, 1H, Ar); MS *m/z* 396 (M^+, 11), 275 (base); Anal. ($C_{20}H_{14}Cl_2N_4O$) C, H, N.

***N*-Benzyl-[2-(4-chlorophenyl)-6,8-dichloroimidazo[1,2-*a*]pyridin-3-yl]acetamide (30):** IR (KBr): 3300, 1630 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 3.90 (s, 2H, CH_2CO), 4.32 (d, J = 6.5 Hz, 2H, CH_2−Ar), 7.1−7.4 (m, 8H, Ar), 7.76 (d, J = 8.2 Hz, 2H, Ar), 8.37 (d, J = 6.5 Hz, 1H, Ar), 8.5−8.6 (m, 1H, NH); MS *m/z* 443 (M^+, 10), 309 (base); Anal. ($C_{22}H_{16}Cl_3N_3O$) C, H, N.

***N*-*n*-Benzyl-*N*-methyl-(2-(4-chlorophenyl-6,8-dichloroimidazo[1,2-*a*]pyridin-3-yl)acetamide (37):** IR (KBr): 1633 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 2.89 (s, 1.5H, CH_3N), 3.05 (s, 1.5H, CH_3N), 4.05 (s, 1H, CH_2CO), 4.08 (s, 1H, CH_2CO), 4.45 (s, 1H, CH_2), 4.59 (s, 1H, CH_2), 6.9−7.0 (m, 1H, Ar), 7.2−7.6 (m, 9H, Ar), 8.13 (d, J = 1.9 Hz, 0.5H, Ar), 8.18 (d, J = 1.9 Hz, 0.5H, Ar); MS *m/z* 457 (M^+, 15), 309 (base); Anal. ($C_{23}H_{18}Cl_3N_3O$) C, H, N.

Preparation of *N,N*-Di-*n*-propyl-2-[2-(4-chlorophenyl)-6,8-dichloroimidazo[1,2-*a*]pyridin-3-yl)]propanamide (31). Method D. To a stirred solution of *N,N*-di-*n*-propyl-[2-(4-chlorophenyl)-6,8-dichloroimidazo[1,2-*a*]pyridin-3-yl)]acetamide[26] (0.2 g, 0.46 mmol) in DMF (10 mL) and cooled at 0 °C (ice bath) was added NaH (20 mg, 0.83 mmol). After 10 min, CH_3I (0.1 g, 0.69 mmol) was dropwise added and stirring was prolonged for further 30 min at room temperature. Then, the reaction mixture was treated with 10 mL of water and the organic phase extracted with $CHCl_3$ (3 × 5 mL) and dried over Na_2SO_4. Evaporation of the solvent gave a residue which resulted the essentially pure amide **31**: IR (KBr):1630 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 0.31 (t, 3H, J = 6.7 Hz, CH_3), 0.71 (t, 3H, J = 6.7 Hz, CH_3), 1.0−1.2 (m, 2H, CH_2), 1.3−1.4 (m, 2H, CH_2), 1.69 (d, J = 7.0 Hz, 3H, *CH_3*CH), 2.4−2.6 (m, 1H, CH_2N), 2.6−2.8 (m, 1H, CH_2N), 2.9−3.0 (m, 1H, CH_2N), 3.3−3.5 (m, 1H, CH_2N), 4.48 (q, J = 7.0 Hz, 1H, CHCO), 7.28 (d, J = 7.0 Hz, 1H, Ar), 7.46 (d, J = 7.0 Hz, 2H, Ar), 7.64 (d, J = 7.0 Hz, 2H, Ar), 8.78 (d, J = 7.0 Hz, 1H, Ar); MS *m/z* 451 (M^+, 7), 323 (base); Anal. ($C_{22}H_{24}Cl_3N_3O$) C, H, N.

In a similar way was prepared compound ***N*-benzyl,*N*-methyl-2-[2-(4-chlorophenyl-6,8-dichloroimidazo[1,2-*a*]-pyridin-3-yl)]propanamide (33)** starting from *N*-benzyl,*N*-methyl-[2-(4-chlorophenyl)-6,8-dichloroimidazo[1,2-*a*]pyridin-3-yl)]acetamide (**30**): IR (KBr):1640 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 1.66 (d, J = 7.1 Hz, 1.5H, CH_3), 1.73 (d, J = 7.1 Hz, 1.5H, CH_3), 2.40 (s, 1.5H, CH_3N), 2.93 (s, 1.5H, CH_3N), 4.00 (q, J = 17.6 Hz 1H, CHCO), 4.3−4.7(m, 2H, CH_2), 6.3−6.4 (m, 1H, Ar), 6.9−7.7 (m, 9H, Ar), 8.68 (d, J = 1.8 Hz 0.5H, Ar), 8.75 (d, J = 1.8 Hz 0.5H, Ar); MS *m/z* 471 (M^+, 5), 323 (base); Anal. ($C_{24}H_{20}Cl_3N_3O$) C, H, N.

Preparation of *N*-Cyclohexyl,*N*-methyl-2-[2-(4-chlorophenyl)-6,8-dichloroimidazo[1,2-*a*]pyridin-3-yl)]propanamide (32). Method E. A solution of *N*-cyclohexyl-[2-(4-chlorophenyl)-6,8-dichloroimidazo[1,2-*a*]pyridin-3-yl)]acetamide **28** (0.7 g, 1.6 mmol) in DMF (5 mL) maintained at room temperature and under stirring was treated with a solution of potassium *tert*-butoxide (0.36 g, 2 mmol), and after 10 min to the resulting mixture was dropwise added CH_3I (0.3 g, 2.1 mmol). Stirring was prolonged for 30 min and then the reaction mixture poured with caution into 10 mL of water. The organic phase was extracted with $CHCl_3$ (3 × 5 mL), dried (Na_2SO_4), and evaporated under reduced pressure. The resulting residue was the essentially pure compound **32:** IR (KBr):1635 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 1.1−1.6 (m, 10H, CH_2), 1.6−1.8 (m, 3H, CH_3),

2.31 (s, 1.2H, CH_3N), 2.70 (s, 1.8H, CH_3N), 2.9−3.1 (m, 0.6H, CHN), 4.3−4.4 (m, 0.4H, CHN), 4.45 (q, J = 7.1 Hz 0.4H, CHCO), 4.60 (q, J = 7.1 Hz, 0,6H, CHCO), 7.2−7.3 (m, 1H, Ar), 7.4−7.8 (m, 4H, Ar), 8.68 (d, J = 1.6 Hz 0.4H, Ar), 8.76 (d, J = 1.6 Hz 0.6H, Ar); MS *m/z* 463 (M^+, 7), 323 (base); Anal. ($C_{23}H_{24}Cl_3N_3O$) C, H, N.

Preparation of *N-n*-Butyl,*N*-methyl-[2-(4-chlorophenyl)-6,8-dichloroimidazo[1,2-*a*]pyridin-3-yl)]acetamide (34). Method F. To a solution of *N-n*-butyl-[2-(4-chlorophenyl)-6,8-dichloroimidazo[1,2-*a*]pyridin-3-yl)]acetamide **27** (0.2 g, 0.49 mmol) in anydrous THF (3 mL), cooled at −45 °C with a liquid nitrogen bath, was dropwise added a solution of *n*-BuLi 2.5 M in hexane (200 μL, 0.5 mmol), and the mixture was stirred for 10 min.. Then, methyl triflate (0.1 g, 1.4 mmol) was slowly added, and stirring was prolonged for 30 min. The progress of reaction was monitored by HPLC analysis. Then, to the mixture was added water (5 mL), and the organic phase was extracted with $CHCl_3$ (3 × 10 mL). The organic layer was separated and dried over Na_2SO_4. Solvent was evaporated under reduced pressure, and the resulting residue was purified by silica gel column chromatography [$CHCl_3$/acetone 97/3 (v/v) as eluent)] to give the required compound **34**.

High-performance liquid chromatography (HPLC) analyses were performed with a Waters (Waters Corp., Milford, MA) 600 pump equipped with a Waters 990 variable wavelength UV detector. HPLC mobile phase was prepared from HPLC-grade methanol. For analysis, a reversed phase Simmetry C_{18} (25 cm × 4.6 mm; 5 μm particles) column in conjunction with a precolumn module was eluted with methanol:deionized water (75:25) (injection volume 20 μL) in isocratic mode. The flow rate of 1 mL/min was maintained, and the column effluent was monitored continuously at 254 nm.

In a similar way were prepared compounds **35** and **36**.

Biological Methods. Materials. Male Sprague−Dawley CD rats at 30 days of age, immediately after weaning, were housed for 30 days either in groups of eight per cage. They were maintained under an artificial 12-h-light, 12-h-dark cycle (light on 0800 to 2000 hours) at a constant temperature of 23° ± 2 °C and 65% humidity. Food and water were freely available until the time of the experiment. Animal care and handling throughout the experimental procedures were in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). All experiments were performed between 10:00 a.m. 1:00 p.m. CB 34 was dissolved by adding one drop of Tween 80 per 5 mL of physiological saline and was administered intraperitoneally in a volume of 0.3 mL per 100 g of body mass.

In Vitro Receptor Binding Assays. [^{3}H]Flunitrazepam Binding. Cerebral cortex was homogenized with a Polytron PT 10 in 50 volumes of ice-cold 50 mM Tris-HCl (pH 7.4), and the homogenate was centrifuged twice at 20 000*g* for 10 min. The final pellet was reconstituted in 50 volumes of Tris-HCl buffer and used for the binding assay. [^{3}H]Flunitrazepam binding was determined in a final volume of 1000 μL, comprising 400 μL of membrane suspension (0.4 to 0.5 mg of protein), 400 μL of Tris-HCl buffer, 100 μL of [^{3}H]-flunitrazepam (74 Ci $mmol^{-1}$; New England Nuclear), and 100 μL of drug solution or solvent. Incubations were performed for 60 min at 0 °C and were terminated by rapid filtration through glass-fiber filter strips (Whatman GF/B). The filters were then rinsed with ice-cold Tris-HCl buffer, and filter-bound radioactivity 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.

[^{3}H]PK 11195 Binding. After killing, the brain was rapidly removed from rats, the cerebral cortex was dissected and all tissues were stored at −80 °C until assayed. 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 40 000*g* for 30 min, and the resulting pellet was resuspended in 50 volumes of PBS and recentrifuged. The new pellet was resuspended in 10 volumes of PBS and used for the assay. [^{3}H]PK 11195 binding was determined in a final volume of 500 μL, comprising 50 μL of membrane suspension (0.15−0.20 mg protein), 50 μL of [^{3}H]PK 11195 (85.5 Ci/mmol, New England Nuclear; final assay concentration 1M), and 400 μL of PBS buffer (pH 7.4 at 25 °C). Incubation (25 °C) was initiated by the addition of membranes and were terminated 90 min later by rapid filtration through glass-fiber filter strips (Wathaman GF/B), that had been presoaked with 0.3% polyethyleneimine and placed in a Cell Harvester manifold (Brandel). The filters were rinsed five times with 4 mL of ice-cold PBS buffer, after which filter-bound radioactivity was quantified by liquid scintillation spectrometry. Nonspecific binding was defined as the binding in the presence of 10 μM of unlabeled PK 11195 (Sigma).

Functional in Vitro Studies. Electrophysiological Studies Using *Xenopus* Oocytes. Complementary DNAs encoding the human α1, β2, and γ2L $GABA_A$ 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, WI) 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 γ2L subunit cDNAs (total of 1.5 ng of cDNA in 30 nL in a 1:1:1 ratio) was injected into the oocytes 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 M 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 microelectrodes (resistance between 0.5 and 3 MΩ) filled with filtered 3 M KCl, and the voltage was clamped at −70 mV with an Axoclamp 2-A amplifier (Axon Instruments, Burlingame, CA). Currents were continuously recorded on a strip-chart recorder. Resting membrane potential usually varied between −25 and −45 mV. GABA (dissolved in MBS) and drugs (dissolved in dimethyl sulfoxide and then diluted in MBS) were perfused for 20 s (7 to 10 s were required to reach equilibrium in the recording chamber). Intervals of 5−10 min were allowed between drug applications. Modulation of GABA-evoked Cl^- currents by drugs is expressed as percentage change, $[(I'/I) - 1] \times 100\%$, where I is the average of control responses obtained before drug application and after drug washout, and I' is the average of the agonist-induced responses obtained from the same cell in the presence of drug.

Functional in Vivo Studies. Effect of Compounds 2, 3, 7, 14, 18, 34, and 35 on Plasma and Brain Concentration of Neuroactive Steroids. Drug Administration. Compounds **2**, **3**, **7**, **14**, **18**, **34**, and **35** were dissolved by adding three drops of Tween 80 per 5 mL of saline and were administered intraperitoneally in a volume of 0.3 mL per 100 g of body mass.

Extraction and Assay of Steroids. Rats were killed either with a guillotine (for measurement of plasma steroids) or by focused microwave irradiation (70 W/cm^2 for 4 s) of the head (for measurement of brain steroids). This latter procedure results in a virtually instantaneous inactivation of brain enzymes,[38] thus minimizing postmortem steroid metabolism. The brain was rapidly (<1 min) removed from the skull, and the cerebral cortices were dissected and then frozen at −20 °C until steroid extraction. Steroids were extracted and purified as previously described.[39] In brief, steroids present in tissue homogenates [600 mg of cortex in 4 mL of phosphate-buffered saline (pH 7.0)] were extracted three times with ethyl

acetate, and the combined organic phases were dried under vacuum. The resulting residue was dissolved in 5 mL of *n*-hexane and applied to a SepPak silica cartridge (Waters), and components were eluted with *n*-hexane and 2-propanol (7:3, vol/vol). Steroids were separated and further purified by HPLC on a 5-μm Lichrosorb-diol column (250 by 4 mm; Phenomenex) with a discontinuous gradient of 2-propanol (0 to 30%) in *n*-hexane. Progesterone, which coelutes with cholesterol, was further purified by washing the corresponding dried HPLC fractions first with 200 μL of dimethyl sulfoxide and then with 400 μL of water. Progesterone was extracted from the aqueous phase twice with 1.5 mL volumes of *n*-hexane. The recovery (70 to 80%) of steroids through the extraction and purification procedures was monitored by adding a trace amount (6000 to 8000 cpm; 20 to 80 Ci/mmol) of tritiated standard to the brain homogenate.

Blood was collected from the trunk of killed rats into heparinized tubes and centrifuged at 900*g* for 20 min at room temperature. The resulting plasma was frozen (−80 °C) until assayed for steroids. Steroids were extracted from plasma three times with 1.5 mL of ethyl acetate.

Steroids from both brain tissue and plasma were quantified by radioimmunoassay as previously described[39,40] with specific antibodies to progesterone (ICN, Costa Mesa, CA), 3α,5α-THDOC, and 3α,5α-THPROG (generated in rabbits and sheep, respectively, and characterized as previously described).[40]

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

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Supporting Information Available: Microanalytical data. This material is available free of charge via the Internet at http://pubs.acs.org.

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