# Synthesis, Structure−Activity Relationships at the $GABA_A$ Receptor in Rat Brain, and Differential Electrophysiological Profile at the Recombinant Human $GABA_A$ Receptor of a Series of Substituted 1,2-Diphenylimidazoles

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A series of new 1,2-diphenylimidazole derivatives (**1a**−**x**) were synthesized and evaluated for their ability to potentiate $\gamma$-aminobutyric acid (GABA)-evoked currents in *Xenopus laevis* oocytes expressing recombinant human $GABA_A$ receptors. Many of these compounds enhanced GABA action with potencies ($EC_{50}$ = 0.19−19 $\mu$M) and efficacies (maximal efficacies of up to 640%) similar to or greater than those of anesthetics such as etomidate, propofol, and alphaxalone. Structure−activity relationship analysis revealed that the presence of an ester moiety in the imidazole ring was required for full agonist properties, while modifications made in the phenyl rings affected potency and efficacy, with ethyl 2-(4-bromophenyl)-1-(2,4-dichlorophenyl)-1*H*-4-imidazolecarboxylate showing the highest potency. These compounds potentiated the [$^3$H]-GABA binding to rat brain membranes, suggesting a site of interaction different from that of GABA. As for etomidate, mutation of asparagine-265 in the $\beta$2 subunit of the $GABA_A$ receptor into serine reduced the ability of derivative **1i** to modulate the GABA function.

## Introduction

$\gamma$-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the vertebrate central nervous system and interacts physiologically with two types of receptors, designated $GABA_A$ and $GABA_B$. $GABA_A$ receptors are ligand-activated $Cl^-$ channels, whereas $GABA_B$ receptors are coupled to G proteins.[1,2] Activation of the $GABA_A$ receptor by the binding of GABA results in an increase in the $Cl^-$ conductance of the neuronal membrane, consequent membrane hyperpolarization, and a reduction in neuronal excitability.[3] The $GABA_A$ receptor is a pentamer assembled from a large number of potential subunits ($\alpha$1−6, $\beta$1−3, $\gamma$1−3, $\delta$, $\epsilon$, $\rho$1−3, $\pi$, and $\theta$).[2,4] Most $GABA_A$ receptors are heterooligomers that contain $\alpha$, $\beta$, and $\gamma$ subunits with a stoichiometry of 2:1:2 or 2:2:1.[2]

In addition to the GABA-binding site, $GABA_A$ receptors contain a large number of allosteric modulatory sites for structurally unrelated compounds, including benzodiazepines, barbiturates, steroids, avermectins, picrotoxin, and loreclezole, that regulate receptor affinity for GABA.[5−8] Benzodiazepines exhibit a continuum of intrinsic activity, ranging from full agonists (positive allosteric modulators of GABA-evoked $Cl^-$ currents with anxiolytic, hypnotic, and anticonvulsant activities) through antagonists devoid of intrinsic activity to inverse agonists (negative allosteric modulators of GABA-evoked $Cl^-$ currents with proconvulsant, convulsant, and anxiogenic activities).[9]

The $GABA_A$ receptor is also a major target of several chemically unrelated volatile or injectable general anesthetics such as halothane, isoflurane, pentobarbital, etomidate, propofol, and alphaxalone. These compounds enhance GABA-evoked $Cl^-$ currents at both neuronal and recombinant $GABA_A$ receptors[10−12] and exhibit specific structure−activity relationships (SARs) and stereoselectivity in this regard.[10,13−15] In addition, enhancement of the $GABA_A$ receptor function by many of these compounds may depend on the receptor subunit composition. Moreover, site-directed mutagenesis has revealed that specific amino acids are important determinants of the action of several GABA-ergic drugs and in particular of volatile or intravenous anesthetics.[16]

A variety of other agents, such as cocaine and clozapine, known for their effects at other receptors, also interact with and affect the function of $GABA_A$ receptors at pharmacologically relevant concentrations. These compounds may provide structural leads for the development of more specific $GABA_A$ receptor ligands.[7]

In the course of a research program aimed at obtaining antipsychotic agents with clozapine-like activities, we recently developed a series of (1,2-diphenylimidazolyl)piperazine derivatives that are endowed with substantial affinities for both $D_2$ dopamine receptors as well as 5-$HT_{1A}$ and 5-$HT_{2A}$ serotonin receptors, compound **I** of which is representative (Chart 1). Compound **I** was also found to inhibit in a concentration-dependent manner (0.1−300 $\mu$M) GABA-evoked $Cl^-$ currents in *Xenopus laevis* oocytes expressing recombinant human $GABA_A$ receptors composed of $\alpha$1, $\beta$2, and $\gamma$2L subunits.[17] This finding prompted us to determine whether the 1,2-diphenylimidazole framework of **I** might serve

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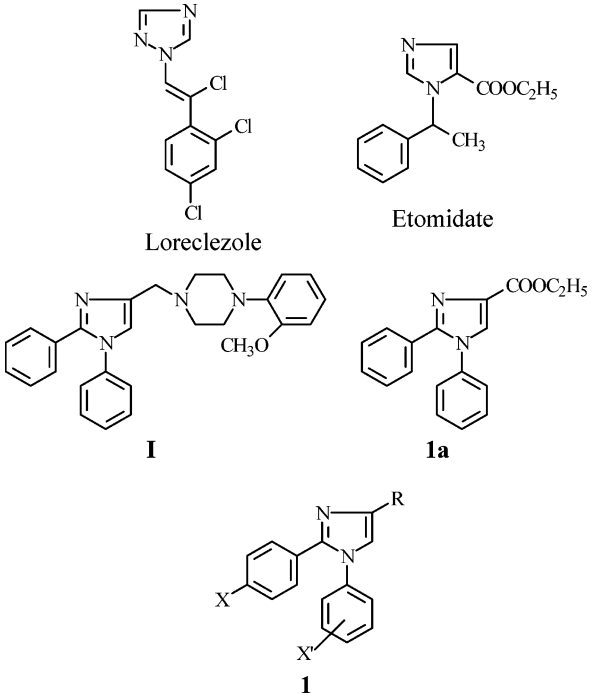
**Chart 1**




as the basis for development of a new series of more specific allosteric modulators of the $GABA_A$ receptor.

Ethyl 1,2-diphenyl-1*H*-4-imidazolecarboxylate (**1a**), an intermediate in our synthesis of **I**, was first selected as a potential template for this investigation. We now show that **1a** and several analogues are effective positive allosteric modulators of human recombinant $GABA_A$ receptors with potencies and efficacies similar to or greater than those of anesthetics such as etomidate, propofol, and alphaxalone.

## Chemistry

Title compounds **1** (Table 1) were synthesized according to the reaction pathways shown in Schemes 1−4. The procedure for preparation of compounds **1b**−**f** and **1h**−**n** (Scheme 1) is the same as that previously described[17] for compounds **1a,g** (Table 1). In brief, the carbinol intermediates **3** were synthesized from appropriate *N*-phenylbenzamidines **2** with ethylbromopyruvate in the presence of $NaHCO_3$ and were then dehydrated in the presence of *p*-toluenesulfonic acid to give the corresponding esters **1** in almost quantitative yields. The alkylation−cyclization reaction yields either only carbinol (**3b**,**e**,**f**) or a mixture of carbinol intermediate and target imidazole (**3h**−**n** and **1h**−**n**, respectively). In the case of **1c** and **1d**, no trace of carbinol was detected and the reaction proceeded directly to the desired imidazole. The amidines **2** were synthesized in satisfactory yields from appropriate benzonitrile and aniline compounds in toluene via trimethylaluminum amide generated in situ.

The nitro-substituted compound **1m** (Scheme 2) was reduced by $SnCl_2$−HCl to aniline derivative **1o**, which was converted either to iodide **1p** by reaction of its diazonium salt with KI or to amide **1q** by treatment with acetic anhydride in tetrahydrofuran (THF).

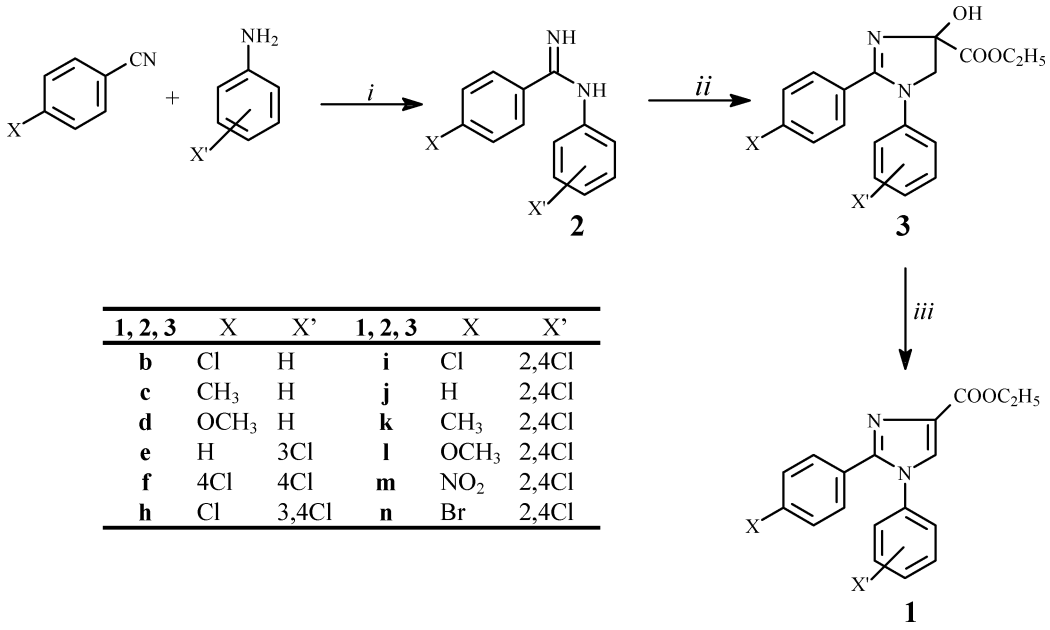
The 1,2-diphenylimidazoles containing H (**1r**) or $CH_3$ (**1s**) at position 4 of the imidazole ring (Table 1) were synthesized in a three-step process (Scheme 3) from commercially available 2-phenylimidazole or 2-phenyl-4-methylimidazole. Aromatic nucleophilic substitution of 4-fluoronitrobenzene with 2-phenylimidazoles in

**Table 1.** Potency ($EC_{50}$) and Maximal Efficacy of **1a-x**, **8**, Propofol, Etomidate, and Alphaxalone for Modulation of GABA-Evoked $Cl^-$ Currents in *Xenopus* Oocytes Expressing Recombinant Human $GABA_A$ Receptors[a]

| compd | R | X | X′ | $EC_{50}$ ($\mu$M) | maximal efficacy (%) |
|---|---|---|---|---|---|
| **1a** | $COOC_2H_5$ | H | H | 6.4 ± 1.9 | 638 ± 7 |
| **1b** | $COOC_2H_5$ | Cl | H | 4.2 ± 1.1 | 458 ± 20 |
| **1c** | $COOC_2H_5$ | $CH_3$ | H | 4.1 ± 1.1 | 625 ± 12 |
| **1d** | $COOC_2H_5$ | $OCH_3$ | H | 9.1 ± 1.1 | 369 ± 25 |
| **1e** | $COOC_2H_5$ | H | 3-Cl | 3.0 ± 1.2 | 350 ± 27 |
| **1f** | $COOC_2H_5$ | Cl | 4-Cl | 1.5 ± 1.1 | 302 ± 9 |
| **1g** | $COOC_2H_5$ | F | 4-F | 3.1 ± 1.2 | 569 ± 38 |
| **1h** | $COOC_2H_5$ | Cl | 3,4-Cl | 2.3 ± 1.7 | 521 ± 33 |
| **1i** | $COOC_2H_5$ | Cl | 2,4-Cl | 0.6 ± 1.3 | 390 ± 22 |
| **1j** | $COOC_2H_5$ | H | 2,4-Cl | 1.8 ± 1.7 | 508 ± 32 |
| **1k** | $COOC_2H_5$ | $CH_3$ | 2,4-Cl | 1.0 ± 1.6 | 568 ± 42 |
| **1l** | $COOC_2H_5$ | $OCH_3$ | 2,4-Cl | 1.9 ± 1.5 | 362 ± 27 |
| **1m** | $COOC_2H_5$ | $NO_2$ | 2,4-Cl | 2.4 ± 1.0 | 413 ± 32 |
| **1n** | $COOC_2H_5$ | Br | 2,4-Cl | 0.19 ± 2.1 | 388 ± 57 |
| **1o** | $COOC_2H_5$ | $NH_2$ | 2,4-Cl | 4.3 ± 1.1 | 406 ± 19 |
| **1p** | $COOC_2H_5$ | I | 2,4-Cl | 0.45 ± 1.8 | 180 ± 37 |
| **1q** | $COOC_2H_5$ | $NHCOCH_3$ | 2,4-Cl | 19 ± 1.5 | 209 ± 41 |
| **1r** | H | H | H | ND | −5.3 ± 2 |
| **1s** | $CH_3$ | H | H | ND | −25 ± 20 |
| **1t** | $COOCH_3$ | Br | 2,4-Cl | 3.4 ±1.6 | 258 ± 33 |
| **1u** | $COOC_3H_7$ | Br | 2,4-Cl | 1.7 ± 2 | 537 ± 82 |
| **1v** | $COOCH_2CH_2N(CH_2)_5$ | Br | 2,4-Cl | ND | −14 ± 4 |
| **1w** | $CON(C_2H_5)_2$ | Cl | 2,4-Cl | 4.4 ± 2.8 | 32.4 ± 2 |
| **1x** | $CON(CH_2CH_2)_2NCH_3$ | Cl | 2,4-Cl | 1.3 ± 2.2 | 58.2 ± 12.6 |
| **8** | COOH | Cl | 2,4-Cl | 8.7 ± 2.5 | 256 ± 25 |
| propofol | | | | 11.2 ± 1.2 | 461 ± 39 |
| etomidate | | | | 3.5 ± 1.1 | 501 ± 60 |
| alphaxalone | | | | 2.0 ± 1.5 | 322 ± 38 |
| pentobarbital | | | | 50.0 ± 1.1 | 214.5 ± 25 |

[a] See Chart 1 for the structure of compounds **1**. ND = not determinable.

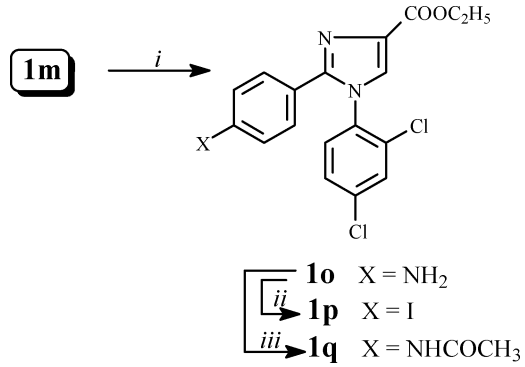
**Scheme 1**[a]



| 1, 2, 3 | X | X' | 1, 2, 3 | X | X' |
|---|---|---|---|---|---|
| **b** | Cl | H | **i** | Cl | 2,4Cl |
| **c** | $CH_3$ | H | **j** | H | 2,4Cl |
| **d** | $OCH_3$ | H | **k** | $CH_3$ | 2,4Cl |
| **e** | H | 3Cl | **l** | $OCH_3$ | 2,4Cl |
| **f** | 4Cl | 4Cl | **m** | $NO_2$ | 2,4Cl |
| **h** | Cl | 3,4Cl | **n** | Br | 2,4Cl |

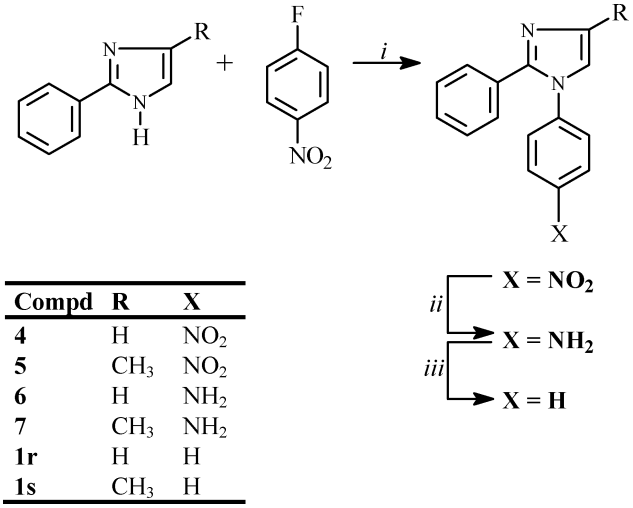
[a] Reagents: (i) $Al(CH_3)_3$, toluene; (ii) $BrCH_2COCOOC_2H_5$, $NaHCO_3$, *i*PrOH; (iii) *p*-TsOH, toluene.

**Scheme 2**[a]



[a] Reagents: (i) $SnCl_2 \cdot 2H_2O$, concentrated HCl, $CH_3OH$; (ii) $NaNO_2$, HCl, KI; (iii) acetic anhydride, THF.

**Scheme 3**[a]

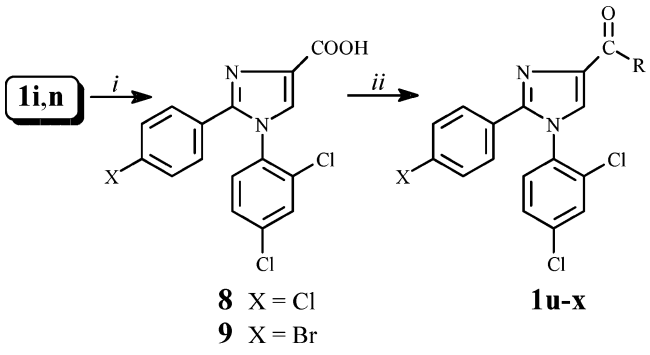


| Compd | R | X |
|---|---|---|
| **4** | H | $NO_2$ |
| **5** | $CH_3$ | $NO_2$ |
| **6** | H | $NH_2$ |
| **7** | $CH_3$ | $NH_2$ |
| **1r** | H | H |
| **1s** | $CH_3$ | H |

[a] Reagents: (i) $K_2CO_3$, DMF; (ii) $SnCl_2 \cdot 2H_2O$, concentrated HCl, $CH_3OH$; (iii) $NaNO_2$, HCl, $H_3PO_2$.

$K_2CO_3$ afforded the nitro intermediates **4** and **5** in high yields. Reduction of the nitro group with $SnCl_2$−HCl gave aniline derivatives **6** and **7**, which were allowed to react with $NaNO_2$ and $H_3PO_2$ to give compounds **1r** and **1s**.

**Scheme 4**[a]



| Compd | R | X |
|---|---|---|
| **1u** | $OC_3H_7$ | Br |
| **1v** | $OCH_2CH_2N$-$(CH_2)_5$ | Br |
| **1w** | $N(C_2H_5)_2$ | Cl |
| **1x** | N-$(CH_2CH_2)_2$-N-$CH_3$ | Cl |

[a] Reagents: (i) NaOH, $H_2O/CH_3OH$; (ii) $(COCl)_2$/DMF, $CH_2Cl_2$, TEA; secondary amine, *n*PrOH, or $(CH_2)_4NCH_2CH_2OH$.

The esters **1i** and **1n** were hydrolyzed, and the resulting acids **8** and **9** (Scheme 4) were converted to the corresponding acid chlorides, which were then reacted with appropriate alcohols or secondary amines to give, respectively, esters **1u** and **1v** and carboxamides **1w** and **1x**. Analogue **1t** (Table 1), which was considered useful for SAR analysis, was obtained by treatment of **1v** with $CHCl_3$−$CH_3OH$. This result can be easily explained assuming a transesterification reaction of **1v**.[18]

## Results and Discussion

**Electrophysiology with *Xenopus* Oocytes Expressing Human $GABA_A$ Receptors.** We first used electrophysiology to screen all compounds for the ability

to modulate GABA-evoked $Cl^-$ currents in *X. laevis* oocytes expressing recombinant human $GABA_A$ receptors ($\alpha2\beta2\gamma2L$). Most of the 1,2-diphenylimidazole derivatives enhanced the action of GABA at the $EC_{10}$ (the concentration of GABA that elicits a response that is 10% of the maximal effect observed with 10 mM GABA) in a concentration-dependent manner, with $EC_{50}$ values ranging from 0.19 to 19 $\mu$M and maximal efficacies of up to 638% (Table 1). In many instances, these values were similar to or greater than those of the anesthetics etomidate, propofol, and alphaxalone, which were included in the screening for comparison purposes. The parent compound (**1a**) thus manifested an efficacy (638%) higher than those of all three of these anesthetics and an $EC_{50}$ (6.4 $\mu$M) most similar to that of etomidate.

For SAR studies, we introduced several substituents at the phenyl groups attached to positions N-1 and C-2 of the imidazole ring of **1a**, exploring the effects of substituents such as halogens (F, Cl, Br, I), $CH_3$, $OCH_3$, $NO_2$, $NH_2$, and $NHCOCH_3$. Congeners **1b**−**d** are *para*-substituted derivatives at the C-2 phenyl ring, whereas **1e** is an example of a *meta* substitution at the N-1 phenyl ring. Among these analogues, compounds **1b**, **1c**, and **1e** possessed a higher potency ($EC_{50}$ of 3.0−4.2 $\mu$M) than did **1a**, whereas that of the *p*-methoxy congener **1d** was reduced ($EC_{50}$ of 9.1 $\mu$M). This finding is indicative of a positive influence of substituent lipophilicity. The *para*-dichlorinated derivative **1f** exhibited a greater potency ($EC_{50}$ of 1.5 $\mu$M) than did **1b**, whereas the difluorinated analogue **1g** possessed a potency ($EC_{50}$ of 3.1 $\mu$M) similar to those of **1b**, **1c**, and **1e**. The introduction into **1f** of an additional chlorine atom in the *ortho* position of the N-1 phenyl ring to give analogue **1i** yielded a further improvement in potency ($EC_{50}$ of 0.6 $\mu$M), whereas moving the same substituent from the *ortho* to the *meta* position, to give positional isomer **1h**, resulted in a decrease in potency ($EC_{50}$ of 2.3 $\mu$M). This result is indicative of a negative influence of the substituent steric effect (influence of the substituent size).

Maintaining the 2,4-dichloro substitution pattern in **1i**, we next investigated the *para* position of the C-2 phenyl ring, either by reintroduction of $CH_3$ or $OCH_3$ groups or by exploring new substituents such as $NO_2$, Br, $NH_2$, I, and $NHCOCH_3$ (**1k**−**q**). The potency of the *para*-unsubstituted congener **1j** ($EC_{50}$ of 1.8 $\mu$M) was reduced compared with that of **1i** but was similar to that of its positional isomer **1f**. The presence of a methyl group in **1k** ($EC_{50}$ of 1.0 $\mu$M) resulted in a slight decrease in potency compared with that of **1i**, whereas the presence of a methoxy group in **1l** ($EC_{50}$ of 1.9 $\mu$M) was greater tolerated than was that in **1d**. The *p*-nitro derivative **1m** exhibited a reduced potency ($EC_{50}$ of 2.4 $\mu$M), whereas the *para*-substituted bromine (**1n**) and iodine (**1p)** compounds showed the highest potencies among all derivatives tested in this study, with $EC_{50}$ values of 0.19 and 0.45 $\mu$M, respectively. Introduction of the hydrophilic and electron-donating amino group (**1o**) resulted in a marked decrease in potency ($EC_{50}$ of 4.3 $\mu$M), whereas the acetylamino derivative **1q** possessed a lower potency ($EC_{50}$ of 19 $\mu$M) than did all of the derivatives **1a**−**p**. Despite its hydrophilic character, both the potency and efficacy of compound **1o** were similar to those of etomidate.

**Table 2.** Potency ($EC_{50}$) of Compounds **1a**, **1b**, **1f**, **1g**, **1i**, and **8** for Enhancement of [$^3$H]GABA Binding to Rat Cerebrocortical Membranes[a]

| compd | $EC_{50}$ (nM) | compd | $EC_{50}$ (nM) |
|---|---|---|---|
| **1a** | 12390 ± 87.3 | **1g** | 2087 ± 102.5 |
| **1b** | 1591 ± 62.8 | **1i** | 87.79 ± 8.9 |
| **1f** | 684 ± 43.4 | **8** | ND |

[a] ND = not determinable.

To evaluate the importance of the ester function of the parent compound (**1a**) for modulatory activity, we synthesized the analogue **1r**, which lacks the 4-$COOC_2H_5$ group, and the 4-$CH_3$ derivative **1s**. Neither of these analogues enhanced GABA-evoked $Cl^-$ currents, although a small inhibitory effect was observed. These results suggest that the carbonyl oxygen might participate in a hydrogen-bond-mediated interaction that is essential for positive modulatory activity. The carboxylic acid derivative **8** retained the ability to enhance $GABA_A$ receptor function. Although this enhancement by **8** occurred with a lower potency ($EC_{50}$ of 8.7 $\mu$M) and efficacy (256%) compared with those of other 1,2-diphenylimidazole derivatives, these values were similar to those of the intravenous anesthetics propofol and alphaxalone.

Homologation of the carboxyethyl function in **1n** ($EC_{50}$ of 0.19 $\mu$M), to give compounds **1t** ($EC_{50}$ of 3.4 $\mu$M) and **1u** ($EC_{50}$ of 1.7 $\mu$M), revealed that the carboxyethyl function is optimal in terms of potency. The introduction into the ester function of **1n** of a basic nitrogen to give the amino ester **1v** abolished positive modulatory activity at the $GABA_A$ receptor, likely because of an unfavorable effect of the protonated site (at physiological pH).

Isosteric replacement of the ester function in **1i** by a carboxamide group to give **1w** was detrimental for modulatory activity (efficacy of 32.4%), and a similar effect was observed for the amino amide derivative **1x**. The lack of activity of the amide **1w** when compared to the ester **1i** could be ascribed to conformational factors; the amide might adopt an unfavorable binding conformation due to the reduced freedom of OC−N bond rotation. Negative steric effects due to the second ethyl group could also be invoked to account for the difference in activity.

**Receptor-Binding Assays.** Given that many of the 1,2-diphenylimidazole derivatives tested in our study were potent positive modulators of GABA-evoked $Cl^-$ currents mediated by recombinant human $GABA_A$ receptors, we also assessed the ability of some of these compounds to affect the binding of [$^3$H]GABA to rat cerebral cortical membranes (Table 2). All compounds tested, with the exception of the carboxylic acid derivative **8**, enhanced [$^3$H]GABA binding, with $EC_{50}$ values ranging from 88 to 12390 nM. The potency of these compounds for enhancement of [$^3$H]GABA binding appeared to correlate with that determined for potentiation of GABA-evoked $Cl^-$ currents, suggesting that these derivatives affect the function of $GABA_A$ receptors in an allosteric manner through interaction with a site distinct from the GABA-binding site.

In the present study, we have thus investigated a series of 1,2-diphenylimidazole derivatives for the ability to modulate GABA-evoked $Cl^-$ currents in *Xenopus* oocytes expressing recombinant human $GABA_A$ receptors, and we found that many of these compounds act

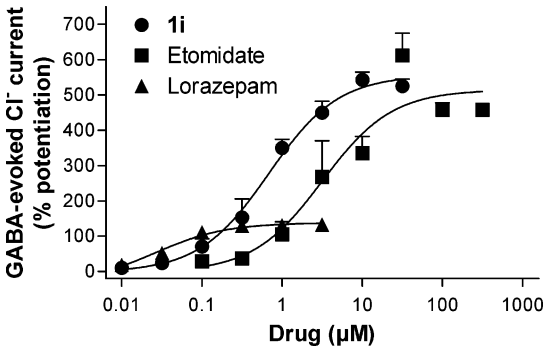



**Figure 1.** Concentration−response curves for the potentiation by compound **1i**, etomidate, and lorazepam of GABA-induced $Cl^-$ currents in *Xenopus* oocytes expressing recombinant human $GABA_A$ receptors. Data are expressed as percentage potentiation of the response induced by GABA at the $EC_{10}$ value and are means ± SEM of values from 8−12 oocytes from two or more batches.

as positive allosteric modulators at these receptors. The potency and efficacy for enhancement of $GABA_A$ receptor function by several of the tested compounds were higher than those of benzodiazepines such as lorazepam as well as those of the general anesthetic etomidate (Figure 1), which, among previously identified $GABA_A$ receptor modulators, is most related structurally to our 1,2-diphenylimidazoles (Chart 1). The 1,2-diphenylimidazole scaffold thus appears to be appropriate for the development of potent and efficacious allosteric modulators of $GABA_A$ receptor function. Our SAR analysis for this class of compounds revealed that the presence of the ester moiety in the imidazole core is required for full agonist properties. Moreover, receptor modulatory activity is greatly affected by the pattern of chemical substitution on the phenyl rings at positions N-1 and C-2 of the imidazole ring. Combination of the 2,4-dichloro substitution pattern on the N-1 phenyl ring with a *p*-bromophenyl group at C-2 (**1n**) gave the highest potency among all derivatives tested.

In vitro radioligand displacement assays with rat brain membranes revealed that the 1,2-diphenylimidazole derivatives do not bind to the GABA recognition site but rather enhance $GABA_A$ receptor function in an allosteric manner through interaction with a distinct binding site. In addition, these compounds did not act at the benzodiazepine-binding site. In fact, flumazenil was not able to block the enhancement of the $GABA_A$ receptor function induced by **1i**.[19] Electrophysiological studies performed in *X. laevis* oocytes expressing $\alpha1\beta1\gamma2L$ and $\alpha1\beta2\gamma2L$ have demonstrated that **1i**, like the general anesthetic etomidate, is selective for $GABA_A$ receptors containing $\beta2$ subunits.[19] In addition, mutation of asparagine-265 of the $\beta3$ subunit of the $GABA_A$ receptor to serine was previously shown to result in a marked reduction in the extent of potentiation of the GABA response by the general anesthetic etomidate.[16] Given that the $\beta$ subunit selectivity of etomidate is determined by a single amino acid, asparagine 265 in $\beta2$ and $\beta3$ and serine in $\beta1$, we mutated asparagine 265, located in the transmembrane domain 2 (TM2),

| | |
|---|---|
| β1 245 | DASAARVALGITTVLTMTTI**S**THLRETL |
| β2 245 | DASAARVALGITTVLTMTTI**N**THLRETL |

of the $\beta2$ subunit to serine, and we expressed recombinant $\alpha1\beta1\gamma2L$, $\alpha1\beta2\gamma2L$, or $\alpha1\beta2(N265S)\gamma2L$ $GABA_A$ receptors in *X. laevis* oocytes. Mutation of asparagine-265 of the $GABA_A$ receptor $\beta2$ subunits to serine greatly reduced the potentiation of the $GABA_A$ receptor function induced by **1i**. This reduction was similar to the one induced in mutated $\alpha1\beta2(N265S)\gamma2L$ $GABA_A$ receptors by the general anesthetic etomidate (Figure 2) and suggests that derivative **1i** may bind to the same binding site of etomidate.

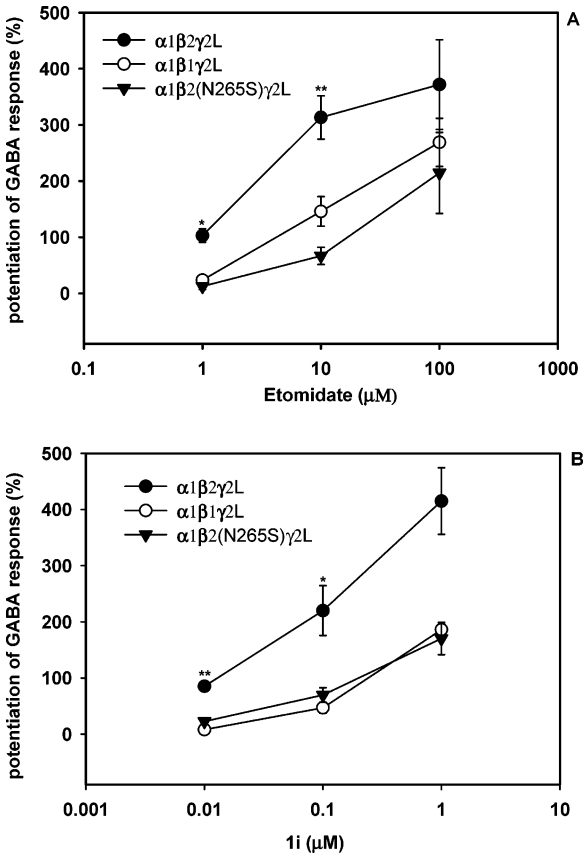



**Figure 2.** Concentration dependence of the potentiation of GABA-induced $Cl^-$ current by etomidate (***A***) or **1i** (B) in *Xenopus* oocytes expressing $\alpha1\beta1\gamma2L$, $\alpha1\beta2\gamma2L$, or $\alpha1\beta2(N265S)\gamma2L$ combinations of $GABA_A$ receptor subunits. Data are expressed as percentage potentiation of the response to GABA at the $EC_{10}$ and are means ± SEM of values from 3−8 oocytes. A single asterisk indicates $p < 0.05$ and double asterisks indicate $p < 0.001$ versus the corresponding values for $\alpha1\beta1\gamma2L$ or $\alpha1\beta2(N265S)\gamma2L$ receptors.

Finally, compound **1i** was chosen to assess the behavioral effects of the 1,2-diphenylimidazole derivatives. This compound induced a reversible loss of the righting reflex (an effect often used to evaluate anesthesia) in *Xenopus* tadpoles with an $EC_{50}$ of 1.2 $\mu$M (Figure 3).This value of $EC_{50}$ is comparable to the one previously found for propofol ($EC_{50}$ of 1.9 ± 0.2 $\mu$M)[20] and for etomidate ($EC_{50}$ of 2.3 ± 0.13 $\mu$M).[15] Again this result suggests that **1i** as well as other 1,2-diphenylimidazole derivatives may constitute a new class of positive modulators of $GABA_A$ receptors with a molecular mechanism comparable to that of etomidate.

Etomidate is one of the most potent general anesthetics employed in clinical practice. The *R*-(+) enantiomer, which is the one used in the clinical formulation,[15] is an order of magnitude more potent than the *S*-(−) enantiomer. This enantioselectivity affords a valuable tool to assess the fundamental mechanisms underlying

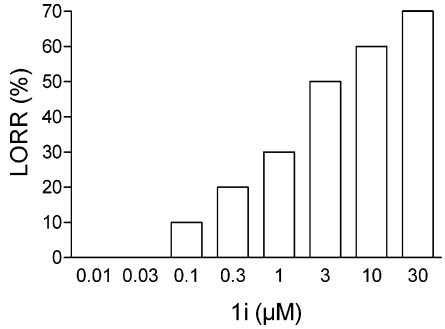



**Figure 3.** Anesthetic action of compound **1i** in *Xenopus* tadpoles. The anesthetic effect of compound **1i** (0.01−30 $\mu$M) was assessed from the percentage of tadpoles ($n$ = 10) that manifested loss of the righting reflex (LORR) at each drug concentration.

anesthesia. However, from a chemical and pharmacological point of view, the development of a single enantiomer is more difficult since it is necessary to prevent possible racemization or it requires extensive pharmacological research.[21] A distinct advantage of the new 1,2-diphenylimidazoles described in this paper is that they do not possess any chiral center, and this feature could facilitate their development.

## Experimental Section

**Chemistry.** Reactions were monitored by TLC with Polygram SIL and ALOX N/$UV_{254}$ precoated plastic sheets (0.2 mm), with detection by exposure to iodine vapor or UV light. Pure compounds yielded a single spot on TLC. Flash chromatography was performed with Merck silica gel type 60 (size 230−240 mesh ASTM). Melting points were determined with a Kofler hot-stage microscope and are uncorrected. Infrared spectra were recorded with a Perkin-Elmer Paragon 500 FT IR spectrophotometer (KBr pellets for solid samples). $^1$H NMR spectra were recorded with the use of a Varian XL 200 FT NMR spectrometer, with $CDCl_3$ as solvent and tetramethylsilane as internal standard. Chemical shifts are expressed in $\delta$ (ppm) downfield from tetramethylsilane, and coupling constants ($J$) are expressed in hertz. Mass spectroscopy was performed with an Agilent 1100 series LC/MSD spectrometer. Measurements were performed in the positive or negative ion mode [($MH^+$) or ($M - H)^-$], with an atmospheric pressure ionization electrospray (API-ES). Electron ionization mass spectra (70 eV) were recorded with a Hewlett-Packard 5790-5970 MSD gas chromatograph−mass spectrometer. Elemental analyses were performed with a Perkin-Elmer 2400 analyzer, and results were within ±0.40% of the theoretical values. All moisture-sensitive reactions were performed under a nitrogen atmosphere, with the use of oven-dried glassware and syringes to transfer solutions. Organic extracts were dried over anhydrous $MgSO_4$ prior to solvent evaporation. All starting materials and reagents were obtained from Aldrich.

**Synthesis of Ethyl 1,2-Diphenyl-1*H*-4-imidazolecarboxylates 1.** Ethyl 1,2-diphenyl-1*H*-4-imidazolecarboxylates **1b**−**f**,**h**−**n** (Scheme 1) were synthesized according to the procedure applied to compounds **1a**,**g**.[17]

**Ethyl 2-(4-Aminophenyl)-1-(2,4-dichlorophenyl)-1*H*-4-imidazolecarboxylate (1o).** To a solution of **1m** (0.8 g, 1.97 mmol) in $CH_3OH$ (12 mL) and concentrated HCl (12 mL) was added at 0 °C $SnCl_2 \cdot 2H_2O$ (2.22 g, 9.75 mmol), and the reaction mixture was stirred for 2 h. The reaction mixture was basified (to pH 14) with a 3 N aqueous solution of NaOH and extracted with AcOEt. The combined extracts were dried and evaporated to give **1o** (0.73 g, 98%) as a white powder. Mp: 217−219 °C ($CHCl_3/CH_3OH$). TLC (AcOEt/petroleum ether, 8:2): $R_f$ = 0.55. IR (KBr): 3424, 3300, 3206, 1679, 1585 cm$^{-1}$. $^1$H NMR ($CDCl_3$): $\delta$ 1.40 (t, $J$ = 7.2 Hz, 3H, $CH_3$), 3.81 (br s, 2H, $ArNH_2$, $D_2O$ exchanged), 4.42 (q, $J$ = 7.2 Hz, 2H, $OCH_2$), 6.52 (d, $J$ = 8.4 Hz, 2H, ArH), 7.15−7.34 (m, 4H, ArH), 7.54 (s, 1H, ArH), 7.67 (s, 1H, CH-5). API-ES: $m/z$ 376.0 ($MH^+$). Anal. ($C_{18}H_{15}Cl_2N_3O_2$) C, H, N.

**Ethyl 1-(2,4-Dichlorophenyl)-2-(4-iodophenyl)-1*H*-4-imidazolecarboxylate (1p).** To a cold solution (0 °C) of amine **1o** (0.3 g, 0.79 mmol) in concentrated HCl (4 mL) and ice (8 g) was added dropwise a 1.0 M solution of $NaNO_2$ (797 $\mu$L, 0,79 mmol). The resulting yellow solution was further stirred at the same temperature for 30 min and then transferred into a solution of KI (0.44 g, 7.95 mmol) in water (4 mL). The mixture was stirred at room temperature for 5 h and then extracted with $CH_2Cl_2$. The combined organic extracts were dried and evaporated in vacuo to leave a residue which was purified by flash silica gel chromatography eluting with AcOEt/petroleum ether (30:70 and 50:50) to give **1p** (0.29 g, 74%) as a white solid. Mp: 197 °C ($CHCl_3/CH_3OH$). TLC (AcOEt/petroleum ether, 4:6): $R_f$ = 0.73. IR (KBr): 1710, 1586 cm$^{-1}$. $^1$H NMR ($CDCl_3$): $\delta$ 1.41 (t, $J$ = 8.0 Hz, 3H, $CH_3$), 4.43 (q, $J$ = 8.0 Hz, 2H, $OCH_2$), 7.12 (d, $J$ = 8.8 Hz, 2H, ArH), 7.24 (d, $J$ = 9.4 Hz, 1H, ArH), 7.38 (d, $J$ = 8.6 Hz, 1H, ArH), 7.56 (s, 1H, ArH), 7.60 (d, $J$ = 8.6 Hz, 2H, ArH), 7.72 (s, 1H, CH-5). API-ES: $m/z$ 486.9 ($MH^+$). Anal. ($C_{18}H_{13}Cl_2IN_2O_2$) C, H, N.

**Ethyl 2-[4-(Acetylamino)phenyl]-1-(2,4-dichlorophenyl)-1*H*-4-imidazolecarboxylate (1q).** To a solution of amine **1o** (0.08 g, 0.21 mmol) in anhydrous THF (2 mL) was added at room temperature acetic anhydride (60 $\mu$L, 0.63 mmol). The resulting solution was stirred at the same temperature for 30 min and then evaporated in vacuo to give **1q** (65 mg, 75%) as a white solid. Mp: 233−234 °C ($CHCl_3/CH_3OH$). TLC (AcOEt/petroleum ether, 8:2): $R_f$ = 0.27. IR (KBr): 3289, 1736, 1684, 1591 cm$^{-1}$. $^1$H NMR ($CDCl_3$): $\delta$ 1.39 (t, $J$ = 7.2 Hz, 3H, $CH_3$), 2.12 (s, 3H, $CH_3$), 4.39 (q, $J$ = 7.2 Hz, 2H, $OCH_2$), 7.25−7.56 (m, 7H, ArH), 7.72 (s, 1H, CH-5), 9.41 (s, 1H, NH, $D_2O$ exchanged). API-ES: $m/z$ 418.1 ($MH^+$). Anal. ($C_{20}H_{17}Cl_2N_3O_3$) C, H, N.

**1-(4-Nitrophenyl)-2-phenyl-1*H*-imidazole (4).** To a solution of 2-phenylimidazole (0.5 g, 3.46 mmol) in anhydrous DMF (28 mL) was added $K_2CO_3$ (0.96 g). After 15 min of stirring at room temperature, 4-fluoronitrobenzene (0.61 g, 4.32 mmol) was added. The resulting mixture was heated at 130 °C for 5 h, cooled to room temperature, and poured onto ice. The mixture was extracted with AcOEt, and the combined extracts were dried and evaporated in vacuo. The residue was subjected to flash silica gel chromatography eluting with AcOEt/hexane (60:40) to give **4** (0.76 g, 84%) as a yellow solid. Mp: 127 °C (triturated with $Et_2O$). TLC (AcOEt/petroleum ether, 1:1): $R_f$ = 0.37. IR (KBr): 1525, 1345 cm$^{-1}$. $^1$H NMR ($CDCl_3$): $\delta$ 7.23−7.40 (m, 9H, ArH), 8.26 (d, $J$ = 9.0 Hz, 2H, ArH). MS: $m/z$ 265.0 ($M^+$, base). Anal. ($C_{15}H_{11}N_3O_2$) C, H, N.

**4-(2-Phenyl-1*H*-1-imidazolyl)aniline (6).** Compound **6** was prepared following the procedure reported for compound **1o**. Yield: 91%. Mp: 178−200 °C (triturated with $Et_2O$). TLC (AcOEt/petroleum ether, 1:1): $R_f$ = 0.27. IR (KBr): 3481, 3443, 1643 cm$^{-1}$. $^1$H NMR ($CDCl_3$): $\delta$ 4.90 (br s, 2H, $ArNH_2$, $D_2O$ exchanged), 6.62−6.66 (m, 2H, ArH), 6.89−6.93 (m, 2H, ArH), 7.12−7.41 (m, 7H, ArH). MS: $m/z$ 235.0 ($M^+$, base). Anal. ($C_{15}H_{13}N_3$) C, H, N.

**1,2-Diphenyl-1*H*-imidazole (1r).**[22] To a cold solution (−10 °C) of amine **6** (0.35 g, 1.49 mmol) in concentrated HCl (10 mL) was added $NaNO_2$ (0.14 g, 2.0 mmol). The resulting yellow solution was further stirred at the same temperature for 1 h and then added dropwise to 50% $H_3PO_2$ (10 mL); stirring was continued for 5 h. The solution was basified with 20% NaOH and then extracted with AcOEt. The combined organic extracts were dried and evaporated in vacuo to leave an oily residue which was purified by flash silica gel chromatography eluting with AcOEt/petroleum ether (70:30) to give **1r** (0.21 g, 65%) as a white solid. Mp: 85−86 °C ($iPr_2O$). TLC (AcOEt/petroleum ether, 1:1): $R_f$ = 0.54. IR (KBr): 3095, 1596, 1498 cm$^{-1}$. $^1$H NMR ($CDCl_3$): $\delta$ 7.16−7.41 (m, 12H, ArH). MS $m/z$ 220.0 ($M^+$, 88), 219.0 (base). Anal. ($C_{15}H_{12}N_2$) C, H, N.

Compounds **5**, **7**, and **1s** were prepared following the procedures reported for compounds **4**, **6**, and **1r**, respectively.

**2-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-1*H*-4-imidazolecarboxylic Acid (8).** A mixture of ester **1i** (1.15 g, 2.90

mmol) in $CH_3OH$ (20 mL) and NaOH (1 N) (20 mL) was refluxed for 1.5 h, cooled, and acidified with concentrated HCl. The precipitate was filtered, washed with water, and dried to give **8** as a white solid (0.9 g, 85%). Mp: 238−239 °C ($CH_3OH$). TLC ($CHCl_3/CH_3OH$, 8:2): $R_f = 0.48$. IR (KBr): 1714, 1562 $cm^{-1}$. $^1H$ NMR ($CDCl_3$): $\delta$ 4.83 (br s, 1H, OH, $D_2O$ exchanged), 7.27−7.57 (m, 7H, ArH), 7.74 (s, 1H, CH-5). API-ES: *m/z* 367.0 ($MH^+$). Anal. ($C_{16}H_9Cl_3N_2O_2$) C, H, N.

Compound **9** was obtained following the procedure reported for compound **8**.

***N*4,*N*4-diethyl-2-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-1*H*-4-imidazolecarboxamide (1w).** To a solution of acid **8** (0.15 g, 0.41 mmol) in $CH_2Cl_2$ (15 mL) was added two drops of DMF. Oxalyl chloride (0.11 mL, 1.23 mmol) was then added and the solution stirred at room temperature for 30 min and then evaporated in vacuo. The acid chloride was solubilized in $CH_2Cl_2$ (15 mL) and cooled at 0 °C (ice bath). At this temperature triethylamine (0.060 mL, 0.43 mmol) and diethylamine (0.037 mL, 0.36 mmol) were added. The reaction mixture was stirred at room temperature for 30 min and then washed with 5% aqueous $NaHCO_3$. The dried organic layer, evaporated in vacuo, gave a crude residue which was purified by flash silica gel chromatography using AcOEt/petroleum ether (8:2) as eluent to give **1w** (0.087 g, 51%) as a white solid. Mp: 289−290 °C ($CHCl_3/CH_3OH$). TLC (AcOEt/petroleum ether, 1:1): $R_f = 0.60$. IR (KBr): 1592, 1570 $cm^{-1}$. $^1H$ NMR ($CDCl_3$): $\delta$ 1.25−1.32 (m, 6H, 2 × $CH_3$), 3.45−3.60 (m, 2H, $CH_2$), 4.02−4.12 (m, 2H, $CH_2$), 7.22−7.39 (m, 6H, ArH), 7.55 (s, 1H, ArH), 7.62 (s, 1H, CH-5). API-ES: *m/z* 422.1 ($MH^+$). Anal. ($C_{20}H_{18}Cl_3N_3O$) C, H, N.

**[2-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-1*H*-4-imidazolyl](4-methylpiperazino]methanone (1x).** Compound **1x** was obtained following the above procedure in the absence of triethylamine. The crude product was purified by recrystallization. Yield: 86%. Mp: 269−270 ($CHCl_3/CH_3OH$). TLC ($CHCl_3/CH_3OH$, 9:1): $R_f = 0.57$. IR (KBr): 1593, 1534 $cm^{-1}$. $^1H$ NMR ($CDCl_3$): $\delta$ 2.35 (s, 3H, $CH_3$), 2.49−2.59 (m, 4H, 2 × $CH_2$), 3.70−3.90 (m, 2H, $CH_2$), 4.31−4.50 (m, 2H, $CH_2$), 7.24−7.39 (m, 6H, ArH), 7.56 (s, 1H, ArH), 7.65 (s, 1H, CH-5). API-ES: *m/z* 449.0 ($MH^+$). Anal. ($C_{21}H_{19}Cl_3N_4O$) C, H, N.

**Propyl 2-(4-Bromophenyl)-1-(2,4-dichlorophenyl)-1*H*-4-imidazolecarboxylate (1u).** Compound **1u** was synthesized using the procedure applied to compound **1w** except that 1-propanol was used instead of diethylamine. The reaction mixture was stirred at room temperature for 6 h. The crude product was purified by flash silica gel chromatography using AcOEt/petroleum ether (7:3) as eluent to give **1u** as a white solid. Yield: 40%. Mp: 142−143 °C ($iPr_2O/CH_2Cl_2$). TLC (AcOEt/petroleum ether, 3:7): $R_f = 0.55$. IR (KBr): 1685, 1588 $cm^{-1}$. $^1H$ NMR ($CDCl_3$): $\delta$ 1.01 (t, $J = 7.6$ Hz, 3H, $CH_3$), 1.80 (m, 2H, $CH_2$), 4.32 (t, $J = 6.8$ Hz, 2H, $CH_2O$), 7.22−7.43 (m, 6H, ArH), 7.55 (s, 1H, ArH), 7.71 (s, 1H, CH-5). MS: *m/z* 454 ($M^+$, 57), 368 (base). Anal. ($C_{19}H_{15}BrCl_2N_2O_2$) C, H, N.

**2-Piperidinoethyl 2-(4-Bromophenyl)-1-(2,4-dichlorophenyl)-1*H*-4-imidazolecarboxylate (1v).** Compound **1v** was synthesized using the procedure applied to compound **1w** except that 1-piperidine ethanol was used instead of diethylamine. The crude product was purified by recrystallization. Yield: 77%. Mp: 142−145 °C ($iPr_2O/CH_2Cl_2$). TLC ($CHCl_3/CH_3OH$, 9:1): $R_f = 0.46$. IR (KBr): 1709, 1550 $cm^{-1}$. $^1H$ NMR ($CDCl_3$): $\delta$ 1.44−1.47 (m, 2H, $CH_2$), 1.61−1.65 (m, 4H, 2 × $CH_2$), 2.51−2.54 (m, 4H, 2 × $CH_2$), 2.78 (t, $J = 6.0$ Hz, 2H, $CH_2N$), 4.49 (t, $J = 6.0$ Hz, 2H, $CH_2O$), 7.22−7.43 (m, 6H, ArH), 7.56 (s, 1H, ArH), 7.74 (s, 1H, CH-5). API-ES: *m/z* 523.8 ($MH^+$). Anal. ($C_{23}H_{22}BrCl_2N_3O_2$) C, H, N.

**Mutagenesis and Expression of $GABA_A$ Receptor Subunits.** Site-directed mutagenesis (asparagine-265 to serine, N265S) of the $\beta$2 subunit of the human $GABA_A$ receptor was performed with the corresponding cDNA subcloned into pCDM8 and a Quik-Change site-directed mutagenesis kit (Stratagene, La Jolla, CA). The mutation was verified by partial sequencing of the sense and antisense DNA strands.

**Expression of Human $GABA_A$ Receptors in *Xenopus* oocytes.** A mixture of plasmids encoding the $\alpha$1, $\beta$1, and $\gamma$2L; $\alpha$1, $\beta$2, and $\gamma$2L; or $\alpha$1, $\beta$2(N265S), and $\gamma$2L receptor subunits (total of 1.5 ng of cDNA in 30 nL in a 1:1:1 ratio) was injected into the animal pole of oocytes with the use of a microdispenser (Drummond Scientific, Broomwall, PA). The injected oocytes were maintained at 15 °C in sterile modified Barth's solution [MBS; 88 mM NaCl, 1 mM KCl, 10 mM HEPES, 0.82 mM $MgSO_4$, 2.4 mM $NaHCO_3$, 0.91 mM $CaCl_2$, and 0.33 mM $Ca(NO_3)_2$, adjusted to pH 7.5] supplemented with streptomycin (10 mg/L), penicillin (10000 U/L), gentamicin (50 mg/L), theophylline (90 mg/L), and pyruvate (220 mg/L).

**Electrophysiology.** Electrophysiological measurements were performed 1−4 days after injection of oocytes with expression plasmids. The oocytes were placed individually in a rectangular chamber (volume ~100 mL) and perifused (1.7 mL/min) with MBS through 18-gauge polyethylene tubing (Clay Adams, Parsippany, NJ) with the use of a roller pump (Cole-Parmer, Chicago, IL). Oocytes were impaled at the animal pole with two glass electrodes (0.5−10 MΩ) filled with 3 M KCl and were clamped at −70 mV with an Axo-Clamp 2A amplifier (Axon Instruments, Union City, CA). Currents were continuously plotted with a chart recorder (Cole-Parmer, Vernon Hills, IL). GABA (Sigma, St. Louis, MO) was dissolved in MBS and applied for 30 s. Test compounds were dissolved in dimethyl sulfoxide (DMSO) at 100 mM and then diluted in MBS to a final DMSO concentration not exceeding 0.1% (DMSO at this concentration had no effect on $Cl^-$ current). Drugs were applied for 60 s either in the absence of GABA or in its presence at the $EC_{10}$. For each experiment, control responses were determined before and 10 min after application of test agents.

**Assay of [$^3$H]GABA Binding.** Male Sprague−Dawley rats with body masses of 200−225 g were killed by decapitation, and their brains were rapidly removed. The fresh cortical tissue was homogenized with a Polytron PT 10 disrupter for 30 s in 10 volumes of ice-cold water, and the homogenate was centrifuged at 48000*g* for 10 min at 0 °C. The resulting pellet was washed once by resuspension and centrifugation in the original volume of a solution containing 20 mM potassium phosphate buffer (pH 7.4) and 50 mM KCl. The membrane pellet was then stored at −20 °C until binding analysis (1−5 days later). On the day of the assay, the membranes were thawed and washed a total of four times by resuspension and centrifugation in the same ice-cold solution.

For the [$^3$H]GABA-binding assay, the membranes were resuspended in 50 volumes of the same solution, and 300 $\mu$L of membrane suspension (~300 $\mu$g of protein) was added to plastic minivials containing the drugs to be tested. The assay was performed in a final volume of 500 $\mu$L, started by the addition of [$^3$H]GABA (specific activity 80−100 Ci/mmol, Perkin-Elmer, Boston, MA) to a final concentration of 10 nM, and stopped after incubation for 10 min at 0 °C by centrifugation at 48000*g* for 10 min at 0 °C. The pellet was washed with 4 mL of ice-cold water and then dissolved in 3 mL of scintillation fluid. The membrane-associated radioactivity was determined with a scintillation counter (TRI-CARB 2100TR, Packard). Drug stock solutions (20 mM) were prepared in DMSO; the concentration of DMSO in the binding reaction mixture did not exceed 0.2% and did not affect [$^3$H]GABA binding. Binding data were corrected for nonspecific binding, which was determined in the presence of 1 mM GABA.

**Behavioral Effects in Tadpoles.** *X. laevis* tadpoles (43−50 days old) were maintained in an aquarium at 20−22 °C. For determination of loss of the righting reflex, each of 10 tadpoles was placed in separate beakers containing 300 mL of tap water with or without **1i** (0.01−30 $\mu$M). With the exception of the tap water control, all beakers contained DMSO at 0.1%, a concentration that did not affect animal behavior. Anesthesia was defined as the absence of a purposeful and sustained swimming response after inversion of the tadpole with a smooth glass rod. The number of anesthetized tadpoles was recorded every 10 min for up to 120 min, after which the tadpoles were returned to fresh tap water. Normal swimming activity was restored within 30 min.

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**Supporting Information Available:** Complete physical and spectral data for new compounds **1b**−**f**,**h**−**n**,**s**,**t** and intermediates **2b**−**f**,**h**−**n**, **3b**,**e**,**f**,**h**−**n**, **5**, **7**, and **9** and elemental analysis (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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