2-Phenyl-imidazo[1,2-*a*]pyridine Compounds Containing Hydrophilic Groups as Potent and Selective Ligands for Peripheral Benzodiazepine Receptors: Synthesis, Binding Affinity and Electrophysiological Studies

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A series of imidazopyridine acetamides were synthesized to evaluate the effects of structural changes at both central (CBRs) and peripheral benzodiazepine receptors (PBRs). These changes include the introduction of polar substituents or ionizable functional groups at the 2- and 8-position of the imidazopyridine skeleton. The results suggest that substituents endowed with hydrogen bonding acceptor and/or donor properties in the *para* position of the phenyl ring lead to high affinity for PBR. In electrophysiological studies, it was found that compounds **9**, **12**, **13**, and **28** markedly enhanced GABA-evoked Cl^- currents in *Xenopus* oocytes expressing $\alpha_1\beta_2\gamma_2$ $GABA_A$ receptors. The capability of flumazenil to reduce the stimulatory effect exerted by compound **9** supports the conclusion that the modulatory effects of the examined compounds occur involving the CBR. The ability of compound **16** to increase $GABA_A$ receptor-mediated miniature inhibitory postsynaptic currents in CA1 pyramidal neurons is indicative of its ability to stimulate the local synthesis and secretion of neurosteroids.

Introduction

During the last decade, the peripheral-type benzodiazepine receptor (PBR[a]) has been the object of extensive studies aimed at defining its biochemical and pharmacological role. In contrast to central-type benzodiazepine receptors (CBRs), which are located primarily in neurons in the central nervous system (CNS), peripheral-type benzodiazepine receptors (PBRs) were detected in the 1970s as benzodiazepine binding sites mainly in several peripheral tissues such as steroidogenic organs and blood cells.

The PBR is a mitochondrial protein of 18 kDa, which can form a trimeric complex with the voltage dependent anion channel VDAC (32 kDa) and the adenine nucleotide carrier ANC (30 kDa). The 18 kDa protein is the minimal functional unit of PBR and recently has been named "translocator protein" regardless of its subcellular localization.[1]

A broad spectrum of functions are associated with the PBR, including the regulation of cholesterol transport and the synthesis of steroid hormones, porphyrin transport, and heme synthesis, apoptosis, cell proliferation, anion transport, regulation of mitochondrial functions, and immunomodulation.[2,3] Among these different functions, of particular interest are those referring to the neurosteroid synthesis and the involvement in apoptosis processes. Thus, it is now well known that steroids synthesized in the brain modify neuronal activity by modulating $GABA_A$ receptor function. Changes in neurosteroid levels are associated with various physiological and pathological conditions including stress, pregnancy, neural development, aging, and postpartum depression. The neurosteroid levels are also altered by psychoactive drugs, including ethanol and antidepressants such as fluoxetine.[4] On the other hand, the PBR is component of the MPTP located at junction sites between the inner and the outer mitochondrial membranes. MPTP opening induced by some PBR ligands increases the mitochondrial membrane permeability, allowing the leakage of mitochondrial pro-apoptotic factors such as caspase-3 and caspase-9.[5] Several experimental evidences also indicate that PBRs are overexpressed in a number of tumor types, and PBR expression appears to be related to the tumor malignancy grade.[6,7] Therefore, these receptors could be the target to selectively increase anticancer drug delivery by using an appropriate PBR ligand−anticancer drug conjugate as well as appropriate PBR ligands could be used as diagnostic imaging agents.[8,9] On the basis of all these functions, there are many potential clinical applications of PBR modulation such as in oncologic, endocrine, neuropsychiatric, and neurodegenerative diseases.

Nowadays, a wide variety of specific ligands with high affinity and selectivity for PBR have been identified (Chart 1). For example, compound **1** (Ro5-4864),[10] 4′-chlorodiazepam, exhibits high affinity for the PBR and very low affinity for the CBR. Compound **2** (PK11195)[11] is an isoquinoline derivative, and

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[a] Abbreviations: ACSF, artificial cerebrospinal fluid; Boc, *tert*-butoxycarbonyl; Boc-Gly-OH, *N*-(*tert*-butoxycarbonyl)glycine; CBRs, central benzodiazepine receptors; CDI, *N,N*′-carbonyldiimidazole; CNS, central nervous system; DCC, *N,N*′-dicyclohexylcarbodiimide; DMAP, 4-(dimethylamino)pyridine; DMF, dimethylformamide; DMSO, dimethylsulfoxide; EEDQ, ethyl 1,2-dihydro-2-ethoxy-1-quinolinecarboxylate; EGTA, ethylenglycol-bis-(2-aminoethyl ether)-*N,N,N*′*N*′-tetracetic acid; HEPES, 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid; MBS, modified Barth's saline; mIPSCs, miniature inhibitory post-synaptic currents; MPTP, mitochondrial permeability transition pore; THF, tetrahydrofuran; PBRs, peripheral benzodiazepine receptors; SAR, structure−activity relationship; SI, selectivity index; TEA, triethylamine; 3α,5α-TH Prog, 5α-pregnan-3α,-ol-20-one; TLC, thin layer chromatography.

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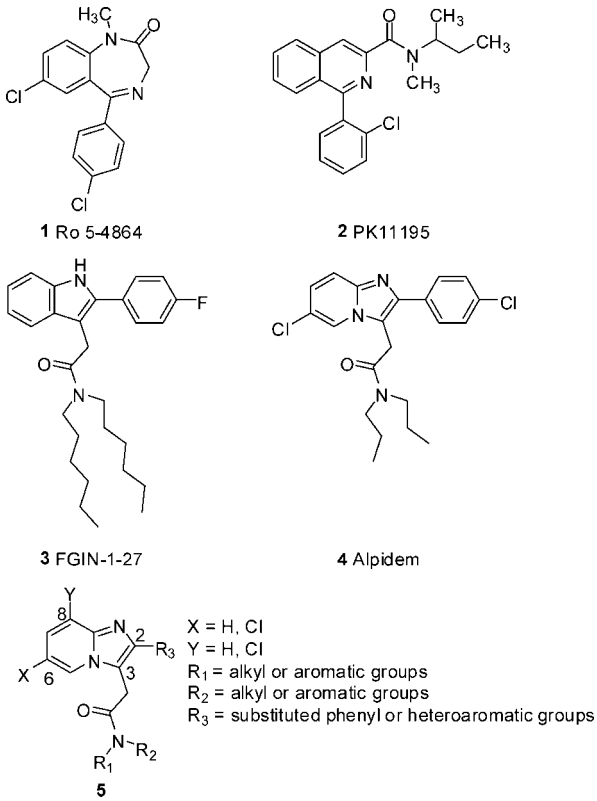
Chart 1. Synthetic Ligands for PBR



currently it is the most widely used specific probe for peripheral benzodiazepine receptors. Compound **3** (FGIN-1-27)[12] is a 2-aryl-3-indoleacetamide derivative and exhibits high affinity for the PBR with high selectivity over the CBR. Compound **4** (i.e., Alpidem)[13] has an imidazopyridine skeleton in its structure and binds with high affinity to both PBRs and CBRs.

We have recently reported a series of potent and selective PBR ligands, designed from Alpidem by introducing several substituents on the imidazopyridine nucleus (Compounds **5**, Chart 1).[14−18] Our structure−activity correlations revealed that substitution at the 8-position of the imidazopyridine nucleus is a key factor for improving affinity and selectivity toward peripheral binding sites. 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.[12] Additionally, the substituents on the acetoamide nitrogen on the 3-position of the imidazopyridine nucleus are responsible for modulation of affinity.[18] Mainly, increasing the branching of the alkyl substituent on the carboxamide nitrogen of the potential PBR ligand may cause unfavorable interaction with the receptor. The presence of aromatic substituents on the carboxamide nitrogen leads to high affinity and selectivity, while introduction of polar substituents in this region is detrimental for binding affinity.

As a continuation of our studies on 2-phenylacetamidoimidazo[1,2-*a*]pyridines as potent and selective PBR ligands, our attention has been focused on the 2- and 8-position of the imidazopyridine nucleus to clarify the effect of substituents in these specific regions on modulation of affinity to PBR. It should be noted that, to our knowledge, very few PBR ligands containing hydrophilic groups have been synthesized so far. Therefore, the need for a greater aqueous solubility for our PBR ligands prompted us to introduce polar substituents or ionizable functional groups. Particularly, the phenyl group on the 2-position of the imidazopyridine skeleton was functionalized with amino-, hydroxy-, and carboxylic-groups. Consequently, these polar substituents could be useful in increasing the hydrophilicity of PBR ligands and, on the other hand, they could be directly conjugated to anticancer drugs or hydrophilic polymers (e.g., polyethylenglicols) with a reversible chemical linkage.

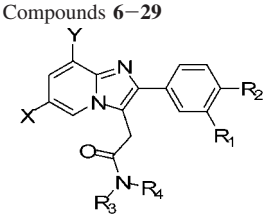
In this paper, we present the synthesis and biological evaluation of new potent and selective PBR ligands mainly characterized by hydrophilic substituents at 2- and 8-positions of the imidazopyridine nucleus. The modulatory action of most compounds evaluated at human $\alpha_1\beta_2\gamma_2$ $GABA_A$ receptors expressed in *Xenopus* oocytes are also discussed.

Chemistry

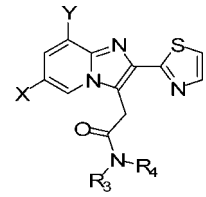
The imidazopyridine acetamides **9**−**11**, **14**, and **16**−**18** (Table 1) were synthesized using synthetic methods outlined in Scheme 1. In particular, condensation in DMF at reflux of suitably substituted 2-aminopyridines **33** with the appropriate bromoketoacids **32** yielded the butyl imidazopyridine acetates **34**. Compounds **32**, in turn, were prepared by treatment of the keto-butyl esters **31** with bromine in carbon tetrachloride. Compounds **34** were hydrolyzed with 1 N NaOH in *n*-butanol to give the corresponding acids **35**. These compounds were then converted to the desired compounds **9**−**11**, **14**, and **16**−**18** by condensation of the appropriate dialkylamines in the presence of EEDQ as dehydrating agent. In particular, the preparation of the nitroimidazopyridines **14** and **16**−**18** was readily accomplished by using the H_2SO_4/HNO_3 mixture. Furthermore, compounds **14**, **16**, **17**, and **18** were smoothly reduced to the corresponding aminoimidazopiridines **15**, **19**, **20**, and **21** by using cyclohexene with 10% Pd/C or Sn in HCl.[19] As shown in Scheme 2, the demethylation of the 2-*p*-methoxyphenylimidazopyridine acetic acid **35a** was accomplished with HBr (48% w/w) at reflux, giving rise to the 2-*p*-hydroxyphenylimidazopyridine acetic acid **36a**. The subsequent condensation of this last compound with the suitable dialkylamine hydrochloride in anhydrous THF in the presence of CDI gave compounds **6**−**8**. Treatment of compound **6** with Boc-Gly-OH in the presence of DCC and the subsequent Boc removal with gaseous HCl led to compound **12**, while treatment of **6** with methyl-4-chloro-4-oxobutanoate in anhydrous THF gave compound **13**. The synthesis of compound **22** was easily accomplished by refluxing a solution of **19** in toluene in the presence of succinic anhydride. The synthesis of compounds **23**−**27** was accomplished by the procedures shown in Scheme 3. Compounds **23**−**25** were obtained by reaction of the known *N,N*-di-*n*-propyl [2-(4-chlorophenyl)-8-amino-imidazo[1,2-*a*]pyridin-3-yl)]acetamide **38**[15] with the appropriate cyclic anhydride, while compound **26** was prepared by condensation of **38** with the *N*-Boc-glycine and the subsequent selective deprotection of the amino group with gaseous HCl. Compounds **28** and **29** were prepared by the sequence of reactions reported in Scheme 4, which involves the synthesis of the methyl bromoester **43** by a reported procedure,[20] followed by its condensation with 3,5-dichloro-2-amino-pyridine **44** to give, after hydrolysis, the thiazolylimidazopyridine-acetic acid **46**. Condensation of this last compound with the appropriate dialkyl- or arylalkyl-amine yielded the desired compounds **28** and **29**.

All compounds were fully characterized by IR, ^{1}H NMR, mass spectra, and elemental analyses (Experimental Section). As already noted in the previous work,[18] an interesting feature of the ^{1}H NMR spectra of these imidazoacetamides concerns the signals associated with the methylene linked at the carbonyl

Table 1. Structure and Physical Properties of Compounds **6**−**29**



6-27

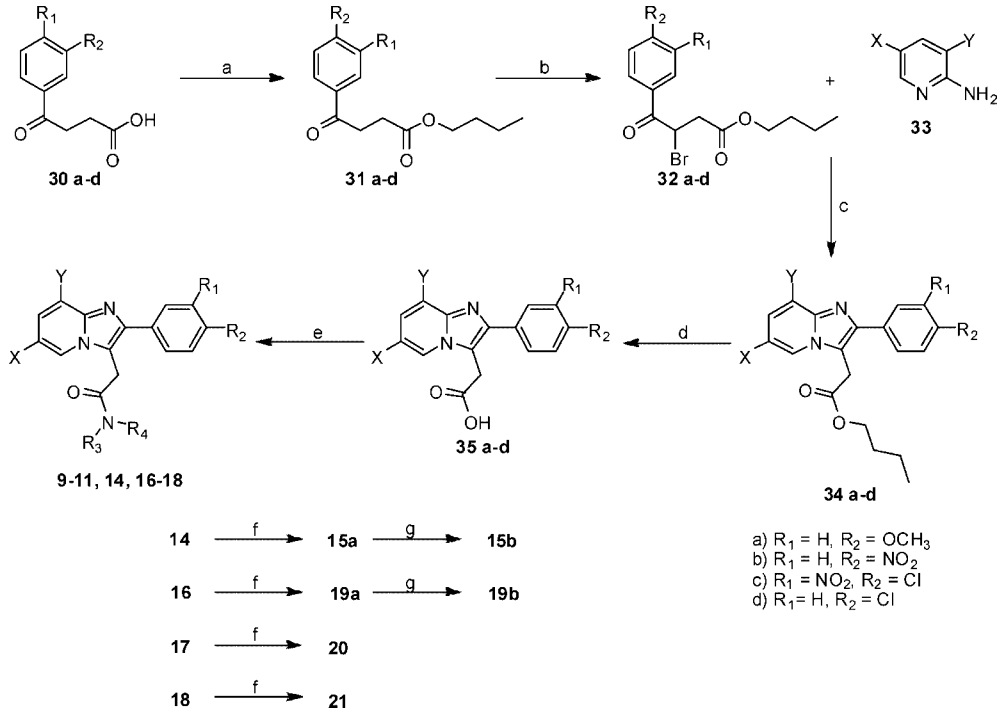


28-29

compd	X	Y	R_1	R_2	R_3	R_4	yield (%)	mp (°C)
6	Cl	Cl	H	OH	*n*-C_3H_7	*n*-C_3H_7	43	212−216
7	Cl	Cl	H	OH	CH_3	*n*-C_4H_9	45	200−203
9	Cl	Cl	H	OCH_3	*n*-C_3H_7	*n*-C_3H_7	85	123−125
10	Cl	Cl	H	OCH_3	CH_3	*n*-C_4H_9	83	114−117
11	Cl	Cl	H	OCH_3	CH_3	C_6H_5	65	201−203
12	Cl	Cl	H	$OCOCH_2NH_2 \cdot HCl$	*n*-C_3H_7	*n*-C_3H_7	94	N.D.[a]
13	Cl	Cl	H	$OCO(CH_2)_2COOCH_3$	*n*-C_3H_7	*n*-C_3H_7	95	199−201
14	Cl	Cl	H	NO_2	*n*-C_3H_7	*n*-C_3H_7	55	218−220
15a	Cl	Cl	H	NH_2	*n*-C_3H_7	*n*-C_3H_7	68	200 dec
15b	Cl	Cl	H	NH_2 HCl	*n*-C_3H_7	*n*-C_3H_7	95	165 dec
16	Cl	Cl	NO_2	Cl	*n*-C_3H_7	*n*-C_3H_7	74	177−180
17	Cl	Cl	NO_2	Cl	CH_3	*n*-C_4H_9	59	147−150
18	H	Cl	NO_2	Cl	CH_3	*n*-C_4H_9	68	155−157
19a	Cl	Cl	NH_2	Cl	*n*-C_3H_7	*n*-C_3H_7	62	218−220
19b	Cl	Cl	NH_2 HCl	Cl	*n*-C_3H_7	*n*-C_3H_7	88	N.D.[a]
20	Cl	Cl	NH_2	Cl	CH_3	*n*-C_4H_9	54	223−225
21	H	Cl	NH_2	Cl	CH_3	*n*-C_4H_9	65	217−220
22	Cl	Cl	NH-$CO(CH_2)_2COOH$	Cl	*n*-C_3H_7	*n*-C_3H_7	84	159 dec
23	H	$NHCOCH_2COOH$	H	Cl	*n*-C_3H_7	*n*-C_3H_7	77	230 dec
24	H	$NHCO(CH_2)_2COOH$	H	Cl	*n*-C_3H_7	*n*-C_3H_7	82	258 dec
25	H	$NHCO(CH_2)_3COOH$	H	Cl	*n*-C_3H_7	*n*-C_3H_7	87	203 dec
26	H	$NHCOCH_2NH_2 \cdot HCl$	H	Cl	*n*-C_3H_7	*n*-C_3H_7	97	N.D.[a]
27	H	$NHCO(CH_2)_2CO_2C_2H_5$	H	Cl	*n*-C_3H_7	*n*-C_3H_7	72	253−256
28	Cl	Cl			*n*-C_3H_7	*n*-C_3H_7	21	158−163
29	Cl	Cl			CH_3	C_6H_5	22	172−175

[a] N.D., not determined.

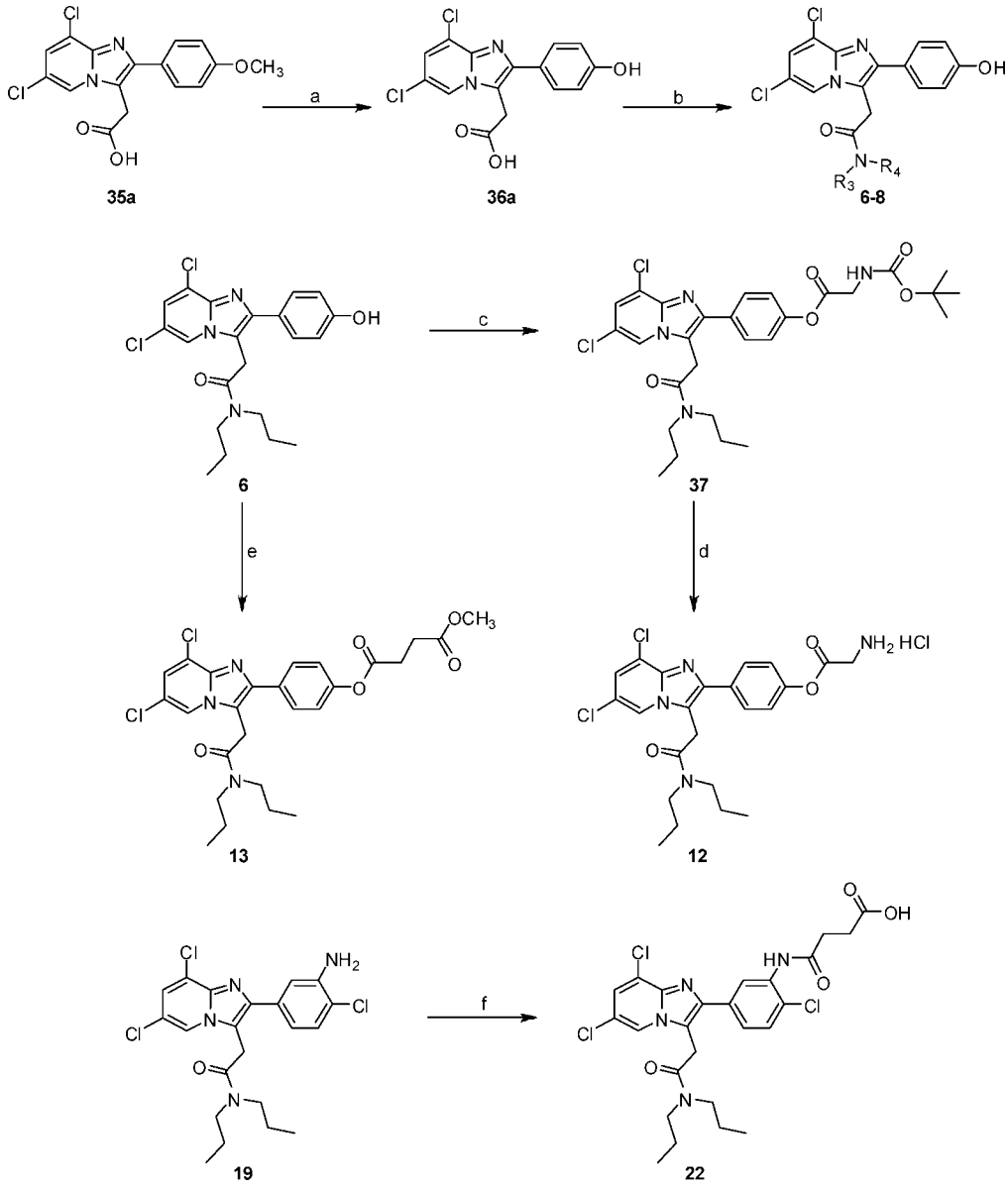
Scheme 1. Preparation of the Imidazopyridine Acetamides **9**−**11**, **14**−**21**[a]



[a] Reagents and conditions: (a) *n*-butanol/H_2SO_4; (b) Br_2/CCl_4; (c) *n*-butanol, Δ; (d) *n*-butanol/ NaOH 1N; (e) CDI, anhydrous solvent, TEA; (f) EtOH/HCl, granular tin, Δ; (g) anhydrous THF/$HCl_{(g)}$.

group and the methyl and methylene groups characterizing the alkyl chains of CO-N < R_3R_4. The $-CH_2CO-$ gives rise to a singlet in the range 3.7−4.6 δ only when the R_3 and R_4 groups are of similar size. When these two groups are very different in size (e.g., in the case of compounds **7**, **10**, **17**, **18**, **20**, and **21**), the signals of the CH_2CO and those of the protons in the R_3

Scheme 2. Preparation of the Imidazopyridine Acetamides **6**−**8**, **12**, **13**, **22**[a]



[a] Reagents and conditions: (a) conc HBr, Δ; (b) CDI, anhydrous THF, TEA; (c) *N*-Boc-Gly, DCC, anhydrous THF, TEA; (d) 0 °C, $CH_2Cl_2/HCl_{(g)}$; (e) methyl-4-chloro-4-oxobutyrate, anhydrous THF; (f) succinic anhydride, toluene, Δ.

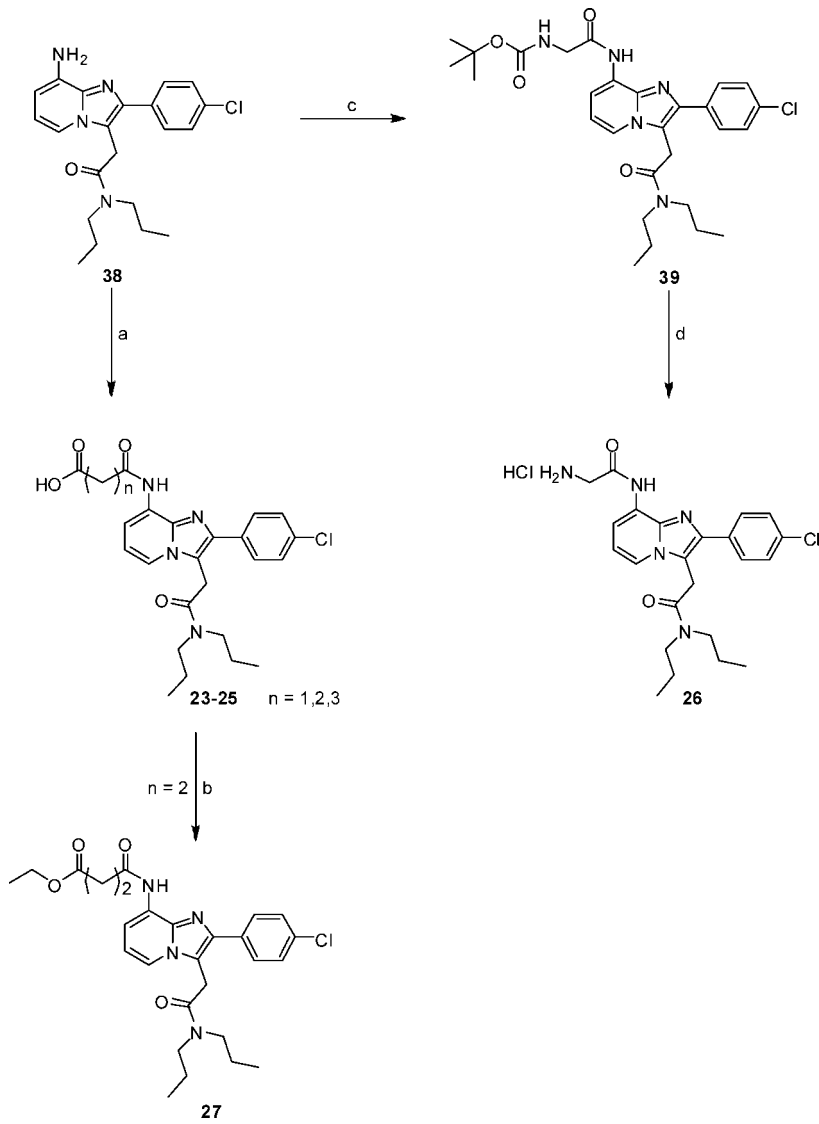
and R_4 groups are duplicated due to the partial double bond character of the amide bond. Physical data for new compounds are reported in Table 1.

Results and Discussion

Affinities of Imidazopyridine Derivatives for Peripheral and Central Benzodiazepine Receptors. The affinities of the 26 tested compounds for PBR and CBR were determined by measuring their ability to displace compound [^{3}H]-**2** and [^{3}H]-flunitrazepam (5-(2-fluorophenyl)-1-methyl-7-nitro-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one) from binding to membrane preparations obtained from rat cerebral cortex. Their effects were compared with those of unlabeled **2**, a selective ligand for PBR.[11] The measured binding affinity for PBR and CBR expressed as K_i values are shown in Table 2. The structural analysis of PBR ligands **6**−**29** suggests that they may share the pharmacophore components proposed by Bourguignon[21] and Anzini[22] (δ1, LA, PAR, FRA in Figure 1) in which a single H-bonding acceptor moiety (δ1, usually a suitably oriented amide carbonyl group) plays a key role in the interaction with PBR.

As above mentioned, to our knowledge, very few PBR ligands containing hydrophilic groups are known. Therefore, the need for a greater aqueous solubility for our PBR ligands as well as the possibility to exploit these hydrophilic groups for a direct conjugation to drugs, especially anticancer drugs or hydrophilic polymers with a reversible chemical linkage, prompted us to introduce polar substituents or ionizable functional groups on the phenyl at the 2 position of the imidazopyridine skeleton. Thus, compounds **6**−**8** as *p*-OH-substituted 2-phenyl-imidazopyridine derivatives were synthesized. Interestingly, it was noted that introduction of a polar substituent such as the −OH group in that position leads to compounds with high affinity for PBR and greater than that of the reference compound **2** (K_i, 1.14 nM); in particular, compound **8** showed an affinity value in the subnanomolar order. Moreover, the selectivity index (SI) for PBR versus CBR, expressed as K_i CBR/K_i PBR ratio, for

Scheme 3. Preparation of the Imidazopyridine Acetamides **23**−**27**[a]

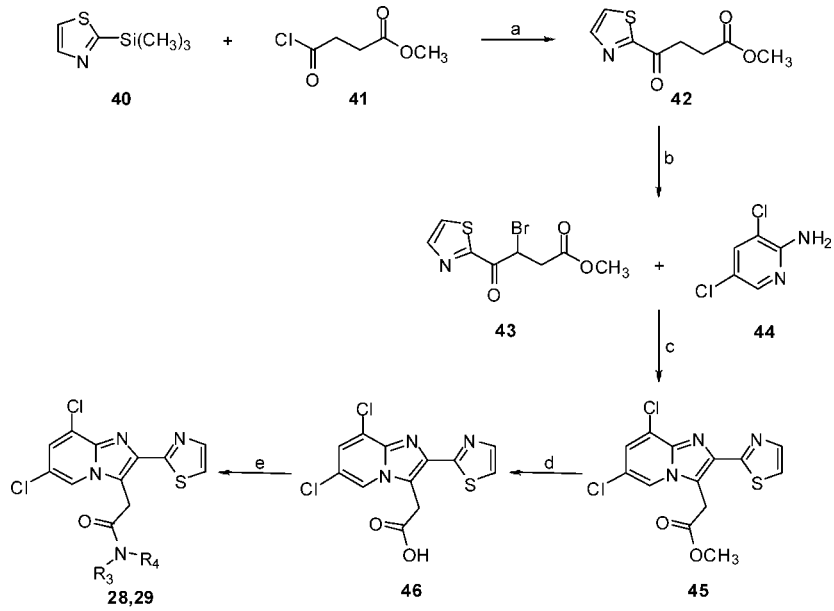


[a] Reagents and conditions: (a) Meldrum's acid, anhydrous THF (**23**); succinic or glutaric anhydride, toluene, Δ (**24**, **25**); (b) 50 °C, EtOH/conc H_2SO_4; (c) *N*-Boc-Gly, DCC, anhydrous CH_2Cl_2, DMAP, TEA; (d) anhydrous $CH_2Cl_2/HCl_{(g)}$.

compounds **6** and **8** was very high (>10^3). A pairwise comparison of binding data demonstrated that replacement of two *n*-propyl groups on the carboxamide nitrogen in **6** with methyl/butyl or methyl/phenyl groups (i.e., **7** and **8**, respectively) improved the affinity. Methylation of the *p*-OH groups of compounds **6**−**8** gave the methylethers **9**−**11**, respectively. Interestingly, the latter compounds retained good affinity and selectivity for PBR even at subnanomolar level (Table 2). These results suggest that the presence of substituents endowed with hydrogen bonding acceptor properties in the *para* position of the phenyl ring seems to be favorable for high affinity and selectivity. Esterification of compound **6** with glycine and methyl emisuccinate led to compounds **12** and **13**, and both of these compounds showed high affinity and selectivity. These data suggest that the presence of an ionizable group such as the amino group of the glycinate **12** on the *para*-position of the phenyl ring seems to be favourable for high affinity and selectivity. To further examine the behavior of an ionizable group, compounds **15a**,**b** (i.e., *p*-NH_2− and *p*-NH_2·HCl substituted 2-phenyl-imidazopyridine compounds) were synthesized. Again, high affinity for the PBR was observed even though it was slightly lower than that of the *p*-OH derivative **6**. However, compounds **15a**,**b** showed a modest selectivity for this receptor (SI of 111 and 371, respectively). The high affinity of compound **15b** suggests that a substituent endowed with hydrogen bonding donor properties in the *para* position of the phenyl ring should favorably interact with the corresponding complementary site of the receptor. Therefore, it may be concluded that substitution with groups characterized by hydrogen bonding acceptor and/or donor properties in the *para* position leads to high affinity for PBR.

We next examined the biological effects of the *p*-Cl-*m*-NH_2 and *p*-Cl-*m*-NH_2·HCl disubstituted compounds **19a**, **20**, **21**, and **19b**, respectively. Interestingly, compounds **19a**,**b**, **20**, **21** showed high affinity and, in particular **19a**,**b**, together with the nitro-compounds **16**−**18**, proved to be among the most active (at subnanomolar level) compounds in this series. However, compounds **20** and **21** exhibited a modest selectivity for PBR with SI of 686 and 187, respectively. In contrast, the carboxylic acid **22** showed a high selectivity for PBR but an affinity value (i.e., K_i 14.4 nM) about 20-fold lower than those of compounds **20** and **21.** These data support the conclusion that the substitution with groups possessing hydrogen bonding acceptor and/or donor

Scheme 4. Preparation of the Imidazopyridine Acetamides **28**, **29**[a]



[a] Reagents and conditions: (a) 0 °C; (b) $PyH^+Br_3^-$, THF; (c) toluene, Δ; (d) dioxane, HCl 1N; (e) EEDQ, THF.

Table 2. Affinities of Compounds **6**−**29** for CBR and PBR from Rat Cerebral Cortex and Their Modulatory Action at Human $\alpha_1\beta_2\gamma_2$ $GABA_A$ Receptors Expressed in *Xenopus* Oocytes

compd	K_i (nM) CBR[a]	K_i (nM) PBR[a]	change ± SEM of GABA induced Cl^- current (%) at 1 μM[b]	change ± SEM of GABA induced Cl^- current (%) at 10 μM[b]
6	$>10^5$	1.31	−3.9 ±5.3	23.8 ± 6.6
7	2930	0.87	7.9 ± 34.0	−23.6 ± 19.9
8	5130	0.32	−5.9 ± 16.8	6.7 ± 3.2
9	$>10^5$	0.31	8.8 ± 3.5	55.9 ±8.4
10	$>10^5$	0.73	24.8 ± 14.0	−16.5 ± 21.0
11	1000	0.23	−8.1 ± 4.7	−8.0 ± 11.0
12	$>10^4$	1.52	26.6 ±6.2	71.0 ± 15.6
13	$>10^4$	1.69	16.6 ±4.2	37.5 ± 8.9
14	$>10^4$	0.15	−4.2 ± 10.3	−16.6 ± 9.3
15a	246	2.22	8.1 ± 2.6	23.9 ±6.9
15b	1024	2.76	29.9 ±6.4	−7.4 ± 2.8
16	$>10^4$	0.24	14.9 ±3.6	9.6 ± 4.0
17		0.30	−10.2 ± 0.2	−9.6 ± 1.3
18	$>10^5$	1.16	12.1 ± 12.2	−20.0 ± 10.9
19a	$>10^4$	0.33	−6.2 ± 5.4	−38.7 ± 10.5
19b	$>10^4$	0.45	−2.9 ± 13.7	−2.2 ± 4.8
20	535.0	0.78	N.D.	N.D.
21	194.0	1.04	9.5 ± 15.6	10.7 ± 13.3
22	$>10^4$	14.4	11.5 ± 11.0	10.0 ± 7.8
23	$>10^4$	193.1	9.6 ± 6.9	19.8 ± 14.8
24	$>10^4$	285.3	1.3 ± 24.0	12.3 ± 0.3
25	$>10^4$	117.7	7.3 ± 25.1	−1.9 ± 30.7
26	$>10^4$	14.2	N.D.	N.D.
27	$>10^4$	0.86	N.D.	N.D.
28	$>10^5$	2.07	6.3 ± 2.5	60.6 ± 12.3
29	$>10^5$	1.44	−1.2 ± 10.6	19.4 ± 4.6
2	$>10^5$	1.14	4.9 ± 5.3	9.4 ± 4.8

[a] Data are means of three separate experiments performed in duplicate which differed by less than 10%. [b] 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 10 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 five to ten different oocytes) ± SEM; N.D., not determined.

properties in *meta* position of the phenyl ring leads to high affinity binding for the PBR.

To improve our understanding of the structure−activity relationships (SAR) at the 2-position of the imidazopyridine skeleton, compounds **28** and **29**, characterized by a thiazole ring

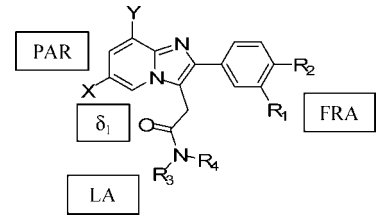


Figure 1. The suggested four components of the pharmacophore model for PBR ligands: FRA, freely rotating aromatic ring region; δ1, electron rich zone; PAR, planar aromatic region; LA, lipophilic area.

at 2-position, were also evaluated. The observed high affinity and selectivity of these compounds indicates that introduction of an aromatic ring different from the benzene one is well tolerated, and this information could have value for molecular design of further PBR ligands in the imidazopyridine-acetamide series.

Among the very few examples known of PBR ligands containing hydrophilic groups, there is the 8-amino imidazopyridine **38**.[15] To evaluate whether further polar substituents or ionizable functional groups could be introduced in this position, compounds **23**−**27** were synthesized and examined. Data suggest that introduction of a −COOH group led to a significant decrease in affinity for PBR, as observed for the set of compounds **23**−**25** (i.e., K_i 193.1, 285.3, and 117.7 nM, respectively). A pairwise comparison of binding data between compounds **24** and **27** showed that esterification of carboxylic acid function led to a significant increase in affinity and selectivity for PBR (i.e, K_i from 285.3 to 0.86 nM). Finally, in contrast with that observed for carboxylic acids **23**−**25**, the presence of the ionizable $-NH_2$ group as in **26** brought about good affinity (i.e., K_i 14.2 nM) and selectivity for PBR.

Overall, all of these findings could be consistent with the pharmacophore mentioned above.[22] While the SAR studies highlighted the main physicochemical factors eliciting the binding of imidazopyridines herein examined to PBR, we further explored the pharmacological profile of these compounds by measuring their modulatory effects on the $GABA_A$ receptors.

Functional in Vitro Study. a. Electrophysiology in *Xenopus* Oocytes. To gain further information on the selectivity of

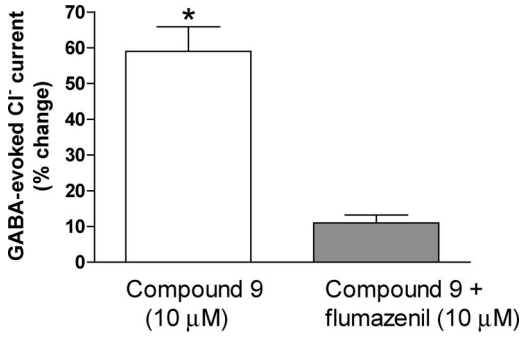


Figure 2. Antagonism by flumazenil of the potentiation of GABA-evoked Cl^- currents by compound **9** at human $\alpha_1\beta_2\gamma_2$ $GABA_A$ receptors expressed in *Xenopus* oocytes. Values are expressed as percentage change of GABA responses induced by compound **9** (10 μM) in the absence and presence of flumazenil (10 μM). Data are means (from four different oocytes) ± SEM.

the different compounds to PBR with respect to CBR, we next examined their capacity to modulate the function of the $GABA_A$ receptor, a pentameric ligand-gated ion channel complex on which the CBR is located. Thus, we applied the voltage-clamp technique to measure the modulatory effects of compounds **6**−**19**, **21**−**25**, **28**, and **29** on GABA-evoked Cl^- currents in oocytes expressing human $\alpha_1\beta_2\gamma_2$ receptors. The different compounds were tested at the concentration of 1 and 10 μM in comparison with compound **2**. Inspection of the data reported in Table 2 shows that the whole set of compounds examined, although most endowed with high affinity and selectivity for PBR did not produce similar modulatory effects on $GABA_A$ receptors. In particular, compounds **6**−**8**, **10**−**12**, **14**−**18**, **19b**, **21**−**25**, and **29** did not significantly modify GABA-evoked Cl^- currents, with a percentage change within ±30% over the control response. The reference compound **2** induced a very weak effect of $GABA_A$ receptor function. In contrast, compounds **9**, **12**, **13**, and **28** markedly enhanced GABA-evoked Cl^- currents, particularly at the concentration of 10 μM. On the other hand, compound **19a** inhibited GABA-evoked Cl^- currents by about 40%. To determine whether the modulatory action of these compounds on GABA-evoked Cl^- currents could be mediated by the central benzodiazepine recognition sites (CBR), we tested the co-application of the specific benzodiazepine receptor antagonist flumazenil ((ethyl 8-fluoro-5-methyl-6-oxo-5,6-dihydro-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylate). At the concentration of 10 μM, flumazenil completely antagonized the potentiation of GABA-evoked Cl^- currents induced by compound **9** (10 μM) (Figure 2), supporting the conclusion that the modulatory effects of the examined compounds occur involving the CBR. The results clearly show that there is no correlation between PBR affinity/selectivity and modulatory activity on $GABA_A$ receptors. Data also revealed that, similarly to what occurs for CBR acting compounds,[23] the transition from active to inactive compounds is consequent to very small structural changes. Thus, replacement of the two *n*-propyl groups on the carboxamide nitrogen of **9** with methyl and *n*-butyl or methyl and phenyl groups to give compounds **10** and **11**, respectively, brought about the change from the potentiation of GABA-evoked Cl^- currents occurring in **9** to the inhibition of such currents observed for **10** and **11**. Similarly, substitution of two *n*-propyl groups on the carboxamide nitrogen of **28** with methyl phenyl ones to give compound **29** led to a decrease of about 40% in potentiation of GABA-evoked Cl^- currents at 10 μM.

b. Electrophysiology in Rat Hippocampal Slices. As mentioned above, the neurosteroid synthesis, occurring in the brain in both glia and neurons, is mediated by the activation of PBR and such steroids act as allosteric modulators of the $GABA_A$ receptors. Our previous work has demonstrated that in vitro application of the PBR selective compound *N,N*-di-*n*-propyl-[2-(4-chlorophenyl)-6,8-dichloroimidazo[1,2-*a*]-pyridin-3-yl)]acetamide (CB34)[15,16] on rat hippocampal slices resulted in an increased tissue concentration of the neurosteroid 3α,5α-TH Prog and in a consequent potentiation of the function of $GABA_A$ receptors in CA1 pyramidal neurons.[24] We thus applied a similar experimental protocol in order to test the ability of these derivatives to modulate $GABA_A$ receptor function and the involvement of neurosteroidogenesis. By using the whole-cell patch clamp technique, we recorded $GABA_A$ receptor-mediated miniature inhibitory postsynaptic currents (mIPSCs) in CA1 pyramidal neurons present in rat hippocampal slices. Perfusion of compound **16** (30 μM) for 30 min, which does not directly modulate $GABA_A$ receptor function when tested in oocytes (Table 2), induced a significant increase in both amplitude and decay time of mIPSCs (Figure 3). To test whether such effects were mediated by an increased local secretion of neurosteroids, we co-applied finasteride (4a*R*,4b*S*,6a*S*,7*S*,9a*S*,9b*S*,11a*R*)-*N*-(*tert*-butyl)-4a,6a-dimethyl-2-oxo-2,4a,4b,5,6,6a,7,8,9,9a,9b,10,11,11a-tetradecahydro-1*H*-indeno[5,4-*f*]quinoline-7-carboxamide) (1 μM), an inhibitor of 5α-reductase, together with compound **16**. Finasteride alone did not alter the kinetic characteristics of $GABA_A$ receptor-mediated mIPSCs but abolished the increase in both amplitude and decay time of these currents induced by compound **16**.

Conclusions

New potent and selective PBR ligands mainly characterized by hydrophilic substituents at 2- and 8-positions of the 2-phenylimidazopyridine nucleus have been synthesized. Biological evaluation of these compounds revealed that, in sharp contrast to that previously observed with the substitution on the amide nitrogen, introduction of polar and ionisable substituents on the *para* and *meta* positions of the phenyl ring leads to compounds with high affinity and selectivity for PBR even at subnanomolar level and greater than that of the reference compound **2**. SAR analysis indicated that the hydrogen bonding acceptor and/or donor ability of the substituents in the *para*/*meta* positions of the phenyl ring together with their lipophilic character are the properties mainly responsible for modulation of affinity. In addition, bulkiness of the substituents in *para*/*meta* positions seems to be detrimental to the affinities for PBR.

Introduction of hydrophilic substituents such as amido-ester, amido-amine, and amido-acid groups at the 8-position of the 2-phenylimidazopyridine skeleton leads to compounds endowed with variable affinity and selectivity ranging from high (i.e., amino, amido-ester-, and amido-amine-derivatives) to moderate (i.e., amido-acid-derivatives) affinity and selectivity.

In electrophysiological studies, while most of the tested derivatives were devoid of any significant modulatory efficacy on *Xenopus* oocytes expressing $\alpha_1\beta_2\gamma_2$ $GABA_A$ receptors, compounds **9**, **12**, **13**, and **28** markedly enhanced GABA-evoked Cl^- currents, particularly at the concentration of 10 μM. The capability of flumazenil to reduce the stimulatory effect exerted by compound **9** supports the conclusion that the modulatory effects of the examined compounds occur involving the CBR. This peculiar property found in these compounds may likely represent a residual characteristic of imidazopyridines such as compound **4**.[13] As for the possible reasons for the difference between the negative results from CBR binding studies (low affinity) and voltage-clamp experiments (modulation of GABA-

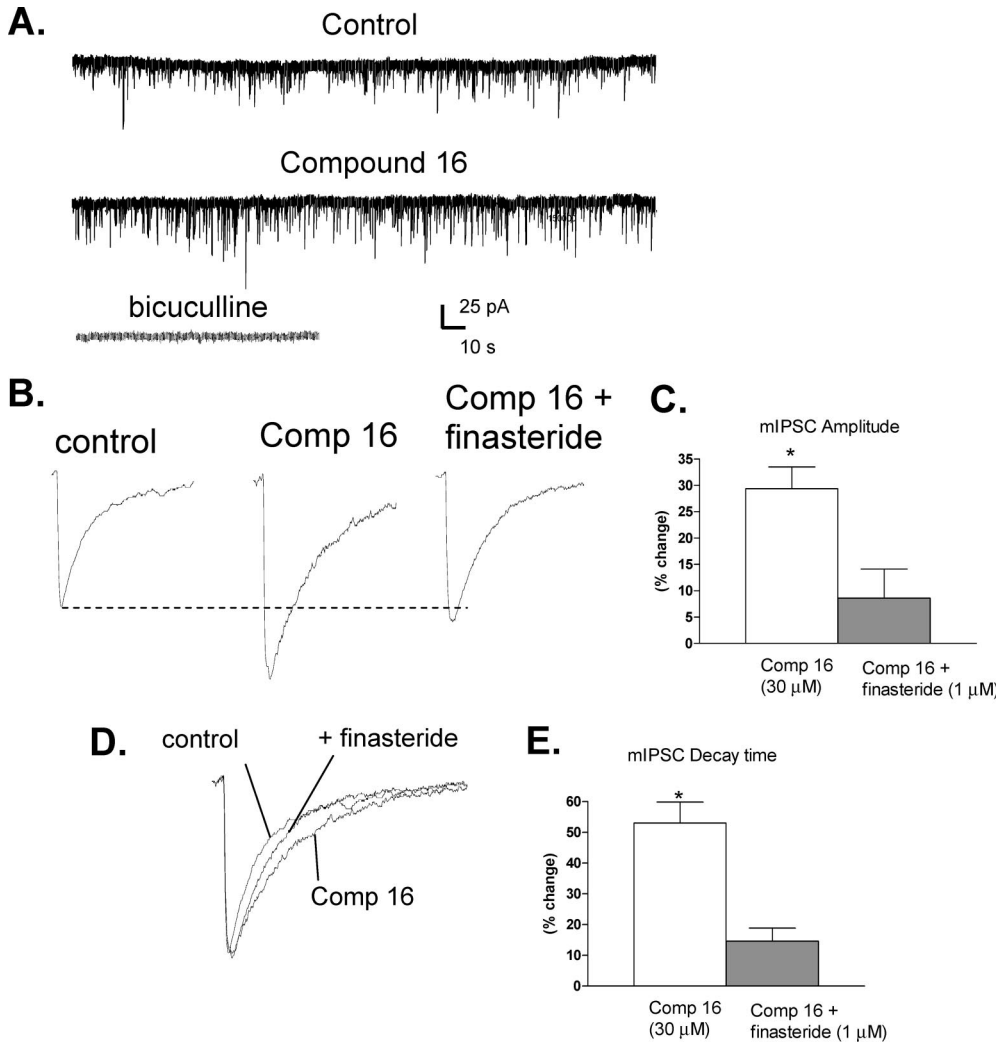


Figure 3. Compound **16** increases $GABA_A$ receptor-mediated mIPSCs in rat CA1 pyramidal neurons in a neurosteroid-dependent manner. (A) Sample traces of mIPSCs recorded before (control), after 30 min of continuous bath application of rat hippocampal slices with compound **16** (30 μM), or in the presence of the $GABA_A$ receptor antagonist bicuculline (20 μM). (B) Averaged traces of multiple mIPSCs recorded during a 3 min period before (control), after 30 min of application of compound **16**, and after 30 min of co-application of compound **16** and finasteride (1 μM). (C) Values represent the mean percent change ± SEM ($n = 6$) in mIPSC amplitude induced by compound **16** with or without the application of finasteride. (D) Averaged traces of multiple mIPSCs showing the increase in decay time induced by compound **16** and the reversal of this effect by finasteride. (E) Values represent the mean percent change ± SEM ($n = 6$) in mIPSC decay time constant induced by compound **16** with or without the application of finasteride. *$p < 0.05$ vs control.

evoked Cl^- currents) of compounds **9**, **12**, **13**, and **28**, it should be considered that, firstly, the concentration of 10 μM, effective in the functional assay, might be still compatible with an estimated affinity for CBR in the high micromolar range, and secondly, these compounds, as other imidazopyridines such as zolpidem, may be endowed with selectivity for $\alpha_1\beta_2\gamma_2$ receptors. In the binding studies, the membrane preparation that was used contained a more heterogeneous population of $GABA_A$ receptors and this may result in a reduced binding inhibition compared to pure $\alpha_1\beta_2\gamma_2$ receptors if there is lower affinity to other receptor subtypes. In this respect, it would be also very interesting to further explore whether the effects of this group of compounds, as that of imidazopyridines, are influenced by the subunit composition of the GABA-A receptor.

However, the results clearly show that there is no correlation between PBR affinity/selectivity and modulatory effects on the function of recombinant $\alpha_1\beta_2\gamma_2$ $GABA_A$ receptors. Data also reveal that the transition from active to inactive compounds is consequent to very small structural changes.

In the hippocampal slice model, the ability of compound **16** to increase $GABA_A$ receptor-mediated mIPSCs in CA1 pyramidal neurons is indicative of its ability to stimulate, through binding to PBR, local synthesis and secretion of neurosteroids such as 3α,5α-TH Prog.[24] In fact, the co-application of finasteride, by inhibiting the formation of neurosteroids from progesterone, prevents this effect. Taken together, these results indicate that the modulatory effects on the function of $GABA_A$ receptors might be due to release of neurosteroids via the activation of 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 IR Fourier transform spectrophotometer in KBr pellets or in NaCl disks. ^{1}H NMR spectra were determined on a Varian Mercury 300 MHz instrument. Chemical shifts are given in δ values referenced to the solvent. Mass spectra were recorded on a Hewlett-Packard 5995 GC-MS low-resolution spectrometer or were obtained using an Agilent 1100 LC-MSD trap system VL instrument using methanol/ammonium formiate 7 mM 9/1 (v/v). Elemental analyses were carried out with a Carlo Erba model 1106 analyzer, and results were within ±0.40% of theoretical values. Silica gel 60 (Merck 70-230 or 230-400 mesh) was used

for column chromatography. All the following reactions were performed under a nitrogen atmosphere, and the progress of the reactions was monitored by thin-layer chromatography (TLC) on using Kieselgel 60 F254 (Merck) plates.

Materials. 4-(4-Nitrophenyl)-4-oxobutanoic acid (**30b**) and 4-(4-chloro-3-nitrophenyl)-4-oxobutanoic acid (**30c**) were prepared following a literature procedure.[20] The general procedure for preparation of 2-phenylimidazo[1,2-*a*]pyridine 3-acetic acids (**35a**, **35c**−**d**) was accomplished following a reported procedure.[8] Compound [6,8-dichloro-2-(1,3-thiazol-2-yl)imidazo[1,2-*a*]pyridin-3-yl]acetic acid **46** was synthesized using a previously reported method.[25] The starting 2-amino-pyridine compounds (**33**), *N,N′*-dialkylamines, DCC, CDI, EEDQ, DMAP, 3-(4-methoxybenzoyl)-propionic acid, hydrobromic acid 48%, TEA, 2,2-dimethyl-1,3-dioxane-4,6-dione (Meldrum's acid), succinic anhydride, glutaric anhydride, methyl 4-chloro-4-oxobutyrate, Boc-Gly-OH, and anhydrous THF were purchased from Sigma-Aldrich (Italy). Commercial reagent grade chemicals and solvents were used without further purification.

General Procedure for Preparation of (2-Phenyl-imidazo[1,2-*a*]pyridine-3-yl)acetamides 9−11, 14, 16−18. To a stirred solution of the suitable 2-amino-pyridine **33** (13 mmol) in *n*-BuOH (50 mL) the butyl-bromoketo ester **32a**−**d** (10 mmol) was added and the resulting mixture was refluxed for 48 h. The reaction was monitored by TLC and, when it was completed, the solvent was evaporated under reduced pressure. The residue was dissolved in $CHCl_3$ (20 mL), washed with 0.1N HCl, and dried (Na_2SO_4). Evaporation of the solvent gave a residue that was purified by silica gel chromatography (light petroleum ether/ ethyl acetate 8/2 (v/v) as eluent) to give the desired butyl (2-phenyl-imidazo[1,2-*a*]pyridin-3-yl)acetates **34a**−**d**. Next, to a solution of **34** (5 mmol) in *n*-butanol (20 mL) NaOH 1 N (2 mL) was added dropwise. The mixture was stirred at room temperature for 12 h. Then, the solvent was evaporated under reduced pressure and the residue was taken up with water (25 mL) and extracted with $CHCl_3$ (3 × 20 mL). The cooled water phase was acidified to pH 4 with 0.1 N HCl, and the resulting precipitate of the pure acid was collected in good yield by filtration and dried under vacuum. A solution of the prepared acid **35a**−**d** (1 mmol) and CDI (1.3 mmol) in anhydrous THF or DMF (20 mL) was stirred at room temperature. Then, the suitable amine **33** (1.3 mmol) and TEA (1.5 mmol) were added and the mixture was stirred for 8−12 h. Solvent was removed under reduced pressure and the residue was taken up 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 6/4(v/v) as eluent)) to give the required compounds **9**−**11**, **14**, and **16**−**18**.

2-(6,8-Dichloro-2-(4-methoxyphenyl)imidazo[1,2-*a*]pyridin-3-yl)-*N*,*N*-dipropylacetamide (9). IR (KBr): 1628 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.71 (t, J = 7.4 Hz, 3H, CH_3), 0.85 (t, J = 7.4 Hz, 3H, CH_3), 1.4−1.6 (m, 4H, CH_2), 3.07 (t, J = 7.4 Hz, 2H, CH_2N), 3.28 (t, J = 7.4 Hz, 2H, CH_2N), 3.85 (s, 3H, CH_3O), 4.06 (s, 2H, CH_2CO), 7.00 (d, J = 8.5 Hz, 2H, Ar), 7.29 (s, 1H, Ar), 7.58 (d, J = 8.5 Hz, 2H, Ar), 8.31 (s, 1H, Ar). MS *m/z* 433 (M^+, 15), 305 (base). Anal. ($C_{22}H_{25}Cl_2N_3O_2$) C, H, N.

***N*-Butyl-2-(6,8-dichloro-2-(4-methoxyphenyl)imidazo[1,2-*a*]pyridin-3-yl)-*N*-methylacetamide (10).** IR (KBr): 1624 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.7−0.9 (m, 3H, CH_3), 1.0−1.6 (m, 4H, CH_2), 2.89 (s, 1.5H, CH_3N), 2.92 (s, 1.5H, CH_3N), 3.13 (t, J = 7.4 Hz, 1H, CH_2N), 3.38 (t, J = 7.4 Hz, 1H, CH_2N), 3.85 (m, 3H, CH_3O), 4.04 (s, 1H, CH_2CO), 4.07 (s, 1H, CH_2CO), 6.99 (d, J = 8.0 Hz, 2H, Ar), 7.2−7.4 (m, 1H, Ar), 7.5−7.7 (m, 2H, Ar), 8.2−8.4 (m, 1H, Ar). MS *m/z* 419 (M^+, 16), 305 (base). Anal. ($C_{21}H_{23}Cl_2N_3O_2$) C, H, N.

2-(6,8-Dichloro-2-(4-methoxyphenyl)imidazo[1,2-*a*]pyridin-3-yl)-*N*-methyl-*N*-phenylacetamide (11). IR (KBr): 1651 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 3.31 (s, 3H, CH_3N), 3.84 (s, 3H, CH_3O), 3.85 (s, 2H, CH_2CO), 6.90 (d, J = 8.5 Hz, 2H, Ar), 7.17 (d, J = 6.8 Hz, 1H, Ar), 7.2−7.4 (m, 5H, Ar), 7.51 (d, J = 8.5 Hz, 2H, Ar), 8.24 (m, 1H, Ar), 9.70 (bs 1H, OH). MS *m/z* 439 (M^+, 19), 305 (base). Anal. ($C_{23}H_{19}Cl_2N_3O_2$) C, H, N.

2-(6,8-Dichloro-2-(4-nitrophenyl)imidazo[1,2-*a*]pyridin-3-yl)-*N*,*N*-dipropylacetamide (14). IR (KBr): 1644, 1523, 1342 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.8−1.0 (m, 6H, CH_3), 1.5−1.7 (m, 4H, CH_2), 3.22 (t, J = 7.7 Hz, 2H, CH_2N), 3.34 (t, J = 7.7 Hz, 2H, CH_2N), 4.07 (s, 2H, CH_2CO), 7.34 (d, J = 1.6 Hz, 1H, Ar), 7.85 (d, J = 8.8 Hz, 2H, Ar), 8.12 (d, J = 1.6 Hz, 1H, Ar), 8.31 (d, J = 8.8 Hz, 2H, Ar). MS *m/z* 448 (M^+, 10), 320 (28). Anal. ($C_{21}H_{22}Cl_2N_4O_3$) C, H, N.

2-(6,8-Dichloro-2-(4-chloro-3-nitrophenyl)imidazo[1,2-*a*]pyridin-3-yl)-*N*,*N*-dipropylacetamide (16). IR (KBr): 1645, 1540, 1363 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.8-1.0 (m, 6H, CH_3), 1.5−1.7 (m, 4H, CH_2), 3.2−3.4 (m, 4H, CH_2N), 4.05 (s, 2H, CH_2CO), 7.34 (m, 1H, Ar), 7.64 (d, J = 8.5 Hz, 1H, Ar), 7.8−8.0 (m, 2H, Ar), 8.0−8.2 (m, 2H, Ar). MS *m/z* 452 (M^+, 27), 324 (17). Anal. ($C_{21}H_{21}Cl_3N_4O_3$) C, H, N.

***N*-Butyl-2-(6,8-dichloro-2-(4-chloro-3-nitrophenyl)imidazo[1,2-*a*]pyridin-3-yl)-*N*-methylacetamide (17).** IR (KBr): 1651, 1536, 1357 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.9−1.0 (m, 3H, CH_3), 1.2−1.4 (m, 2H, CH_2), 1.5−1.7 (m, 2H, CH_2), 2.99 (s, 1.5H, CH_3N), 3.11 (s, 1.5H, CH_3N), 3.34 (t, J = 7.7 Hz, 1H, CH_2N), 3.44 (t, J = 7.7 Hz, 1H, CH_2N), 4.04 (s, 1H, CH_2CO), 4.06 (s, 1H, CH_2CO), 7.34 (s, 1H, Ar), 7.63 (d, J = 8.2 Hz, 2H, Ar), 7.8-8.0 (m, 1H, Ar), 8.0-8.2 (m, 1H, Ar). MS *m/z* 468 (M^+, 14), 356 (15);. Anal. ($C_{20}H_{19}Cl_3N_4O_3$) C, H, N.

***N*-Butyl-2-(8-chloro-2-(4-chloro-3-nitrophenyl)imidazo[1,2-*a*]pyridin-3-yl)-*N*-methylacetamide (18).** IR (KBr): 1623 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.9-1.0 (m, 3H, CH_3), 1.2−1.4 (m, 2H, CH_2), 1.5−1.7 (m, 2H, CH_2), 2.92 (s, 1.5H, CH_3N), 2.98 (s, 1.5H, CH_3N), 3.2-3.5 (m, 2H, CH_2N), 4.08 (s, 1H, CH_2CO), 4.10 (s, 1H, CH_2CO), 6.8-7.0 (m, 2H, Ar), 7.3−7.7 (m, 3H, Ar), 7.9−8.2 (m, 1H, Ar). MS (ESI) *m/z* 432.9 $[M - H]^-$. Anal. ($C_{20}H_{20}Cl_2N_4O_3$) C, H, N.

General Procedure for Conversion of 14 and 16−18 to Compounds 15a, 15b, 19a, 19b, 20, and 21. To a stirred solution of the nitro compounds **14** and **16**−**18** (0.67 mmol) in ethanol (50 mL) and conc HCl (20 mL), granular tin (13.4 mmol) was added. The mixture was refluxed for 2 h and then diluted with 50 mL of water and filtered through a pad of celite. The solvent was evaporated under reduced pressure, and the residue was taken up with 25 mL of 20% $NaHCO_3$, extracted with ethyl acetate (3 × 20 mL), and dried (Na_2SO_4). Evaporation of the solvent gave a residue that was purified by silica gel chromatography (light petroleum ether/ ethyl acetate 1/1 (v/v) as eluent). Compounds **15b** and **19b** were prepared by bubbling gaseous HCl into a solution of the amine **15a** and **19a**, respectively, in anydrous THF for 15 min. The excess of HCl was removed under a stream of nitrogen, and the solvent was evaporated under reduced pressure to give the corresponding hydrochlorides as white solids.

2-(2-(4-Aminophenyl)-6,8-dichloroimidazo[1,2-*a*]pyridin-3-yl)-*N*,*N*-dipropylacetamide (15a). IR (KBr): 3442, 3346, 1634 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.71 (t, J = 7.3 Hz, 3H, CH_3), 0.83 (t, J = 7.3 Hz, 3H, CH_3), 1.4−1.6 (m, 4H, CH_2), 3.26 (t, J = 7.7 Hz, 2H, CH_2N), 3.29 (t, J = 7.7 Hz, 2H, CH_2N), 3.80 (br, 2H, NH_2), 4.08 (s, 2H, CH_2CO), 6.75 (d, J = 8.8 Hz, 2H, Ar), 7.2−7.3 (m, 1H, Ar), 744 (d, J = 8.8 Hz, 2H, Ar), 8.29 (d, J = 1.8 Hz, 1H, Ar).MS *m/z* 418 (M^+, 18), 290 (base). Anal. ($C_{21}H_{24}Cl_2N_4O$) C, H, N.

2-(2-(3-Amino-4-chlorophenyl)-6,8-dichloroimidazo[1,2-*a*]pyridin-3-yl)-*N*,*N*-dipropylacetamide (19a). IR (KBr): 3447, 3354, 1633 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.77 (t, J = 7.4 Hz, 3H, CH_3), 0.85 (t, J = 7.4 Hz, 3H, CH_3), 1.4-1.6 (m, 4H, CH_2), 3.11 (t, J = 7.4 Hz, 2H, CH_2N), 3.28 (t, J = 7.4 Hz, 2H, CH_2N), 4.05 (s, 2H, CH_2CO), 4.14 (br, 2H, NH_2), 6.84 (m, 1H, Ar), 7.15 (m, 1H, Ar), 7.2−7.4 (m, 1H, Ar), 8.25 (m, 1H, Ar). MS *m/z* 452 (M^+, 27), 324 (93). Anal. ($C_{21}H_{23}Cl_3N_4O$) C, H, N.

2-(2-(3-Amino-4-chlorophenyl)-6,8-dichloroimidazo[1,2-*a*]pyridin-3-yl)-*N*-butyl-*N*-methylacetamide (20). IR (KBr): 3340, 3355, 1633 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.9−1.0 (m, 3H, CH_3), 1.2−1.4 (m, 2H, CH_2), 1.5−1.7 (m, 2H, CH_2), 3.01 (s, 1.5 H, CH_3N), 3.11 (s, 1.5 H, CH_3N), 3.34 (t, J = 7.7 Hz, 1H, CH_2N), 3.44 (t, J = 7.7 Hz, 1H, CH_2N), 4.04 (s, 1H, CH_2CO), 4.04 (br, 2H, NH_2), 4.06 (s,

1H, CH_2CO), 6.84 (m, 1H, Ar), 7.15 (m, 1H, Ar), 7.2−7.4 (m, 1H, Ar), 8.25 (m, 1H, Ar). MS *m/z* 440 (M^+, 20), 321 (base). Anal. ($C_{20}H_{21}Cl_3N_4O$) C, H, N.

2-(2-(3-Amino-4-chlorophenyl)-8-chloroimidazo[1,2-*a*]pyridin-3-yl)-*N*-butyl-*N*-methylacetamide (21). IR (KBr): 3451, 3365, 1646 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.9−1.0 (m, 3H, CH_3), 1.2−1.4 (m, 2H, CH_2), 1.5−1.7 (m, 2H, CH_2), 2.85 (s, 1.5H, CH_3N), 2.89 (s, 1.5H, CH_3N), 3.12 (t, $J = 7.7$ Hz, 1H, CH_2N), 3.34 (t, $J = 7.7$ Hz, 1H, CH_2N), 4.08 (s, 1H, CH_2CO), 4.10 (s, 1H, CH_2CO), 4.2 (br, 2H, NH_2), 6.76 (t, $J = 6.9$ Hz, 1H, Ar), 6.8−6.9 (m, 1H, Ar), 7.2−7.4 (m, 3H, Ar), 8.1−8.2 (m, 1H, Ar). (ESI) *m/z* 405 $[M + H]^+$. Anal. ($C_{20}H_{22}Cl_2N_4O$) C, H, N.

Synthesis of the Emisuccinamide 22. A mixture of **19** (1 g, 1.8 mmol) and succinic anhydride (324 mg, 3.2 mmol) was refluxed in toluene (50 mL) overnight. Then the reaction mixture was allowed to cool at room temperature and the resulting precipitate, corresponding to essentially pure acid, was recovered by filtration, washed with toluene, and then dried under vacuum.

4-(2-Chloro-5-(6,8-dichloro-3-(2-(dipropylamino)-2-oxoethyl)imidazo[1,2-*a*]pyridin-2-yl)phenylamino)-4-oxobutanoic acid (22). IR (KBr): 3339, 1686, 1617 cm^{-1}. ^1H NMR (DMSO-d_6) δ: 0.7−0.9 (m, 6H, CH_3), 1.4−1.6 (m, 4H, CH_2), 2.4−2.7 (m, 4H, CH_2CH_2COO), 3.20 (t, $J = 7.4$ Hz, 2H, CH_2N), 3.33 (t, $J = 7.4$ Hz, 2H, CH_2N), 4.26 (s, 2H, CH_2CO), 7.39 (d, $J = 8.5$ Hz, 1H, Ar), 7.55 (d, $J = 8.5$ Hz, 1H, Ar), 7.66 (s, 1H, Ar), 7.97 (s, 1H, Ar), 8.57 (s, 1H, Ar), 9.65 (s, 1H, NH), 12.16 (br, 1H, COOH). MS (ESI) *m/z* 551.2 $[M - H]^-$. Anal. ($C_{25}H_{27}Cl_3N_4O_4$) C, H, N.

General Procedure for Preparation of (2-Phenyl-imidazo[1,2-*a*]pyridine-3-yl)acetamides 6−8. A stirred solution of compound **35a** (1g, 2.8 mmol) in conc HBr (50 mL) was refluxed for 4 h. The reaction mixture was then cooled and poured in water (100 mL). The resulting precipitate **36a** was collected by filtration, washed several times with water, and dried under vacuum. Next a solution of the acid **36a** (500 mg, 1.49 mmol) and CDI (313 mg, 1.93 mmol) in anhydrous THF (25 mL) was stirred at room temperature. After 30 min, the suitable dialkylamine (1.9 mmol) and TEA (2.2 mmol) were added and the stirring was prolonged for 12 h. Then the reaction mixture was taken up with water, extracted with $CHCl_3$, 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 1/1 (v/v) as eluent) to give the desired compounds.

2-(6,8-Dichloro-2-(4-hydroxyphenyl)imidazo[1,2-*a*]pyridin-3-yl)-*N*,*N*-dipropylacetamide (6). IR (KBr): 1638, cm^{-1}. ^1H-NMR (DMSO-d_6) δ: 0.7−0.9 (m, 6H, CH_3), 1.4−1.7 (m, 4H, CH_2), 3.2−3.3 (m, 4H, CH_2NCO), 4.20 (s, 2H, CH_2CON), 6.83 (d, $J =$ 8.5 Hz, 2H, Ar), 7.41(d, $J = 8.5$ Hz, 2H, Ar), 7.60 (d, $J = 1.6$ Hz, 1H, Ar), 8.55 (d, $J = 1.6$ Hz, 1H, Ar), 9.65 (s, 1H, OH). MS (ESI) *m/z* 420.2 $[M + H]^+$. Anal. ($C_{21}H_{23}Cl_2N_3O_2$) C, H, N.

2-(6,8-Dichloro-2-(4-hydroxyphenyl)imidazo[1,2-*a*]pyridin-3-yl)-*N*-buthyl-*N*-methylacetamide (7). IR (KBr): 1631 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.7−1.0 (m, 3H, CH_3), 1.0−1.6 (m, 4H, CH_2), 2.89 (s, 1.5H, CH_3N), 2.92 (s, 1.5H, CH_3N), 3.14 (t, $J = 7.4$ Hz, 1H, CH_2N), 3.38 (t, $J = 7.4$ Hz, 1H, CH_2N), 4.01 (s, 1H, CH_2CO), 4.04 (s, 1H, CH_2CO), 6.7−6.9 (m, 2H, Ar), 7.3−7.5 (m, 3H, Ar), 8.2−8.4 (m, 1H, Ar), 9.69 (br 1H, OH). MS *m/z* 405 (M^+, 2), 291 (base). Anal. ($C_{20}H_{21}Cl_2N_3O_2$) C, H, N.

2-(6,8-Dichloro-2-(4-hydroxyphenyl)imidazo[1,2-*a*]pyridin-3-yl)-*N*-methyl-*N*-phenylacetamide (8). IR (KBr): 1648 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 3.31 (s, 3H, CH_3N), 3.83 (s, 2H, CH_2CO), 6.76 (d, $J =$ 8.5 Hz, 2H, Ar), 7.17 (d, $J = 6.9$ Hz, 2H, Ar), 7.2−7.5 (m, 6H, Ar), 8.23 (d, $J = 1.4$ Hz, 1H, Ar), 9.65 (br 1H, OH). MS (ESI) *m/z* 425.9 $[M + H]^+$. Anal. ($C_{22}H_{17}Cl_2N_3O_2$) C, H, N.

Preparation of (2-Phenyl-imidazo[1,2-*a*]pyridine-3-yl)acetamides 12, 13. Compound **12** was prepared as follows: A solution of Boc-Gly-OH (248 mg, 1.42 mmol) and DCC (177 mg, 0.85 mmol) in anhydrous THF (25 mL) was stirred for 30 min at 0 °C by using an ice bath. After this time, the compound **6** (300 mg, 0.71 mmol) and TEA (0.15 mL, 1.06 mmol) were added and the mixture was stirred overnight at room temperature. Solvent was evaporated under reduced pressure, and the residue was dissolved in $CHCl_3$ (20 mL), washed with 5% $NaHCO_3$, and dried (Na_2SO_4). Solvent was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (CH_2Cl_2/ acetone 9/1 (v/v) as eluent) to give the Boc-protected product **37**. Next, to the stirred and ice-cooled solution of **37** (200 mg, 0.35 mmol) in anhydrous CH_2Cl_2 (20 mL), gaseous HCl was bubbled for 30 min. Evaporation of the solvent under reduced pressure gave the corresponding Boc-deprotected compound **12** as hydrochloride salt in good yield.

4-(6,8-Dichloro-3-(2-(dipropylamino)-2-oxoethyl)imidazo[1,2-*a*]pyridin-2-yl)phenyl 2-Aminoacetate (12). IR (KBr): 3406, 1640, 1629 cm^{-1}. ^1H NMR (D_2O) δ: 0.6-0.8 (m, 6H, CH_3), 1.4−1.6 (m, 4H, CH_2), 3.1−3.3 (m, 4H, CH_2N), 4.15 (s, 2H, CH_2CO), 4.17 (s, 2H, NCH_2COO), 7.29 (d, $J = 8.8$ Hz, 2H, Ar), 7.51 (d, $J = 8.8$ Hz, 2H, Ar), 7.76 (d, $J = 1.6$ Hz, 1H, Ar), 8.31 (d, $J = 1.6$ Hz, 1H, Ar). MS (ESI) *m/z* 477.2 $[M + H]^+$. Anal. ($C_{23}H_{26}Cl_2N_4O_3$) C, H, N.

4-(6,8-Dichloro-3-(2-(dipropylamino)-2-oxoethyl)imidazo[1,2-*a*]pyridin-2-yl)phenyl Methyl Succinate (13). To a stirred solution of **6** (200 mg, 0.48 mmol) in anhydrous THF (20 mL) methyl 4-chloro-4-oxobutyrate (152 μL, 0.95 mmol) was added dropwise at room temperature. Then after 1 h, the solvent was evaporated under reduced pressure and the residue was dissolved in EtOAc (20 mL), washed with 5% $NaHCO_3$, and dried (Na_2SO_4). Evaporation of the solvent gave a residue that was purified by silica gel column chromatography (light petroleum ether/ethyl acetate 1/1 (v/v) as eluent). IR (KBr): 1757, 1744, 1626 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.73 (t, $J = 7.4$ Hz, 3H, CH_3), 0.81 (t, $J = 7.4$ Hz, 3H, CH_3), 1.4−1.6 (m, 4H, CH_2), 2.5−2.9 (m, 4H, $OCOCH_2CH_2COO$), 3.21 (t, $J = 7.1$ Hz, 2H, CH_2N), 3.29 (t, $J = 7.1$ Hz, 2H, CH_2N), 3.98 (s, 3H, CH_3OCO), 4.03 (s, 2H, CH_2CO), 7.16 (d, $J = 8.8$ Hz, 2H, Ar), 7.2-7.3 (m, 1H, Ar), 7.60 (d, $J = 8.8$ Hz, 2H, Ar), 8.19 (d, $J = 1.6$ Hz, 1H, Ar). MS (ESI) *m/z* 534.2 $[M + H]^+$. Anal. ($C_{26}H_{29}Cl_2N_3O_5$) C, H, N.

Preparation of (2-Phenyl-imidazo[1,2-*a*]pyridine-3-yl)acetamides 23−25. A solution of **38** (500 mg, 1.3 mmol) and 2,2-dimethyl-1,3-dioxane-4,6-dione (Meldrum's acid, 281 mg, 1.95 mmol) in anhydrous THF (25 mL) was stirred at room temperature for 48 h. Solvent was evaporated under reduced pressure, and the residue was dissolved in ethyl acetate (20 mL), washed with 0.1 N HCl, and dried (Na_2SO_4). The organic solvent was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography ($CHCl_3$/CH_3OH 9/1 (v/v) as eluent) to give the acid **23**. Compounds **24** and **25** were prepared as follows: A mixture of **38** (1 g, 2.6 mmol) and succinic or glutaric anhydride (5.2 mmol) was refluxed in toluene (50 mL) overnight. Then, the reaction mixture was allowed to cool at room temperature and the resulting precipitate, corresponding to essentially pure acid, was recovered by filtration, washed with toluene, and then dried under vacuum.

3-(2-(4-Chlorophenyl)-3-(2-(dipropylamino)-2-oxoethyl)imidazo[1,2-*a*]pyridin-8-ylamino)-3-oxopropanoic acid (23). IR (KBr): 1722, 1607, 1591 cm^{-1}. ^1H NMR (DMSO-d_6) δ: 0.77−0.85 (m, 6H, CH_3), 1.4−1.6 (m, 4H, CH_2), 3.1−3.5 (m, 6H, CH_2N + $COCH_2COO$), 4.20 (s, 2H, CH_2CO), 6.84 (t, $J = 7.0$ Hz, 1H, Ar), 7.51 (d, $J = 8.4$ Hz, 2H, Ar), 7.69 (d, $J = 8.4$ Hz, 2H, Ar), 7.85 (m, 1H, Ar), 8.08 (m, 1H, Ar), 10.0 (s, 1H, NH), 12.8 (br, 1H, COOH). MS (ESI) *m/z* 469.0 $[M - H]^-$. Anal. ($C_{24}H_{27}ClN_4O_4$) C, H, N.

4-(2-(4-Chlorophenyl)-3-(2-(dipropylamino)-2-oxoethyl)imidazo[1,2-*a*]pyridin-8-ylamino)-4-oxobutanoic Acid (24). IR (KBr): 1720, 1640 cm^{-1}. ^1H NMR (DMSO-d_6) δ: 0.7−0.9 (m, 6H, CH_3), 1.4−1.6 (m, 4H, CH_2), 2.42 (t, $J = 6.6$ Hz, 2H, CH_2CONH), 2.48 (t, $J = 6.6$ Hz, 2H, CH_2COO), 3.20 (t, $J = 7.7$ Hz, 2H, CH_2N), 3.30 (t, $J = 7.7$ Hz, 2H, CH_2N), 4.18 (s, 2H, CH_2CO), 6.8-6.9 (m, 1H, Ar), 7.49 (d, $J = 8.5$ Hz, 2H, Ar), 7.6−8.0 (m, 3H, Ar), 8.0−8.4 (m, 1H, Ar), 9.89 (s, 1H, NH), 12.0 (br, 1H, COOH). MS (ESI) *m/z* 483.2 $[M - H]^-$. Anal. ($C_{25}H_{29}ClN_4O_4$) C, H, N.

5-(2-(4-Chlorophenyl)-3-(2-(dipropylamino)-2-oxoethyl)imidazo[1,2-*a*]pyridin-8-ylamino)-5-oxopentanoic Acid (25). IR (KBr): 3287, 1696, 1645, cm^{-1}. ^1H NMR (DMSO-d_6) δ: 0.7−0.9 (m, 6H, CH_3), 1.4−1.6 (m, 4H, CH_2), 1.8−1.9 (m, 2H, CH_2), 2.41 (t, $J =$ 6.6 Hz, 2H, CH_2CONH), 2.57 (t, $J = 6.6$ Hz, 2H, CH_2COO), 3.20

(t, $J = 7.7$ Hz, 2H, CH_2N), 3.30 (t, $J = 7.7$ Hz, 2H, CH_2N), 4.07 (s, 2H, CH_2CO), 6.8-7.0 (m, 1H, Ar), 7.51 (d, $J = 8.5$ Hz, 2H, Ar), 7.6−8.0 (m, 3H, Ar), 8.0−8.4 (m, 1H, Ar), 9.89 (s, 1H, NH), 12.0 (br, 1H, COOH). MS (ESI) *m/z* 497.2 $[M - H]^-$. Anal. ($C_{26}H_{31}ClN_4O_4$) C, H, N.

Preparation of 2-(8-(2-Aminoacetamido)-2-(4-chlorophenyl)imidazo[1,2-*a*]pyridin-3-yl)-*N*,*N*-dipropylacetamide (26). A solution of Boc-Gly-OH (455 mg, 2.6 mmol) and DCC (400 mg, 1.94 mmol) in anhydrous CH_2Cl_2 (25 mL) was stirred for 30 min at 0 °C by using an ice bath. After this time, the compound **38** (500 mg, 1.3 mmol), DMAP (15 mg, 0.13 mmol), and TEA (0.27 mL, 1.94 mmol) were added and the stirring was prolonged overnight at room temperature. Solvent was evaporated under reduced pressure, and the residue was dissolved in $CHCl_3$ (20 mL), washed with 5% $NaHCO_3$, dried (Na_2SO_4), and evaporated. The residue was purified by silica gel column chromatography (CH_2Cl_2/ethyl acetate 7/3 (v/v) as eluent) to give the Boc-protected product **39**. Next, in a stirred and ice-cooled solution of the compound **39** (200 mg, 0.37 mmol) in anhydrous CH_2Cl_2 (20 mL), gaseous HCl was bubbled for 30 min. Evaporation of the solvent under reduced pressure gave the corresponding Boc-deprotected compound **26** as hydrochloride salt in good yield. IR (KBr): 3421, 1635, cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.6−0.8 (m, 6H, CH_3), 1.4−1.6 (m, 4H, CH_2), 3.15 (t, $J = 6.9$ Hz, 2H, CH_2N), 3.23 (t, $J = 6.9$ Hz, 2H, CH_2N), 4.06 (s, 2H, CH_2CO), 4.23 (s, 2H, NCH_2CO), 7.25 (t, $J = 7.1$ Hz, 1H, Ar), 7.43 (d, $J = 8.5$ Hz, 2H, Ar), 7.49 (d, $J = 8.5$ Hz, 2H, Ar), 7.80 (d, $J = 7.7$ Hz, 1H, Ar), 8.17 (d, $J = 7.7$ Hz, 1H, Ar). MS (ESI) *m/z* 442.2 $[M + H]^+$. Anal. ($C_{23}H_{28}ClN_5O_2$) C, H, N.

Preparation of 4-(2-(4-Chlorophenyl)-3-(2-(dipropylamino)-2-oxoethyl)imidazo[1,2-*a*]pyridin-8-ylamino)-4-oxobutanoate (27). A solution of the acid **24** (400 mg, 0.83 mmol) and concentrated H_2SO_4 (2 mL) in ethanol (20 mL) was stirred at 50 °C for 12 h. Then, the solvent was evaporated under reduced pressure and the residue was taken up with 5% $NaHCO_3$ (25 mL), extracted with EtOAc (3 × 25 mL), and dried (Na_2SO_4). Evaporation of the solvent gave a residue that was purified by silica gel column chromatography ($CHCl_3$/ethyl acetate 95/5 (v/v) as eluent). IR (KBr): 1733, 1698, 1639 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.68 (t, $J = 7.4$ Hz, 3H, CH_3), 0.73 (t, $J = 7.4$ Hz, 3H, CH_3), 1.26 (t, $J = 7.4$ Hz, 3H, CH_3), 1.4−1.6 (m, 4H, CH_2), 2.7−2.9 (m, 4H, $OCOCH_2CH_2CON$), 3.10 (t, $J = 7.4$ Hz, 2H, CH_2N), 3.27 (t, $J = 7.4$ Hz, 2H, CH_2N), 4.06 (s, 2H, CH_2CO), 4.15 (q, $J = 7.4$ Hz, 2H, CH_2OCO), 6.8−7.0 (m, 1H, Ar), 7.49 (d, $J = 8.5$ Hz, 2H, Ar), 7.62 (d, $J = 8.5$ Hz, 2H, Ar), 7.7−7.8 (m, 1H, Ar), 7.9−8.0 (m, 1H, Ar), 8.80 (s, 1H, NHCO). MS (ESI) *m/z* 535.2 $[M + Na]^+$. Anal. ($C_{27}H_{33}ClN_4O_4$) C, H, N.

Preparation of (2-Thiazolyl-imidazo[1,2-*a*]pyridine-3-yl)acetamide 29. Compound **29** was prepared following the previously reported method for **28**.[25]

2-(6,8-Dichloro-2-(thiazol-2-yl)imidazo[1,2-*a*]pyridin-3-yl)-*N*-methyl-*N*-phenylacetamide (29). To a stirred solution of **46** (0.25 g, 0.76 mmol) in anhydrous THF (20 mL) at room temperature were added in the order EEDQ (0.28 g, 1.14 mmol), the appropriate dialkylamine (0.92 mmol), and, after 10 min, TEA dropwise (0.16 mL, 1.14 mmol). Stirring was continued for additional 24 h at room temperature and then the solvent was evaporated under reduced pressure. The resulting residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate 7:3 v/v as eluent). IR (KBr):1650 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 3.28 (s, 3H, CH_3N), 4.44 (s, 2H, $-CH_2CON$), 7.2−7.5 (m, 7H, Ar), 7.68 (d, $J = 3.0$ Hz, 1H, Ar), 8.28 (d, $J = 1.6$ Hz, 1H, Ar). MS (ESI): *m/z* 417.0 $[M + H]^+$. Anal. ($C_{19}H_{14}Cl_2N_4OS$) C, H, N.

Biological Methods. Materials. Male Sprague−Dawley CD rats (Charles River, Como, Italy) at 6−8 weeks of age were used for binding experiments. They were housed for at least 7 days before experiments and 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 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).

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) buffer, and the homogenate was centrifuged twice at 20000*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 (0.4−0.5 mg of protein), 400 μL of Tris-HCl buffer, 100 μL of [^{3}H]Flunitrazepam (74 Ci/mmol; New England Nuclear, final concentration 0.5 nM), 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 quantified 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-]-2 Binding. After killing, the brain was rapidly removed from rats, the cerebral cortex was dissected, and all tissue 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 40000*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]-**2** 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]-**2** (85.5 Ci/mmol, New England Nuclear; final assay concentration 1 nM), 350 μL of PBS buffer, and 50 μL of drug solution or solvent. Incubations were initiated by the addition of membranes and were terminated 90 min later by rapid filtration through glass-fiber filter strips (Whatman 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 unlabelled **2** (Sigma).

Functional in Vitro Studies. a. Electrophysiological Recordings Using *Xenopus* Oocytes. Complementary DNAs encoding the human α_1, β_2, and γ_{2L} $GABA_A$ 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 distilled water, divided into portions, and stored at −20°C until used for injection. Stage V and VI oocytes were manually isolated form sections of *Xenopus laevis* ovary, placed in MBS containing 66 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 α_1, β_2, and γ_{2L} 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 micropipette (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 membrane 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 (7−10 s were required to reach equilibrium in the recording chamber). Maximal percentage of dimethyl sufoxide in the final dilution of the compounds was 0.1%, which, alone, did not influence GABA-evoked Cl^- currents. Intervals of 5−10 min were allowed between drug applications. Modulation of GABA-evoked Cl^- current amplitude by drugs is expressed as percentage change, $[(I'/I) - 1]\cdot 100\%]$, where I is the average of control amplitude obtained before drug application and after drug washout and I' is the average of the agonist-induced current amplitude obtained in the same cell in the presence of drug.

A number of 5−10 different oocytes were used for testing each of the two concentrations of the different compounds, and oocytes were obtained from different frog donors.

b. Patch Clamp Recording in Rat Hippocampal Slices. Male Sprague−Dawley rats were anesthetized by intraperitoneal injection of ketamine (250 mg per kilogram of body mass), and the brain was rapidly removed into ice-cold cutting solution (220 mM sucrose, 2 mM KCl, 1.3 mM NaH_2PO_4, 12 mM $MgSO_4$, 0.2 mM $CaCl_2$, 10 mM glucose, 2.6 mM $NaHCO_3$ (pH 7.3) equilibrated with 95% O_2 and 5% CO_2). Coronal slices (thickness, 300 μm) of the hippocampus were cut with a Vibratome 1000 plus (Vibratome, St. Louis, MO) and then ACSF containing 126 mM NaCl, 3 mM KCl, 1.25 mM NaH_2PO_4, 1 mM $MgSO_4$, 2 mM $CaCl_2$, 10 mM glucose, and 26 mM $NaHCO_3$ (pH 7.3), equilibrated with 95% O_2 and 5% CO_2, first for 40 min at 34°C and then for 30 min at room temperature before beginning experiments.

Tissue slices were transferred to a chamber perfused with ACSF at a rate of ∼2 mL/min at room temperature. Whole-cell patch clamp electrophysiological recordings from CA1 pyramidal neurons were performed with an Axopatch 200-B amplifier (Axon Instruments, Union City, CA) and an infrared-differential interference contrast microscope. Patch microelectrodes (borosilicate capillaries with a filament; outer diameter, 1.5 μm) (Sutter Instruments, Novato, CA) were prepared with a two-step vertical puller (Sutter Instruments) and had a resistance of 4−to 6 MΩ. mIPSCs were recorded at a holding potential of −65 mV with an internal solution containing 140 mM CsCl, 2 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM EGTA, 10 mM Hepes-CsOH (pH 7.3), 2 mM adenosine triphosphate (disodium salt), and 5 mM QX-314 (lidocaine *N*-ethyl bromide). Access resistance varied between 20 and 40 MΩ; if it changed by >20% during an experiment, the recording was discarded. Currents through the patch clamp amplifier were filtered at 2 kHz and digitized at 5.5 kHz with commercial software (pClamp 8.2; Axon Instruments). The external solution (ACSF) contained 500 μM lidocaine and 3 mM kynurenic acid. The mIPSCs were analyzed with Mini Analysis 5.4.17 software (Synaptosoft, Decatur, GA). Each event identified was confirmed by visual inspection for each experiment. We evaluated the effects of the various drugs on the amplitude and decay time of mIPSCs in individual neurons by cumulative probability analysis, with statistical significance determined with the Kolmogorov−Smirnov nonparametric two-sample test.

Statistical Analysis. Statistical comparisons of pooled data were performed by one-way analysis of variance followed by Scheffe's post hoc test. In all cases, a P value of < 0.05 was considered statistically significant.

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Supporting Information Available: Table of microanalytical data for compounds **6**−**27**, **29**, and physicochemical and spectral data for compounds **31a**,**c**, **32a**,**c**, **34a**,**c**, **35a**,**b**,**c**, **36a**, **37**, and **39**. This material is available free of charge via the Internet at http://pubs.acs.org.

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