Synthesis and Binding Affinity of 2-Phenylimidazo[1,2-*a*]pyridine Derivatives for both Central and Peripheral Benzodiazepine Receptors. A New Series of High-Affinity and Selective Ligands for the Peripheral Type

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A number of 6-substituted or 6,8-disubstituted alkyl 2-phenylimidazo[1,2-*a*]pyridine-3-carboxylates **5a**−**h**, -acetates **5i**−**s**, **6a**−**g**, and -propionates **5t**, **6h** and of *N*,*N*-dialkyl-2-phenylimidazo[1,2-*a*]pyridine-3-carboxamides **7a**−**d**, -acetamides **7e**−**t** or -propionamide **7u** were prepared following new synthetic methods, and their affinities for both the central (CBR) and the peripheral (PBR) benzodiazepine receptors evaluated. The compounds of the ester series displayed low affinity for both receptor types. Conversely, most of *N*,*N*-dialkyl(2-phenylimidazo[1,2-*a*]pyridin-3-yl)acetamides **7e**−**t** proved to possess high affinity and selectivity for CBR or PBR depending on the nature of substituents at C(6)- and/or C(8) on the heterocyclic ring system. In particular, the 6-substituted compounds **7f**−**n** displayed ratios of IC_{50} values (IC_{50}(CBR)/IC_{50}(PBR)) ranging from 0.32 (**7m**) to 232 (**7k**), while the 6,8-disubstituted compounds **7o**−**t** were more than 1000-fold more selective for PBR versus CBR. Compounds **7f,m** were examined in several different benzodiazepine receptor subtypes. Expression of specific $GABA_A$ receptor subunit assemblies in *Xenopus* oocytes was utilized to evaluate functionally both the efficacy and potency of the positive modulation of GABA-evoked Cl^- currents by **7f** and **7m** in comparison with Zolpidem. The rank order of potencies of these drugs was **7f** ($EC_{50} = 3.2 \times 10^{-8}$ M) > Zolpidem ($EC_{50} = 3.6 \times 10^{-8}$ M) > **7m** ($EC_{50} = 2.2 \times 10^{-7}$ M). The actions of these compounds were also tested on $\alpha_2\beta_2\gamma_{2s}$ receptors. However, the EC_{50} of these compounds was increased, compared to $\alpha_1\beta_2\gamma_{2s}$ receptors, by 30-, 4-, and 5-fold for **7m**, **7f**, and Zolpidem, respectively. Finally, these compounds were almost completely devoid of activity at receptors containing the α_5 subunit.

Introduction

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system of vertebrates. Three types of GABA receptors, denoted $GABA_A$, $GABA_B$, and $GABA_C$, have so far been characterized. The most abundant $GABA_A$ receptors are ligand-gated chloride ion channels and are characterized by the presence of several allosteric modulatory sites that regulate GABA affinity.[1,2] These sites include distinct ones for barbiturates, benzodiazepines (BZs), neurosteroids, and ethanol. Molecular biological studies have demonstrated that several receptor subunits (α_1−α_6, β_1−β_3,γ_1−γ_3, δ) combine to form the $GABA_A$ receptor complex.[3] Of the chemical classes which have binding sites on this macromolecular ionophore, the benzodiazepines are the most widely studied. Although the exact nature of the BZ/chloride ionophore receptor complex remains to be established, expression of α, β, and γ subunits results in a channel assembly that favor ligands of the BZ receptor (BZR) complex. Using the classical BZ1/BZ2 nomenclature,[4,5] the BZ1 receptors are probably formed by the combination of subunits $\alpha_1\beta_2\gamma_2$, whereas a mixture of subunits α_2-, α_3-, and $\alpha_5\beta_2\gamma_2$ represents the BZ2 receptors.[6,7] The third type, namely the BZ3 receptors, constitute the "peripheral" receptors since they have been identified in the brain as well as in a wide range of peripheral tissues; their subcellular location has been reported to be mainly mitochondrial,[8−10] and hence, this receptor is also termed "mitochondrial benzodiazepine receptor".[11] Although the pharmacological role of the BZ3 receptors remains to be fully clarified, some evidence indicates their involvement in important cellular functions such as the production of neurosteroids.[11] In fact, it has been shown that appropriate agonists for this receptor stimulate the cellular synthesis of steroid hormones such as pregnenolone, dehydroepiandrosterone, and others.

In this context, it is clear that improved understanding of the $GABA_A$/BZR complex and subtypes of its components may lead to more selective drugs with improved activity and/or fewer side effects for the treatment of anxiety, sleep disorders, convulsions, and memory deficits. An important goal in the $GABA_A$-receptor research remains the full determination of the distribution and physiological roles of each of these subtypes. Hence, increasingly selective high-affinity agents should be very important as tools for these studies.

Among the known ligands, the *N*,*N*-dialkyl-2-phenylacetamidoimidazo[1,2-*a*]pyridines **1** (Alpidem) and **2** (Zolpidem) (Chart 1) showed both high affinity and selectivity toward non-BZ2 receptors.[12] Thus, Alpidem has high affinity for BZ1 and BZ3 sites while Zolpidem possesses high affinity for BZ1 but neither for BZ2 nor

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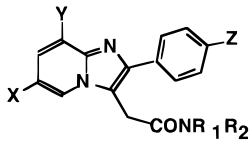
Chart 1



1 Alpidem X = Z = Cl ; Y = H ; $R_1 = R_2 = C_3H_7$

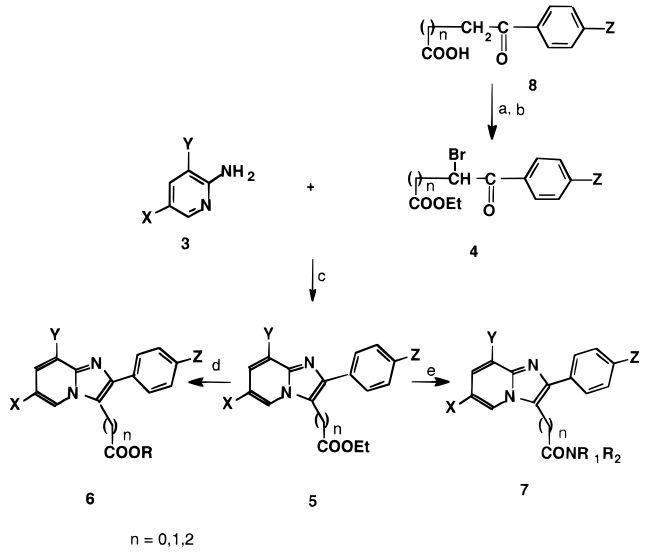
2 Zolpidem X = Z = CH_3; Y = H; $R_1 = R_2 = CH_3$

peripheral sites. To the best of our knowledge, extensive SAR studies on acetamidoimidazo[1,2-*a*]pyridines were confined mainly to evaluating the influence of chlorine, or of the methyl or hydroxymethyl groups at the 6-position of the heterocyclic nucleus for a number of derivatives substituted or unsubstituted on the phenyl ring at C(2).[13] In our search for new non-benzodiazepine BZR ligands, we designed and synthesized analogs of **1** and **2** with the aim of improving the affinity and selectivity for BZR (sub)types. Our synthetic plan was to modify the acetamide moiety and the substituents of **1** and **2** without changing the 2-phenylimidazo[1,2-*a*]-pyridine heterocyclic ring system. In particular, we investigated whether exchanging the acetamide moiety with the acetate group could lead to compounds with improved binding properties, considering that, for most classes of BZ receptor ligands, compounds characterized by the presence of an ester group usually displayed higher affinity than the corresponding amide derivatives.[14] Furthermore, an examination of the importance of the carbon chain length between the heterocyclic nucleus and the amide function, as well as an exploration of the role of substituents at 6- and 6,8-positions of the heterocyclic system, were carried out. Interestingly, some of the 2-phenylacetamidoimidazo[1,2-*a*]pyridine compounds reported herein showed high affinity and selectivity for the peripheral (BZ3) BZRs.

Chemistry

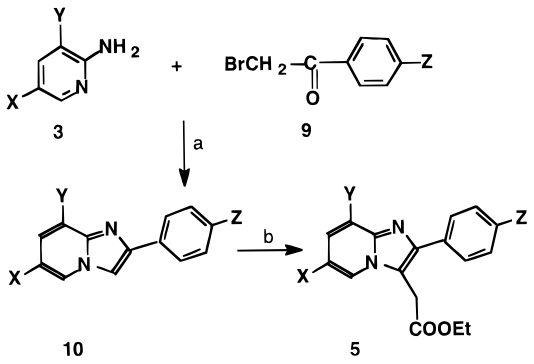
Condensation of suitably substituted 2-aminopyridines **3** (Scheme 1) with the appropriate bromo keto esters **4** in refluxing 1-butanol gave the desired imidazo-[1,2-*a*]pyridines **5** in moderate to good yield. By this procedure (method A), in most cases, the simultaneous formation of the ester derivatives **6** (R = *n*-butyl) was obtained. Treatment of compounds **5** with the appropriate alcohols or amines following standard methods yielded the required esters **6** or amides **7**, respectively. The starting bromo ketoesters **4** were prepared by a three-step procedure involving a Friedel−Crafts acylation of the appropriate aromatic compound with succinic or glutaric anhydride to prepare the corresponding keto acids **8**[15] which, in turn, were converted to the corresponding ethyl esters by standard method. Treatment of these last compounds with bromine in acetic acid gave the required compounds **4**. Moreover, some of the compounds **5** ($n = 1$) were also obtained following a different synthetic approach (method B) as shown in Scheme 2. Thus, according to Tschitschibabin,[16] reaction of the suitably substituted 2-aminopyridines **3** with the appropriate bromoacetophenones **9** in ethanol at 60 °C gave the imidazopyridines **10** in 50−70% yield. By reaction of the compounds **10** with ethyl diazoacetate/Cu[17] in refluxing dry toluene we obtained the ethyl

Scheme 1[a]



[a] Reagents: (a) EtOH, H^+; (b) Br_2/CH_3COOH; (c) method A, *n*-BuOH; (d) ROH; (e) R_1R_2NH.

Scheme 2[a]



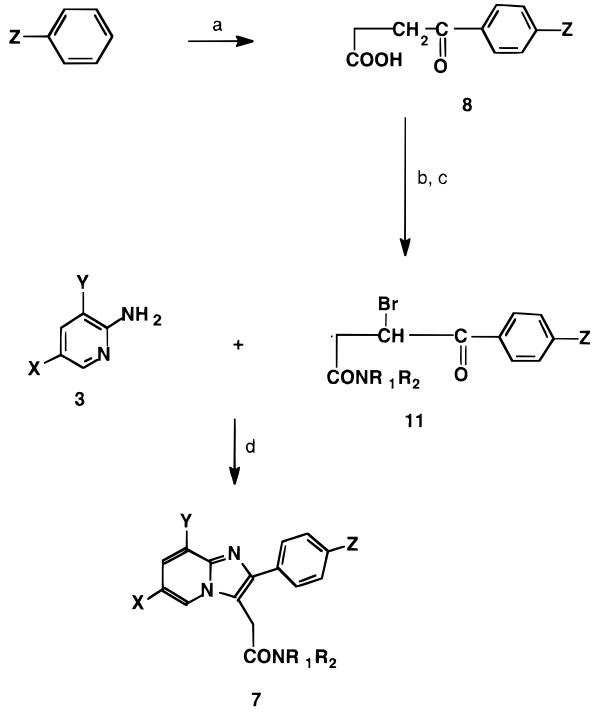
[a] Reagents: (a) EtOH; (b) method B, $N_2CHCOOC_2H_5$/Cu, toluene.

esters **5** ($n = 1$). The preparation of amides **7** by a more straightforward route was accomplished as outlined in Scheme 3. It involves the condensation of suitably substituted 2-aminopyridines **3** with the appropriate bromo keto amides **11** (method C). Compounds of type **11** were prepared by a three-step procedure shown in Scheme 3, namely, Friedel−Crafts acylation of the appropriate aromatic compound with succinic anhydride[15] to prepare the 3-benzoylpropionic acids, which, in turn, were allowed to react with the required dialkylamines in the presence of ethyl 1,2-dihydro-2-ethoxy-1-quinolinecarboxylate (EEDQ) as dehydrating agent.[18] Treatment of the resulting amides with bromine in carbon tetrachloride afforded the desired compounds **11**.

Reduction of the esters **5g,h** by using $(C_2H_5)_3O^+BF_4^-$/$NaBH_4$ in dry methylene chloride followed by acylation of the intermediate alcohol **12** with propionyl chloride gave the propionates **13** and **14**, respectively (Scheme 4).

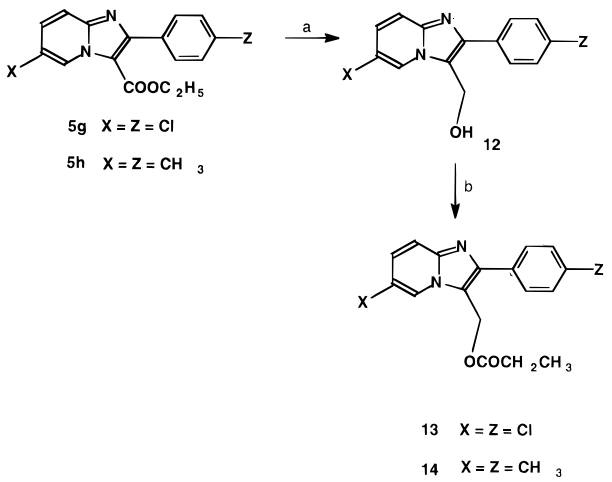
In principle, following methods A or C two regioisomeric compounds **5** and **5′** or **7** and **7′**, respectively, may be produced. The structural assignment to the isolated compounds is based on the analysis of 2D NOESY ^{1}H-NMR spectra which showed the presence of cross peaks between the proton linked at C(5) and the protons of the methylene group at C(3), it being consistent with the **5** and **7** structure only. Further evidence arises

Scheme 3[a]



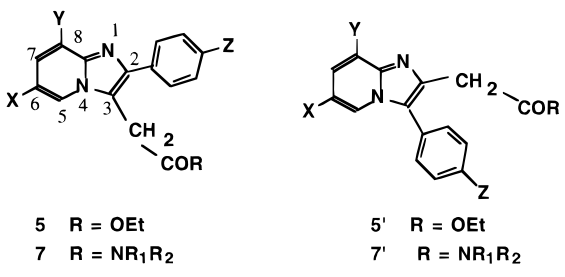
[a] Reagents: (a) succinic anhydride, $AlCl_3$; (b) R_1R_2NH, EEDQ, THF; (c) Br_2/CCl_4; (d) method C, *n*-BuOH.

Scheme 4[a]



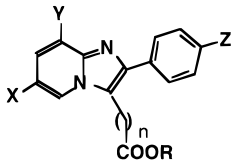
[a] Reagents: (a) $(C_2H_5)_3O^+BF_4^-$, $NaBH_4$; (b) CH_3CH_2COCl.

from the fact that some key intermediate compounds **5** ($n = 1$) prepared following either method A or method B were identical in all respects. Finally, the physical properties of the Alpidem (**1**) prepared according to method C are identical to the ones reported in literature.[13e]



The synthetic methods described in this paper represent an alternative approach to the preparation of compounds **5**−**7**. Analytical and physical data for final compounds are reported in Tables 1 and 2.

Table 1. Structure and Physical Properties of Compounds **5a**−**t** and **6a**−**h**



compd	X	Y	Z	R	*n*	method	mp (°C)	yield (%)
5a	H	H	H	C_2H_5	0	A	*a*	
5b	CH_3	H	H	C_2H_5	0	A	*a*	
5c	Cl	H	H	C_2H_5	0	A	115	31
5d	H	H	CH_3	C_2H_5	0	A	90−93	14
5e	H	H	Cl	C_2H_5	0	A	115−118	44
5f	CH_3	H	Cl	C_2H_5	0	A	121−123	47
5g	Cl	H	Cl	C_2H_5	0	A	151−154	23
5h	CH_3	H	CH_3	C_2H_5	0	A	73−75	23
5i	CH_3	H	H	C_2H_5	1	B	100−102	39
5j	Cl	H	H	C_2H_5	1	A	116−119	29
5k	Br	H	H	C_2H_5	1	A	130−133	33
5l	NH_2	H	H	C_2H_5	1	B	144−146	41
5m	NO_2	H	H	C_2H_5	1	A	oil	15
5n	CH_3	H	CH_3	C_2H_5	1	A	oil	10
5o	Cl	H	Cl	C_2H_5	1	A	157−159	31
5p	Br	H	Cl	C_2H_5	1	A	166−168	29
5q	Cl	Cl	Cl	C_2H_5	1	A	176−178	48
5r	CF_3	Cl	Cl	C_2H_5	1	A	149−151	48
5s	Br	Br	Cl	C_2H_5	1	A	192−194	55
5t	Cl	H	H	C_2H_5	2	A	104−106	19
6a	CH_3	H	H	C_4H_9	1	A	oil	8
6b	Cl	H	H	C_4H_9	1	A	123−125	9
6c	Cl	H	Cl	C_4H_9	1	A	133−135	25
6d	Br	H	Cl	C_4H_9	1	A	140	29
6e	Cl	Cl	H	C_4H_9	1	A	88−91	15
6f	NO_2	H	Cl	C_4H_9	1	A	166−168	15
6g	Cl	H	Cl	$CH(C_2H_5)_2$	1	A	98−100	74
6h	Cl	H	H	C_4H_9	2	A	oil	11

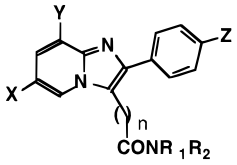
[a] Reference 13d.

Results and Discussion

The ability of the compounds **5**−**7** to interact with the central BZRs (CBR) was investigated by a binding assay using [^{3}H]flunitrazepam as radioligand and membranes from rat brain tissues as receptor source. The percentage of inhibition of specific [^{3}H]flunitrazepam binding was determined using a 40 μM concentration of the tested compounds followed by the determination of IC_{50} only for the most active ones (percentages of inhibition greater that 80%). To evaluate the affinity of compounds **5**−**7** for peripheral BZR (PBR), binding studies were carried out by using Ro 5-4864 as specific radioligand and membranes from renal cells as PBR source. The measured binding affinities for central and peripheral BZRs as well as the ratios of IC_{50} values (CBR/PBR), which can be used as a measure of the *in vitro* selectivity of the prepared compounds, are shown in Table 3. As can be seen from the reported data, the compounds prepared in this study displayed a broad range of binding affinities (IC_{50} values ranging from 4 to $>10^4$ nM), and it is apparent that significant differences in binding affinity and selectivity may exist even between compounds which, at first glance, are structurally very similar.

In all of the cases which we have examined, replacement of the acetamide moiety of the *N,N*-dialkyl-2-phenylacetamidoimidazo[1,2-*a*]pyridines **1** and **2** with

Table 2. Structure and Physical Properties of Compounds **7a−u**



compd	X	Y	Z	R_1	R_2	*n*	method	mp (°C)	yield (%)
7a	H	H	H	C_2H_5	C_2H_5	0	A	oil	29
7b	Cl	H	Cl	C_2H_5	C_2H_5	0	A	163−165	67
7c	Cl	H	Cl	C_3H_7	C_3H_7	0	A	120−123	25
7d	Cl	H	Cl	$-(CH_2)_4-$		0	A	162−163	25
7e	H	H	H	C_3H_7	C_3H_7	1	C	63−66	19
7f	Cl	H	H	C_3H_7	C_3H_7	1	A	153−154	30
7g	Br	H	H	C_3H_7	C_3H_7	1	A	141−144	23
7h	I	H	H	C_3H_7	C_3H_7	1	C	78−80	27
7i	CH_3	H	H	C_3H_7	C_3H_7	1	C	oil	15
7j	CH_3O	H	H	C_3H_7	C_3H_7	1	C	oil	15
7k	NO_2	H	H	C_3H_7	C_3H_7	1	C	189	23
7l	Br	H	Cl	C_3H_7	C_3H_7	1	A	138−140	33
7m	Cl	H	Cl	$-(CH_2)_4-$		1	A	213−215	28
7n	Cl	H	Cl	$-(CH_2)_5-$		1	A	188−190	19
7o	Cl	Cl	H	C_3H_7	C_3H_7	1	A	oil	15
7p	Cl	Cl	Cl	C_3H_7	C_3H_7	1	A	183−185	31
7q	Br	Br	Cl	C_3H_7	C_3H_7	1	A	80 dec	20
7r	CF_3	Cl	Cl	C_3H_7	C_3H_7	1	A	130 dec	23
7s	Br	CH_3	H	C_3H_7	C_3H_7	1	C	105−107	15
7t	CH_3	Br	H	C_3H_7	C_3H_7	1	C	109−114	10
7u	Cl	H	H	C_3H_7	C_3H_7	2	A	oil	44

Table 3. Affinities of Compounds **5**, **6**, and **7** for CBR and PBR[a]

	IC_{50} (nM)			IC_{50} (nM)		ratio IC_{50}(CBR)/
compd	CBR	PBR	compd	CBR	PBR	IC_{50}(PBR)
5c	2120	*b*	**7b**	8380	2040	
5h	1150	*b*	**7c**	8730	426	
5i	1390	*b*	**7d**	2980	*b*	
5j	1170	*b*	**7e**	464	80	5.8
5o	695	*b*	**7f**	86	4	21.5
5t	1110	*b*	**7g**	116	14.5	7.25
6a	3150	*b*	**7h**	286	23	12.4
6b	2500	*b*	**7i**	124.7	34.5	3.6
6c	2780	*b*	**7j**	2830	33.6	84.2
6h	1180	*b*	**7k**	2740	11.8	232
			7l	58	12	4.83
			7m	92	287	0.32
			7n	240	18.7	12.8
			7o	(46%)[c]	12	>1000
			7p	(0%)[c]	20	>1000
			7q	(17%)[c]	55.3	>1000
			7r	(0%)[c]	37	>1000
			7s	(68%)[c]	13.5	>1000
			7t	(78%)[c]	19	>1000
			7u	308	3270	0.09
			Alpidem	26	7.9	3.3
			Zolpidem	48	4700	0.01

[a] The IC_{50} values are the means of at least two experiments performed in triplicate and which differed by less than 15%; compounds **5a,b,d−g,k−n,p−s**, and **6d−g** are characterized by IC_{50} values > 10^4 nM for both CBR and PBR and they are not listed in the present table. [b] IC_{50} value > 10^4 nM. [c] Values in parentheses are the percentages of inhibition of specific [^{3}H]flunitrazepam binding determined at 40 μM concentration of the tested compound.

the ester function (mainly ethyl or butyl esters) resulted in a marked loss of activity both at central and peripheral BZRs. On this basis, it can be concluded that the acetamide moiety appeared to be essential for potent activity. Considering that the interaction of the amide carbonyl with the receptor should be only of hydrogen-bonding acceptor type[19] and since the hydrogen-bonding acceptor capability of amide carbonyl is reported to be roughly comparable to that of the ester group,[20] the difference in activity of the esters when compared to the amides is striking. To account for this finding, it was considered that esters **5** and **6**, unlike amide analogues **7**, could undergo hydrolysis to the corresponding acids which we proved to lack binding affinity (data not shown). Therefore, stability in the assay medium of a number of acetates **5i,o−r, 6d−f**, amides **7e,k,o**, and Alpidem (**1**) itself was checked. The amides were found to be very stable, whereas the esters underwent hydrolysis only to a low extent (5−25%). In light of the observed stabilities, it is reasonable to ascribe the lack of activity of the esters **5** and **6** to intrinsic properties of acetates. For instance, conformational factors could be invoked to account for the difference in activity between amides and esters. It is possible that amides, unlike esters, may adopt favorable binding conformations due to the reduced freedom of OC−N bond rotation.

We next examined other ester compounds such as the propionates of the 2-phenyl-3-(hydroxymethyl)imidazo-[1,2-*a*]pyridine (i.e. **13** and **14**) which also resulted in a marked loss of activity both at central and peripheral BZRs.

Conversely, most of the amides **7** proved to possess high affinity and selectivity for CBR and/or PBR. In the amide series, however, removal of the methylene linker between the imidazopyridine nucleus and the amide group leading to compounds **7a−d** or lengthening the carbon chain between the heterocyclic nucleus and the amide function (**7u**) resulted in a notable decrease in affinity. All of these findings demonstrated the significant advantage for a single methylene unit between the imidazopyridine nucleus and the amide function; this may be explained by taking into account the binding site models proposed for recognition of BZR ligands. In fact, hydrogen bond accepting sites with appropriate geometries are a common feature of most models for BZR pharmacophores reported in the literature.[19] In our case, the specific hydrogen bond accepting sites are the carbonyl oxygen atom and the heterocyclic nitrogen atom N(1). It is likely that compounds which do not possess a single methylene unit as a spacer such as **7a−d,u** do not fulfil the mentioned geometric requirements.

We next evaluated substituent effects on the pyridine moiety of the acetamidoimidazo[1,2-*a*]pyridine derivatives **7** (Table 3). Our attention was focused mainly on the dipropylamide congeners **7e−l,o−t** since alkylation of the acetamide nitrogen with two *n*-propyl groups, as a confirmation of literature data on other classes of benzodiazepine receptors ligands,[11a] strongly favors binding affinity. Using compound **7e** as a reference, substitution with electron withdrawing and hydrophobic halogens at C(6), namely, 6-chloro (**7f**), 6-bromo (**7g**), 6-iodo (**7h**), resulted in an increase of both CBR and PBR affinities, the rank order of binding potency being Cl > Br > I. The presence of a methyl group at 6-position (**7i**) was again found to increase, with respect to **7e**, the binding affinity for both receptor types. Among the 6-substituted compounds, the highest selectivities for peripheral BZR sites were observed for the 6-methoxy (**7j**) and the 6-nitro (**7k**) congeners, [IC_{50}(CBR)/IC_{50}(PBR) = 84.2, and 232, respectively]. Compound **7k** is more than 200-fold more selective for the

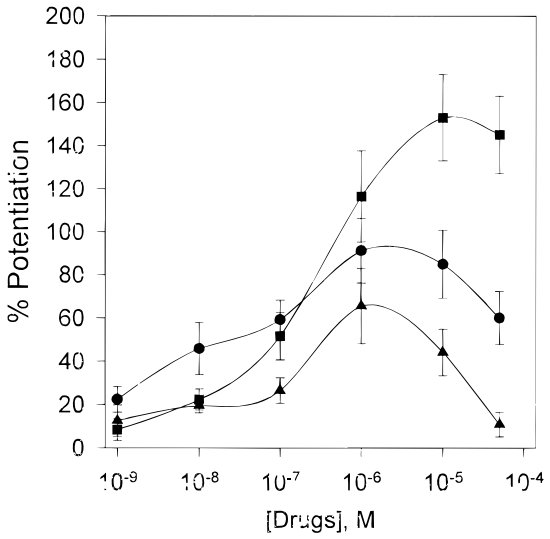


Figure 1. Modulatory actions of **7f,m** and Zolpidem at human recombinant $GABA_A$ receptors expressed in *Xenopus* oocytes. Values represent the mean ± SEM (six to nine different oocytes) percent potentiation of the control response to $GABA_A$ (EC_{20}) by various concentrations of **7m** (■), **7f** (▲), and Zolpidem (●) measured in oocytes expressing $\alpha_1\beta_2\gamma_{2s}$ receptors. Actual EC_{20} concentrations of GABA for $\alpha_1\beta_2\gamma_{2s}$ receptors ranged from 2 to 10 μM.

peripheral versus central receptors. Introduction of a further chlorine at the *para* position of the phenyl ring at C(2) led to an enhancement of affinity for CBR (116 nM for **7g** compared to 58 nM for **7l** and 86 nM for **7f** compared to 26 nM for Alpidem, **1**). In addition, the ring size of the cycloalkyl group on the amide nitrogen of **7m** and **7n** was found to notably influence CBR versus PBR selectivity (compare the affinity for CBR and PBR of **7m** and **7n**, respectively).

Two 6-substituted compounds, **7f,m**, were also examined in several different central benzodiazepine receptor subtypes. Compound **7m** was selected since it possesses high selectivity for the CBR, as indicated by the ratio of IC_{50} values [IC_{50}(CBR)/IC_{50}(PBR) = 0.32]. Compound **7f** was included in these experiments because it was almost equipotent with **7m** in interacting with the CBR, and moreover it was the most active compound for the PBR. Compounds **7f,m** potentiated human $GABA_A$ receptor-mediated ion current. Expression of specific $GABA_A$ receptor subunit assemblies in *Xenopus* oocytes was utilized to evaluate functionally both the efficacy and potency of the positive modulation of GABA-evoked Cl^- currents by **7f** and **7m** in comparison with Zolpidem. The effects of all three compounds (10^{-9} to 10^{-5} M) at receptors formed by $\alpha_1\beta_2\gamma_{2s}$ subunits is shown in Figure 1. GABA-evoked currents were potentiated in the presence of all these compounds. Potentiation by each compound was concentration-dependent and reversible following washout. The rank order of maximal efficacies (defined as maximal potentiation of peak GABA-current amplitude) was **7m** (153 ± 11%) > Zolpidem (91.3 ± 15%) > **7f** (66 ± 17%). The rank order of potencies of these drugs was **7f** ($EC_{50} = 3.2 \times 10^{-8}$ M) > Zolpidem ($EC_{50} = 3.6 \times 10^{-8}$ M) > **7m** ($EC_{50} = 2.2 \times 10^{-7}$ M). Thus, compound **7m** had a greater efficacy than the other two compounds although it was comparatively less potent. The actions of these compounds were also tested on $\alpha_2\beta_2\gamma_{2s}$ receptors, and the data are reported in Table 4. Compared to the results obtained with receptors

Table 4. Effects of **7f**,**m** and Zolpidem on GABA-Evoked Cl^- Currents in Oocytes Expressing $\alpha_2\beta_2\gamma_{2s}$ $GABA_A$ Receptors[a]

compound	maximal efficacy (%)	EC_{50}
7f	139 ± 22	1.3×10^{-7}
7m	71 ± 17	6.6×10^{-6}
Zolpidem	150 ± 20	1.8×10^{-7}

[a] Maximal potentiation is expressed as the percentage increase in the current induced by GABA at EC_{20}. Data are means ± SEM obtained from five to eight different oocytes.

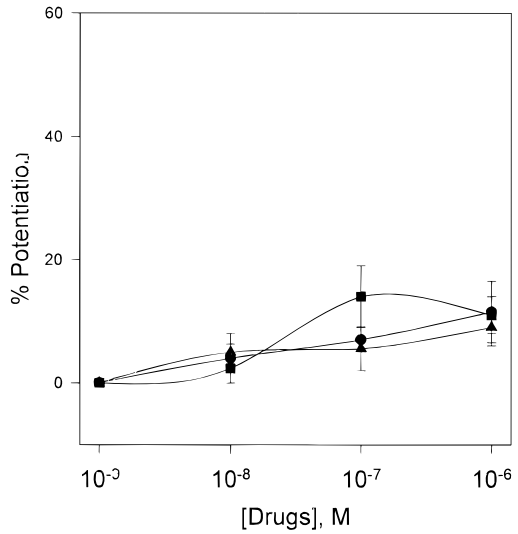


Figure 2. Modulatory actions of **7f,m** and Zolpidem at $GABA_A$ receptors containing the α_5 subunit. Values represent the potentiation of Cl^- currents induced by GABA by various concentrations of **7m** (■), **7f** (▲), and Zolpidem (●) measured in oocytes expressing $\alpha_5\beta_2\gamma_{2s}$ receptors and are expressed as mean (±SEM from three to five oocytes) percent increase of the control response obtained with GABA (EC_{20}).

containing the α_1 subunit, maximal efficacies of the three compounds were found to be similar at receptors containing the α_2 subunit. However, the EC_{50} of these compounds was increased, compared to $\alpha_1\beta_2\gamma_{2s}$ receptors, by 30-, 4-, and 5-fold for **7m**, **7f**, and Zolpidem, respectively. Finally, these compounds were almost completely devoid of activity at receptors containing the α_5 subunit (Figure 2).

Even more interesting for the effects on selectivity toward peripheral receptors was the double substitution on the pyridine ring. Thus, introduction of a further chlorine at the 8-position of **7f**, affording **7o**, had a positive influence in determining high affinity and selectivity for peripheral receptors. We were pleased to find that high affinity and selectivity for peripheral receptors was observed also for compound **7p** which is similarly disubstituted at the 6- and 8-positions. The same effect was observed, once again, for the 6,8-disubstituted compounds **7q**−**t**. Hence, interestingly, compounds **7o**−**t** emerged as high-affinity and selectivity for the peripheral receptor ligands based on radioligand binding assays and constitute new examples of the few high-affinity and selective ligands for this receptor described to date.[21c] These results clearly indicate that disubstitution at 6- and 8-positions on the pyridine moiety with dichloro (**7o,p**), dibromo (**7q**), CF_3,-chloro (**7r**), bromo,CH_3 (**7s**), and CH_3,Br (**7t**) substituents, respectively, is a key factor promoting BZ3 receptor selectivity of the acetamidoimidazo[1,2-*a*]pyridine compounds. It is remarkable that even slight changes on the pyridine nucleus can have notable influence on

binding affinity and receptor selectivity. Similar results have been observed in the benzodiazepine series where substitution with halogens and alkyl groups at 4′- and 1-positions, respectively, of the 5-phenyl-1,4-benzodiazepine heterocyclic ring system leads to high affinity and selectivity for the peripheral-type receptor.[22] In addition, it must be pointed out that even though recent reports from the literature[21] highlight some structural features which should play an important role in determining the affinity for the peripheral receptors, at present it is difficult to make any conclusion concerning the role played by the substituents at C(6) and C(6)−C(8) on the acetamidoimidazo[1,2-*a*]pyridine structure. Further work is in progress aimed at clarifying this point.

In conclusion, new methods were developed to synthesize 2-phenylimidazo[1,2-*a*]pyridine derivatives endowed with high affinity for peripheral benzodiazepine receptor types. Our structure−activity correlations revealed considerable substituent effects at C(6) and C(6)−C(8) on the 2-phenylacetamidoimidazo[1,2-*a*]pyridine heterocyclic ring system. From these studies the 6,8-disubstituted acetamidopyridines **7o**−**t** were found both to show high affinity and to be selective ligands for the peripheral BZR type. In view of this high affinity and selectivity together with their potential ability to stimulate the production of neurosteroids, it appears that these molecules might be useful tools for elucidating the physiological and pharmacological role of peripheral benzodiazepine receptors. More detailed pharmacological investigation of members of the series reported herein, together with an expansion of our SAR and molecular modeling efforts aimed at obtaining insights into the structural requirements for central and peripheral benzodiazepine receptors, will be reported in due course.

Experimental Section

Chemistry. Melting points were determined in open capillary tubes with a Büchi apparatus and are uncorrected. IR spectra were obtained on a Perkin-Elmer 283 spectrophotometer (KBr pellets for solid or nujol for liquid). ^{1}H NMR spectra were determined on a Varian 390 or XL-200 or Bruker 300 MHz (NOESY experiment) instrument. Chemical shifts are given in δ values downfield from Me_4Si as internal standard. Mass spectra were recorded on a Hewlett-Packard 5995c GC−MS low-resolution spectrometer. All compounds showed appropriate IR, ^{1}H NMR, and mass spectra. Elemental analyses were carried out with a Carlo Erba model 1106 analyzer, and results were within ±0.40% of the theoretical values. Silica gel 60 (Merck 70−230 mesh) was used for column chromatography. All the following reactions were performed under a nitrogen atmosphere. The starting 2-aminopyridine compounds are commercially available except for 2-amino-5-iodopyridine and 2-amino-5-methoxypyridine prepared as follows, respectively. The preparation of ethyl benzoylpropionates or -butyrates was accomplished following a reported procedure.[15]

2-Amino-5-iodopyridine. This compound was prepared following a previously published procedure.[23] Full characterization of this compound is reported here: IR (KBr) 3285, 3370 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.57 (s,2H, NH_2), 6.37 (d, J = 8 Hz, 1H, C(3)-H), 7.66 (dd, J = 8 and 2 Hz, 1H, C(4)-H), 8.25 (d, J = 2 Hz, 1H, C(6)-H); MS m/z 220 (base M$^+$). Anal. ($C_5H_5IN_2$) C, H, N.

2-Amino-5-methoxypyridine. A mixture of 2-amino-5-iodopyridine[23] (3 g, 14 mmol), Na (96 mg), and copper powder (1.2 g) in methanol (60 mL) was heated in a sealed tube at 160 °C for 48 h. After cooling, the reaction mixture was filtered, and the filtrate was evaporated under reduced pressure. The residue was purified by silica gel column chromatography [light petroleum ether/ethyl acetate, 2/8 (v/v), as eluent] to give 0.80 g (51% yield) of the title compound: IR (Nujol) 3290, 3332 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.83 (s,3H, OCH_3), 6.53 (d, J = 3 Hz, 1H, C(3)-H), 7.16 (dd, J = 9 and 3 Hz, 1H, C(4)-H), 7.80 (d, J = 3 Hz, 1H, C(6)-H); MS m/z 124 (base M$^+$). Anal. ($C_6H_8N_2O$) C, H, N.

General Procedure for Preparation of Bromo Keto Esters 4. A solution of bromine (68 mmol) in 25 mL of acetic acid was added slowly to a solution of the appropriate keto ester (68 mmol) in 25 mL of acetic acid over a 1 h interval. After addition was complete, the solution was stirred under nitrogen overnight, at which point it was pale yellow. Evaporation of the solvent gave a residue which was treated with 5% $NaHCO_3$. After being washed with water, the organic phase was extracted with $CHCl_3$ (20 mL) and dried (Na_2SO_4) and the solvent removed by rotatory evaporation. Purification of the crude product was accomplished by distillation or silica gel column chromatography [light petroleum ether/ethyl acetate, 9/1 (v/v), as eluent].

Ethyl 3-benzoyl-3-bromopropionate: IR (Nujol) 1730, 1680 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.20 (t, 3H, CH_3), 2.9−3.6 (m, 2H, CH_2CO), 4.13 (q, 2H, CH_2O), 5.4−5.6 (m, 1H, CHBr), 7.2−7.7 (m, 3H, arom), 7.9−8.3 (m, 2H, arom); signals attributable to enol form are also present; MS m/z 284 (1, M$^+$), 105 (base). Anal. ($C_{12}H_{13}BrO_3$) C, H, N.

Ethyl 3-bromo-3-(4-chlorobenzoyl)propionate: IR (Nujol) 1730, 1680 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.23 (t, 3H, CH_3), 2.9−3.6 (m, 2H, CH_2CO), 4.16 (q, 2H, CH_2O), 5.3−5.5 (m, 1H, CHBr), 7.3−7.6 (m, 2H, arom), 7.9−8.2 (m, 2H, arom); signals attributable to enol form are also present; MS m/z 318 (16, M$^+$), 139 (base). Anal. ($C_{12}H_{12}BrClO_3$) C, H, N.

Ethyl 3-bromo-3-(*p*-toluoyl)propionate: IR (Nujol) 1722, 1680 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.20 (t, 3H, CH_3), 2.43 (s, 3H, CH_3), 2.9−3.6 (m, 2H, CH_2CO), 4.20 (q, 2H, CH_2O), 5.4−5.6 (m, 1H, CHBr), 7.2−7.5 (m, 2H, arom), 7.8−8.1 (m, 2H, arom); signals attributable to enol form are also present; MS m/z 298 (16, M$^+$), 119 (base). Anal. ($C_{13}H_{15}BrO_3$) C, H, N.

Ethyl 4-benzoyl-4-bromo-butyrate: IR (Nujol) 1725, 1680 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.26 (t, 3H, CH_3), 2.3−2.7 (m, 4H, CH_2), 4.16 (q, 2H, CH_2O), 5.3−5.5 (m, 1H, CHBr), 7.3−7.6 (m, 3H, arom), 7.9−8.1 (m, 2H, arom); signals attributable to enol form are also present. Anal. ($C_{13}H_{15}BrO_3$) C, H, N

General Procedure for Preparation of Alkyl 2-Phenylimidazo[1,2-*a*]pyridine-3-carboxylates, -acetates, -propionates 5 and 6 (Method A). To a solution of the suitably substituted 2-aminopyridine **3** (14 mmol) in *n*-BuOH (50 mL) was added the appropriate bromo keto ester **4** (15.4 mmol). The mixture was refluxed under stirring for 6−20 h. The progress of reaction was monitored by TLC. 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). Evaporation of the solvent gave a residue corresponding to a mixture of compounds **5** and **6** which was purified by silica gel column chromatography [light petroleum ether/ethyl acetate, 8/2 (v/v), as eluent]. Physical data are summarized in Table 1. Spectral data for the representative compounds **5c** and **6a** are as follows. Compounds **5d**−**h,j,k,m**−**t**, **6b**−**f,h** show very similar spectral features.

Ethyl 2-phenyl-6-chloroimidazo[1,2-*a*]pyridine-3-carboxylate (5c): IR (KBr) 1680 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.23 (t, 3H, CH_3), 4.33 (q, 2H, OCH_2), 7.2−7.9 (m, 7H, arom), 9.60 (s, 1H, arom); MS, m/z 300 (63, M$^+$), 228 (base). Anal. ($C_{16}H_{13}ClN_2O_2$) C, H, N.

***n*-Butyl (2-phenyl-6-methylimidazo[1,2-*a*]pyridin-3-yl)acetate (6a):** IR (Nujol) 1725 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.90 (t, 3H, CH_3), 1.1−1.6 (m, 4H, CH_2), 2.37 (s, 3H, CH_3-arom), 4.03 (s, 2H, CH_2CO), 4.20 (t, 2H, CH_2O), 6.9−8.0 (m, 8H, arom); MS m/z 322 (38, M$^+$), 221 (base). Anal. ($C_{20}H_{22}N_2O_2$) C, H, N.

Isopentyl (2-(4-Chlorophenyl)-6-chloroimidazo[1,2-*a*]-pyridin-3-yl)acetate (6g). A solution of **5o** (2 g, 5.7 mmol) in aqueous EtOH containing NaOH (20 mL) was refluxed for 0.5 h. After cooling, the mixture was acidified with 10% HCl solution, and the corresponding precipitated acid was filtered off. A mixture of the crude acid (1g, 3.1 mmol) and boron

trifluoride etherate (0.71 g, 5 mmol) in 3-pentanol (20 mL) was refluxed for 24 h. Evaporation of the solvent under reduced pressure gave a residue wich was dissolved in $CHCl_3$ (30 mL). The solution was washed with 10% $NaHCO_3$ solution and water and then evaporated to dryness to give 0.9 g of **6g**: IR (KBr) 1713 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.83 (t, 6H, CH_3), 1.3−1.8 (m, 4H, CH_2), 4.00 (s, 2H, CH_2CO), 4.80 (quintuplet, 1H, CHO), 7.1−7.9 (m, 6H, arom), 8.26 (d, J = 2 Hz, 1H, arom); MS m/z 390 (31, M^+), 275 (base). Anal. ($C_{20}H_{20}Cl_2N_2O_2$) C, H, N.

2-(4-Chlorophenyl)-3-(hydroxymethyl)-6-chloroimidazo-[1,2-*a*]pyridinyl Propionate (13). A solution of **5g** (2 g, 6.5 mmol) and triethyloxonium fluoborate (1.4 g, 7.2 mmol) in dry CH_2Cl_2 (25 mL) was stirred for 20 h at room temperature. Then, the solvent was evaporated under reduced pressure and the residue was dissolved in absolute ethanol. $NaBH_4$ (4.3 g, 0.114 mol) was added portionwise to the stirred solution at 0 °C; when the addition was complete, stirring was continued for 18 h at room temperature. The solution was poured into 250 mL of water and extracted with $CHCl_3$ (3 × 30 mL). The combined extracts were washed with water, dried (Na_2SO_4), and evaporated. Evaporation of the solvent under reduced pressure gave a residue wich was purified by silica gel column chromatography [light petroleum ether/ethyl acetate, 1/1 (v/v) as eluent] to give 0.2 g (yield 11%) of 2-(4-chlorophenyl)-3-(hydroxymethyl)-6-chloroimidazo[1,2-*a*]pyridine (**12**). A mixture of this compound (0.2 g) and propionyl chloride (25 mL) was stirred at room temperature overnight. Then, after being washed with water and 5% $NaHCO_3$, the organic phase was extracted with $CHCl_3$ (20 mL) and dried (Na_2SO_4) and the solvent removed by rotatory evaporation. The residue was purified by preparative thin layer chromatography [light petroleum ether/ethyl acetate, 8/2 (v/v), as eluent] to give 0.1 g (yield 42%) of compound **13**: mp 116−118 °C; IR (KBr) 1710 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.17 (t, 3H, CH_3), 2.42 (q, 2H, CH_2O), 5.47 (s, 2H, CH_2), 7.2−7.8 (m, 6H, arom), 8.37 (d, J = 2 Hz, 1H, arom); MS m/z 348 (23, M^+), 275 (base). Anal. ($C_{17}H_{14}Cl_2N_2O_2$) C, H, N. In similar way was prepared compound **14** (24%): mp 95−98 °C; IR (Nujol) 1630 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.24 (t, 2H, CH_2), 2.35 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 3.57 (q, 2H, CH_2), 4.86 (s, 3H, CH_2), 7.0−7.7 (m, 6H, arom), 7.9−8.00 (m, 1H, arom); MS m/z 280 (26, M^+), 235 (base). Anal. ($C_{19}H_{20}N_2O_2$) C, H, N.

Preparation of Alkyl (2-Phenylimidazo[1,2-*a*]pyridin-3-yl)acetates 5 (Method B). To a boiling solution of the appropriate 2-phenylimidazo[1,2-*a*]pyridine **10**[16] (9.6 mmol) in dry toluene (30 mL) was added ethyl diazoacetate (9.6 mmol) in small amounts, each addition followed by small portion of copper powder (total amount 0.5 g).[17] The mixture was refluxed under stirring for 0.5 h. After cooling, evaporation of the solvent gave a residue which was purified by silica gel column chromatography [light petroleum ether/ethyl ether, 1:1 (v/v), as eluent].

Ethyl (2-phenyl-6-methylimidazo[1,2-*a*]pyridin-3-yl)-acetate (5i): IR (KBr) 1723 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.26 (t, 3H, CH_3), 2.37 (s, 3H, CH_3-Ar), 4.03 (s, 2H, CH_2CO), 4.23 (q, 2H, CH_2), 7.0−8.3 (m, 8H, arom); MS m/z 294 (28, M^+), 221 (base). Anal. ($C_{18}H_{18}N_2O_2$) C, H, N.

Ethyl (2-phenyl-6-aminoimidazo[1, 2-*a*]pyridin-3-yl)-acetate (5l): IR (KBr) 1735 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.20 (t, 3H, CH_3), 3.70 (s, 2H, CH_2CO), 4.16 (q, 2H, CH_2O), 6.73 (dd, J = 9 and 2 Hz, 1H, arom), 7.1−7.8 (m, 6H, arom), 7.90 (d, J = 2 Hz, 1H, arom); MS m/z 295 (78, M^+), 222 (base). Anal. ($C_{17}H_{17}N_3O_2$) C, H, N.

General Procedure for Preparation of *N,N*-Dialkyl-2-phenylimidazo[1,2-*a*]pyridine-3-carboxamides, -acetamides, or -propionamides 7 (Method A). To a solution of the suitable ester **5** or **6** (2.6 mmol) in 80% EtOH (50 mL) was added 1 N NaOH (20 mL). The mixture was heated under stirring at 60°C with a water bath for 1 h. Evaporation of the solvent under reduced pressure gave a residue wich was acidified with dilute HCl, and the precipitate acid was filtered off. To a cooled and stirred solution of the crude acid (3.3 mmol) in dry CH_2Cl_2 (20 mL) was dropwise added $SOCl_2$ (5.6 mmol) dissolved in dry CH_2Cl_2 (20 mL). The mixture was refluxed for 2 h, and then, after cooling with an ice bath, the appropriate dialkylamine (3.5 mL) dissolved in dry CH_2Cl_2 (10 mL) was added. After addition was complete, the mixture was stirred at room temperature overnight. Evaporation of the solvent under reduced pressure gave a residue which was purified by silica gel column chromatography [light petroleum ether/ethyl acetate, 8/2 (v/v), as eluent] to give the required amide.

***N,N*-Diethyl 2-phenylimidazo[1,2-*a*]pyridine-3-carboxamide (7a):** IR (Nujol) 1630 cm^{-1}; ^1H NMR recorded at −30 °C ($CDCl_3$) δ 0.68 (t, 3H, CH_3), 1.25 (t, 3H, CH_3), 2.7−2.8 (m, 1H, CH_2), 3.2−3.3 (m, 2H, CH_2), 3.9−4.0 (m, 1H, CH_2), 6.8−6.9 (m, 1H, arom), 7.2−7.7 (m, 7H, arom), 8.28 (dd, J = 6 and 2 Hz, 1H, arom); MS m/z 293 (23, M^+), 194 (base). Anal. ($C_{18}H_{19}N_3O$) C, H, N.

***N,N*-Diethyl-2-(4-chlorophenyl)-6-chloroimidazo[1,2-*a*]-pyridine-3-carboxamide (7b):** IR (KBr) 1620 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.75 (t, 3H, CH_3), 1.27 (t, 3H, CH_3), 2.9−3.1 (m, 2H, CH_2), 3.5−3.7 (m, 2H, CH_2), 7.2−7.8 (m, 6H, arom), 8.32 (d, J = 3 Hz, 1H, arom); MS m/z 361 (39, M^+), 262 (base). Anal. ($C_{18}H_{17}Cl_2N_3O$) C, H, N.

***N,N*-Di-*n*-propyl-2-(4-chlorophenyl)-6-chloroimidazo-[1,2-*a*]pyridine-3-carboxamide (7c):** IR (KBr) 1600 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.45 (t, 3H, CH_3), 0.95 (t, 3H, CH_3), 1.1−1.2 (m, 4H, CH_2), 1.5−1.8 (m, 4H, CH_2), 7.2−7.8 (m, 6H, arom), 8.32 (d, J = 1 Hz, 1H, arom); MS m/z 389 (24, M^+), 262 (base). Anal. ($C_{20}H_{21}Cl_2N_3O$) C, H, N.

2-(4-Chlorophenyl)-3-(pyrrolidinocarbonyl)-6-chloroimidazo[1,2-*a*]pyridine (7d): IR (KBr) 1650 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.6−1.7 (m, 2H, CH_2), 1.8−1.9 (m, 2H, CH_2), 2.8−2.9 (m, 2H, CH_2), 3.6−3.7 (m, 2H, CH_2), 7.2−7.8 (m, 6H, arom), 8.62 (d, J = 2 Hz, 1H, arom); MS m/z 359 (62, M^+), 262 (base). Anal. ($C_{18}H_{15}Cl_2N_3O$) C, H, N.

***N,N*-Di-*n*-propyl-(2-phenyl-6-chloroimidazo[1,2-*a*]pyridin-3-yl)acetamide (7f):** IR (KBr) 1636 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.68 (t, 3H, CH_3), 0.83 (t, 3H, CH_3), 1.4−1.6 (m, 4H, $2CH_2$), 3.07 (t, 2H, CH_2N), 3.24−3.29 (m, 2H, CH_2N), 4.07 (s, 2H, CH_2CO), 7.2−7.6 (m, 7H, arom), 8.32 (d, J = 1 Hz, 1H, arom); MS m/z 369 (29, M^+), 241 (base). Anal. ($C_{21}H_{24}ClN_3O$) C, H, N.

***N,N*-Di-*n*-propyl-(2-phenyl-6-bromoimidazo[1,2-*a*]pyridin-3-yl)acetamide (7g):** IR (KBr) 1635 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.72 (t, 3H, CH_3), 0.84 (t, 3H, CH_3), 1.4−1.6 (m, 4H, $2CH_2$), 3.10 (t, 2H, CH_2N), 3.28 (t, 2H, CH_2N), 4.08 (s, 2H, CH_2CO), 7.4−7.7 (m, 7H, arom), 8.43 (d, J = 2 Hz, 1H, arom); MS m/z 413 (21, M^+), 285 (base). Anal. ($C_{21}H_{24}BrN_3O$) C, H, N.

***N,N*-Di-*n*-propyl-[2-(4-chlorophenyl)-6-bromoimidazo-[1,2-*a*]pyridin-3-yl]acetamide (7l):** IR (KBr) 1618 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.86 (t, 6H, $2CH_3$), 1.3−2.1 (m, 4H, CH_2), 3.58 (t, 2H, CH_2N), 3.70 (t, 2H, CH_2N), 4.07 (s, 2H, CH_2CO), 7.3−7.8 (m, 6H, arom), 8.40 (s, 1H, arom); MS m/z 447 (16, M^+), 321 (base). Anal. ($C_{21}H_{23}BrClN_3O$) C, H, N.

2-(4-Chlorophenyl)-3-[(pyrrolidinocarbonyl)methyl]-6-chloroimidazo[1,2-*a*]pyridine (7m): IR (KBr) 1620 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.7−1.9 (m, 4H, $2CH_2$), 3.2−3.5 (m, 4H, CH_2N), 3.90 (s, 2H, CH_2CO), 7.0−7.7 (m, 6H, arom), 8.23 (s, 1H, arom); MS m/z 373 (22, M^+), 275 (base). Anal. ($C_{19}H_{17}Cl_2N_3O$) C, H, N.

2-(4-Chlorophenyl)-3-[(piperidinocarbonyl)methyl]-6-chloroimidazo[1,2-*a*]pyridine (7n): IR (KBr) 1620 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.3−1.4 (m, 2H, CH_2), 1.4−1.5 (m, 2H, CH_2), 1.5−1.6 (m, 2H, CH_2), 3.30 (t, 2H, CH_2N), 3.54 (t, 2H, CH_2N), 4.03 (s, 2H, CH_2CO), 7.1−7.6 (m, 6H, arom), 8.24 (s, 1H, arom); MS m/z 387 (20, M^+), 275 (base). Anal. ($C_{20}H_{19}Cl_2N_3O$) C, H, N.

***N,N*-Di-*n*-propyl-(2-phenyl-6,8-dichloroimidazo[1,2-*a*]-pyridin-3-yl)acetamide (7o):** IR (Nujol) 1620 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.70 (t, 3H, CH_3), 0.84 (t, 3H, CH_3), 1.4−1.6 (m, 4H, $2CH_2$), 3.07 (t, 2H, CH_2N), 3.27 (t, 2H, CH_2N), 4.08 (s, 2H, CH_2CO), 7.4−7.7 (m, 6H, arom), 8.34 (s, 1H, arom); MS m/z 403 (29, M^+), 275 (base). Anal. ($C_{21}H_{23}Cl_2N_3O$) C, H, N.

***N,N*-Di-*n*-propyl-[2-(4-chlorophenyl)-6,8-dichloroimidazo[1,2-*a*]pyridin-3-yl]acetamide (7p):** IR (KBr) 1636 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.75 (t, 3H, CH_3), 0.84 (t, 3H, CH_3), 1.4−1.6 (m, 4H, CH_2), 3.10 (t, 2H, CH_2N), 3.28 (t, 2H, CH_2N), 4.02 (s, 2H, CH_2CO), 7.28 (d, J = 1 Hz, 1H, arom), 7.42 (d, J

= 9 Hz, 2H, arom), 7.58 (d, J = 9 Hz, 2H, arom), 8.22 (d, J = 1 Hz, 1H, arom); MS m/z 437 (29, M^+), 309 (base). Anal. ($C_{21}H_{22}Cl_3N_3O$) C, H, N.

***N,N*-Di-*n*-propyl-[2-(4-chlorophenyl)-6,8-dibromoimidazo[1,2-*a*]pyridin-3-yl]acetamide (7q):** IR (KBr) 1625 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.74 (t, 3H, CH_3), 0.84 (t, 3H, CH_3), 1.4−1.6 (m, 4H, CH_2), 3.09 (t, 2H, CH_2N), 3.28 (t, 2H, CH_2N), 4.01 (s, 2H, CH_2CO), 7.40 (d, J = 3 Hz, 2H, arom), 7.54 (d, J = 0.5 Hz, 1H, arom), 7.58 (d, J = 3 Hz, 2H, arom), 8.34 (d, J = 0.5 Hz, 1H, arom); MS m/z 525 (8, M^+), 399 (base). Anal. ($C_{21}H_{22}Br_2ClN_3O$) C, H, N.

***N,N*-Di-*n*-propyl-[2-(4-chlorophenyl)-6-(trifluoromethyl)-8-chloroimidazo[1,2-*a*]pyridin-3-yl]acetamide (7r):** IR (KBr) 1612 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.78 (t, 3H, CH_3), 0.84 (t, 3H, CH_3), 1.5−1.6 (m, 4H, $2CH_2$), 3.13 (t, 2H, CH_2N), 3.29 (t, 2H, CH_2N), 4.07 (s, 2H, CH_2CO), 7.4−7.5 (m, 3H, arom), 7.5−7.6 (m, 2H, arom), 8.52 (d, J = 1 Hz, 1H, arom); MS m/z 471 (20, M^+), 128 (base). Anal. ($C_{22}H_{22}Cl_2F_3N_3O$) C, H, N.

***N,N*-Di-*n*-propyl-3-(2-phenyl-6-chloroimidazo[1,2-*a*]pyridin-3-yl)propionamide (7u):** IR (Nujol) 1625 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.85 (t, 3H, $2CH_3$), 1.2−1.7 (m, 4H, CH_2), 2.63 (t, 2H, CH_2), 3.03 (t, 2H, CH_2), 3.26 (t, 2H, CH_2N), 3.50 (t, 2H, CH_2N), 7.1−7.9 (m, 7H, arom), 8.26 (s, 1H, arom); MS m/z 383 (46, M^+), 241 (base). Anal. ($C_{22}H_{26}ClN_3O$) C, H, N.

***N,N*-Di-*n*-propyl 3-benzoyl-propionamide.** A solution of the 3-benzoylpropionic acid (1 g, 6 mmol), *n*-dipropylamine (0.67 g, 6.6 mmol), triethylamine (0.91 g, 9 mmol), and EEDQ (1.78 g, 7.2 mmol) in THF (50 mL) was refluxed for 6 h. After cooling, evaporation of the solvent under reduced pressure gave a residue which was acidified with dilute HCl and neutralized with dilute NaOH. After washing with water, the organic phase was extracted with $CHCl_3$ (20 mL) and dried (Na_2SO_4) and the solvent removed by rotatory evaporation. The residue was purified by silica gel column chromatography [light petroleum ether/ethyl acetate, 8/2 (v/v), as eluent] to give 1.44 g (90%yield) of the required amide: IR (Nujol) 1685, 1636 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.86 (t, 3H, CH_3), 1.00 (t, 3H, CH_3), 1.1−1.8 (m, 4H, CH_2), 2.76 (t, 2H, CH_2), 3.1−3.5 (m, 6H, CH_2), 7.3−7.6 (m, 3H, arom), 7.9−8.1 (m, 2H, arom); MS m/z 261 (4, M^+), 161 (base). Anal. ($C_{16}H_{23}NO_2$) C, H, N. In similar way was prepared the ***N,N*-Di-*n*-propyl 3-(4-chloro)-benzoyl-propionamide**.Yield 98%. IR (Nujol) 1677, 1630 cm^{-1};^1H NMR ($CDCl_3$) δ 0.84(t, 3H, CH_3), 0.93 (t, 3H, CH_3), 1.3−1.8 (m, 4H, CH_2), 2.74 (t, 2H, CH_2), 3.1−3.4 (m, 6H, CH_2), 7.3−7.5 (m, 2H, arom), 7.8−8.1 (m, 2H, arom); MS m/z 295 (6, M^+), 195 (base). Anal. ($C_{16}H_{22}ClNO_2$) C, H, N.

***N,N*-Di-*n*-propyl-3-benzoyl-3-bromopropionamide.** To an ice-cooled solution of *N,N*-dipropyl-3-benzoylpropionamide (0.9 g, 3.4 mmol) in CCl_4 (40 mL) was slowly added a solution of bromine (0.5 g, 3.4 mmol) in CCl_4 (10 mL). After addition was complete, the solution was stirred at 0 °C under nitrogen for 1 h, and then at room temperature overnight at which point it was pale yellow. Evaporation of the solvent gave a residue which was purified by silica gel column chromatography [light petroleum ether/ethyl acetate, 9/1 (v/v), as eluent] to give 0.89 g (76% yield) of the title compound: IR (Nujol) 1681, 1622 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.84 (t, 3H, CH_3), 0.99 (t, 3H, CH_3), 1.4−1.8 (m, 4H, CH_2), 2.9−3.8 (m, 6H, CH_2), 5.6−5.8 (m, 1H, CHBr), 7.4−7.6 (m, 3H, arom), 8.0−8.2 (m, 2H, arom); MS m/z 339 (0.4, M^+), 105 (base). Anal. ($C_{16}H_{22}BrNO_2$) C, H, N. In similar way the ***N,N*-Di-*n*-propyl-3-bromo-3-(4-chlorobenzoyl)propionamide** was prepared: yield 93%; IR (Nujol) 1631, 1689 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.76 (t, 3H, CH_3), 0.91 (t, 3H, CH_3), 1.3−1.7 (m, 4H, CH_2), 2.8−3.7 (m, 6H, CH_2), 5.5−5.7 (m, 1H, CHBr), 7.3−7.6 (m, 2H, arom), 7.9−8.1 (m, 2H, arom) MS m/z 373 (0.5, M^+), 139 (base). Anal. ($C_{16}H_{21}BrClNO_2$) C, H, N.

General Procedure for Preparation of *N,N*-Dialkyl-(2-phenylimidazo[1,2-*a*]pyridin-3-yl)acetamides 7 (Method C). To a solution of the suitably substituted 2-aminopyridine **3** (11 mmol) in *n*-BuOH (50 mL) was added the appropriate *N,N*-di-*n*-propyl-3-bromo-3-benzoylpropionamide **11** (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. 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 **7**. In the cases **1** and **7i,k**, the condensation reaction was carried out in refluxing DMF. Physical data are summarized in Table 2.

***N,N*-Di-*n*-propyl-(2-phenylimidazo[1,2-*a*]pyridin-3-yl)-acetamide (7e):** IR (KBr) 1620 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.61 (t, 3H, CH_3), 0.79 (t, 3H, CH_3), 1.3−1.4 (m, 2H, CH_2), 1.4−1.5 (m, 2H, CH_2), 3.01 (t, 2H, CH_2N), 3.23 (t, 2H, CH_2N), 4.12 (s, 2H, CH_2CO), 6.8−6.9 (m, 1H, arom), 7.2−7.3 (m, 1H, arom), 7.3−7.5 (m, 1H, arom), 7.6−7.7 (m, 1H, arom), 8.3−8.4 (m, 1H, arom); MS m/z 335 (16, M^+), 207 (base). Anal. ($C_{21}H_{25}N_3O$) C, H, N.

***N,N*-Di-*n*-propyl-(2-phenyl-6-iodoimidazo[1,2-*a*]pyridin-3-yl)acetamide (7h):** IR (Nujol) 1620 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.69 (t, 3H, CH_3), 0.83 (t, 3H, CH_3), 1.1−1.7 (m, 4H, CH_2), 3.10 (t, 2H, CH_2N), 3.29 (t, 2H, CH_2N), 4.10 (s, 2H, CH_2CO), 7.2−7.7 (m, 7H, arom), 8.53 (d, J = 2 Hz, 1H, arom); MS m/z 461 (15, M^+), 333 (base). Anal. ($C_{21}H_{24}IN_3O$) C, H, N.

***N,N*-Di-*n*-propyl-(2-phenyl-6-methylimidazo[1,2-*a*]pyridin-3-yl)acetamide (7i):** IR (Nujol) 1643 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.50 (t, 3H, CH_3), 0.80 (t, 3H, CH_3), 1.1−1.7 (m, 4H, CH_2), 2.35 (t, 3H, CH_3), 2.80 (t, 2H, CH_2N), 3.10 (t, 2H, CH_2N), 4.00 (s, 2H, CH_2CO), 6.8−7.7 (m, 7H, arom), 8.10 (d, J = 2 Hz, 1H, arom); MS m/z 349 (12, M^+), 221 (base). Anal. ($C_{22}H_{27}N_3O$) C, H, N.

***N,N*-Di-*n*-propyl-(2-phenyl-6-methoxyimidazo[1,2-*a*]-pyridin-3-yl)acetamide (7j):** IR (KBr) 1638 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.67 (t, 3H, CH_3), 0.82 (t, 3H, CH_3), 1.4−1.6 (m, 4H, CH_2), 3.06 (t, 2H, CH_2N), 3.27 (t, 2H, CH_2N), 3.90 (s, 3H, OCH_3), 4.15 (s, 2H, CH_2CO), 7.32 (d, J = 9 Hz, 1H, arom), 7.4−7.5 (m, 3H, arom), 7.6−7.7 (m, 2H, arom), 8.02 (s, 1H, arom), 8.10 (d, J = 9 Hz, 1H, arom); MS m/z 365 (27, M^+), 237 (base). Anal. ($C_{22}H_{27}N_3O_2$) C, H, N.

***N,N*-Di-*n*-propyl-(2-phenyl-6-nitroimidazo[1,2-*a*]pyridin-3-yl)acetamide (7k):** IR (KBr) 1637 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.66 (t, 3H, CH_3), 0.73 (t, 3H, CH_3), 1.2−1.6 (m, 4H, CH_2), 3.06 (t, 2H, CH_2N), 3.16 (t, 2H, CH_2N), 4.03 (s, 2H, CH_2-CO), 7.3−7.7 (m, 5H, arom), 7.53 (d, J = 9 Hz, 1H, arom), 7.86 (dd, J = 9 and 2 Hz, 1H, arom), 9.23 (d, J = 2 Hz, 1H, arom); MS m/z 380 (57, M^+), 252 (base). Anal. ($C_{21}H_{24}N_4O_3$) C, H, N.

***N,N*-Di-*n*-propyl-(2-phenyl-6-bromo-8-methylimidazo-[1,2-*a*]pyridin-3-yl)acetamide (7s):** IR (KBr) 1612 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.70 (t, 3H, CH_3), 0.83 (t, 3H, CH_3), 1.2−1.8 (m, 4H, CH_2), 2.66 (s, 3H, CH_3-Ar), 3.10 (t, 2H, CH_2N), 3.30 (t, 2H, CH_2N), 4.06 (s, 2H, CH_2CO), 7.13 (d, J = 2 Hz, 1H, arom), 7.2−7.8 (m, 5H, arom), 8.36 (d, J = 2 Hz, 1H, arom); MS m/z 427 (15, M^+), 299 (base). Anal. ($C_{22}H_{26}BrN_3O$) C, H, N.

***N,N*-Di-*n*-propyl-(2-phenyl-6-methyl-8-bromoimidazo-[1,2-*a*]pyridin-3-yl)acetamide (7t):** IR (Nujol) 1623 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.66 (t, 3H, CH_3), 0.83 (t, 3H, CH_3), 1.2−1.6 (m, 4H, CH_2), 2.36 (s, 3H, CH_3-arom), 3.03 (t, 2H, CH_2N), 3.30 (t, 2H, CH_2N), 4.10 (s, 2H, CH_2CO), 7.2−7.8 (m, 6H, arom), 8.20 (d, J = 2 Hz, 1H, arom); MS m/z 427 (39, M^+), 275 (base). Anal. ($C_{22}H_{26}N_3O$) C, H, N.

***N,N*-Di-*n*-propyl-[2-(4-chlorophenyl)-6-chloroimidazo-[1,2-*a*]pyridin-3-yl]acetamide (1, alpidem):** mp 137−140 °C (lit.[13e] mp 138−142 °C; IR (Nujol) 1615 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.75 (t, 3H, CH_3), 0.83 (t, 3H, CH_3), 1.4−1.6 (m, 4H, CH_2), 3.11 (t, 2H, CH_2N), 3.27 (t, 2H, CH_2N), 4.02 (s, 2H, CH_2-CO), 7.2−7.6 (m, 6H, arom), 8.22 (d, J = 2 Hz, 1H, arom); MS m/z 403 (39, M^+), 275 (base). Anal. ($C_{21}H_{23}Cl_2N_3O$) C, H, N.

Stability Studies of 5i,o−r, 6d−f, 7e,k,o, and Alpidem 1. These experiments were carried out at 4 ± 0.5 °C by using brain membranes from male rats (180−200 g). A 50 μL suspension of rat brain preparation was incubated in triplicate with 40 μM solutions of each tested compound for 90 min in 50 mM Tris-HCl buffer (500 μL final volume). Then, the samples were diluted with 500 μL of cold acetonitrile and centrifugated at 12000*g* for 5 min. Aliquots of the supernatants were subjected to HPLC analysis. Compounds **5i,o−r**, **6d−f**, **7e,k,o**, and **1** were dissolved in 50% ethanol/Tris-HCl buffer. Zero-time determinations were performed by extracting the mixture with acetonitrile immediately following the mixture preparation. The degradation of the ester compounds

was quantified by measuring the peaks areas in relation to those of the initial peak at zero time. Average values of two separate experiments were reported. The stationary phase used was Novapack C18 (15 cm × 3.9 mm, 4 μm); methanol/water (80:20) was used as mobile phase with a flow rate of 1.0 mL/min. Detection and quantitation was performed at 250 nm. The degree of hydrolysis for **5i,o,p,q,r**, **6d,e,f** was 11%,-14%, 25%, 5%, 7%, 12%, 8%, 16%, respectively. The amides were found to be very stable (percent of hydrolysis < 5%).

Biological Methods. Radioligand Binding Assays. [^{3}H]Flunitrazepam Binding. [^{3}H]Flunitrazepam (New England Nuclear, Boston, MA) had a specific activity of 84.3 Ci/mmol and a radiochemical purity >99%. Male Wistar rats (180−200 g) (Charles River, Italy) were killed by decapitation, and whole brains (excluding cerebellum and pons medulla) were quickly removed. The brains were homogenized in 20 volumes of ice-cold 0.32 M sucrose with a Potter homogenizer. The homogenate was centrifuged for 5 min at 2000*g* at 4 °C, and the supernatant was centrifuged for 10 min at 40000*g* at 4 °C. The pellet was suspended in 30 mL of 50 mM Tris-HCl cold buffer, pH 7.4, and centrifuged for 30 min at 40000*g* at 4 °C. This pellet was suspended in 8−10 mL of Tris-HCl cold buffer. BZR binding activity was determined as follows: 50 μL of membrane suspension were incubated in triplicate with 0.67 nM [^{3}H]flunitrazepam and with 40 μM solutions of each tested compound for 90 min at 4 °C in 50 mM Tris-HCl cold buffer (500 μL final volume). After this incubation time, the samples were diluted with 5 mL of Tris-HCl cold buffer and immediately filtered under reduced pressure through glass-fiber filter disks (Wathman, GF/C) with a vacuum filtration manifold (Millipore, model 1225). The filters were washed with 5 mL of the same cold buffer, and the retained radioactivity was counted in pico vials in 4 mL of Ready Protein Beckman liquid scintillation cocktail. Compounds **5**−**7** were dissolved in 50% ethanol/Tris-HCl buffer and added to the assay mixture. Nonspecific binding was determined by incubating membranes and [^{3}H]flunitrazepam in the presence of 10 μM diazepam. Specific binding was obtained by subtracting nonspecific binding from total binding and was approximately 95% of the total binding. Six to eight concentrations of the compounds in triplicate were added to samples to determine IC_{50} values.

[^{3}H]Ro 5-4864 Binding. [^{3}H]Ro 5-4864 (New England Nuclear, Boston, MA) had a specific activity of 84.7 Ci/mmol and a radiochemical purity >99%. Male Wistar rats (180−200 g) (Charles River, Italy) were killed by decapitation. The kidneys were removed and homogenized with a Potter in 20 volumes of ice-cold 0.32 M sucrose. The homogenate was centrifuged at 2000*g* for 5 min, and the supernatant was centrifuged at 40000*g* for 10 min at 4 °C. The membranes were suspended and lysed in 30 mL of 50 mM Tris-HCl cold buffer (pH 7.4) and centrifuged at 40000*g* for 30 min at 4 °C.[24] The resulting pellet was suspended in 7−8 mL of 50 mM Tris-HCl cold buffer. Studies of [^{3}H]Ro 5-4864 binding activity of kidney mitochondrial preparation were performed as follows: 50 μL of mitochondrial suspension (200−250 μg of protein) was incubated in triplicate with 0.9 nM [^{3}H]Ro 5-4864 and with 40 μM of each tested compound for 90 min at 4 °C in a total volume of 500 μL of 50 mM Tris-HCl cold buffer. After incubation, samples were diluted with 5 mL of Tris HCl cold buffer and immediately filtered under reduced pressure through glass-fiber filter disks (Wathman, GF/C) with a vacuum filtration manifold (Millipore, model 1225). The filters were rinsed with 5 mL of the same Tris buffer, and the retained radioactivity was determined in pico vials in 4 mL of Ready Protein Beckman liquid scintillation cocktail. Compounds **5**−**7** were dissolved in 50% ethanol/Tris-HCl buffer and added to the assay mixture. Blank sample was carried out in the same conditions to determine the effect of ethanol on the total binding. Non specific binding was defined as binding of [^{3}H]-Ro 5-4864 in the presence of 10 μM diazepam. Specific binding was obtained by subtracting nonspecific binding from the total binding and was approximately 90% of the total binding. Six to eight concentrations of the drugs in triplicate were used to determine IC_{50} values with an iterative curve-fitting program.

Protein concentration was assayed by the method of Lowry[25] with bovine serum as standard. Biochemical data were analyzed using Student's "*t*" test and IC_{50} were determined from displacement curves with the LIGAND program.[26]

Electrophysiological Studies Using *Xenopus* Oocytes. The cDNAs encoding the human α_1, α_2, α_5, β_2 and γ_{2s} $GABA_A$ receptor subunits were subcloned into the pCDM8 expression vector (Invitrogen, San Diego, CA).[27] Plasmids were purified with the Promega Wizard Plus Miniprep DNA Purification System (Madison, Wl) and then resuspended in sterile distilled water, divided into portions, and stored at −20 °C until used for injection. Adult *Xenopus laevis* females were obtained from Dipl. Biol.-Dipl Ing. Horst Kähler (Hamburg, Germany). Oocyte isolation and cDNA microinjection were performed essentially as previously described.[28] Isolated oocytes were 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 $Ca(NO_3)_2$. Various mixtures of $GABA_A$ receptor subunit cDNAs (1.5 ng of each in a total volume of 30 nL) were injected into the nucleus of oocytes by the "blind" method. The injected oocytes were cultured at 19 °C in sterile MBS supplemented with streptomycin (10 μg/mL), penicillin (10 units/mL), gentamicin (50 μg/mL), 0.5 mM theophylline, and 2 mM sodium pyruvate. Recordings were obtained 1−4 days after injection from oocytes placed in a 100-μL rectangular chamber. The animal pole of oocytes was impaled with two glass electrodes (0.5−3 MΩ) fllled with filtered 3 M KCl and the voltage was clamped at −70 mV with an Axoclamp 2-B amplifier (Axon Instrurments, Burlingame, CA). Currents were continuously recorded on a strip-chart recorder. Resting membrane potential usually varied between −30 and −50 mV. Drugs were perfused for 20 s unless otherwise noted. Intervals of 5−10 min were allowed between drug applications.

Statistical Analysis. Currents were expressed as a percentage of the control response (in nanoamps) obtained with GABA alone. A GABA control response was obtained before and after each drug application to take into account possible shifts in the control currents. Oocytes from at least two frogs were used for each experiment, with the total number of oocytes corresponding to the *n* value. Data are presented as means ± SEM and were analyzed by Student's *t* test or by one or two-way analysis of variance (ANOVA) followed by Scheffe's post hoc test.

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