Propofol Analogues. Synthesis, Relationships between Structure and Affinity at $GABA_A$ Receptor in Rat Brain, and Differential Electrophysiological Profile at Recombinant Human $GABA_A$ Receptors

Giuseppe Trapani,*,† Andrea Latrofa,† Massimo Franco,† Cosimo Altomare,† Enrico Sanna,‡ Marcello Usala,‡ Giovanni Biggio,‡ and Gaetano Liso†

Dipartimento Farmaco-Chimico, Facoltà di Farmacia, Università degli Studi di Bari, Via Orabona 4, 70125 Bari, Italy, and Dipartimento di Biologia Sperimentale, Sezione di Neuroscienze, Università di Cagliari, Via Palabanda 12, 09123 Cagliari, Italy

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A number of propofol (2,6-diisopropylphenol) congeners and derivatives were synthesized and their in vitro capability to affect $GABA_A$ receptors determined by the inhibition of the specific [^{35}S]-*tert*-butylbicyclophosphorothionate ([^{35}S]TBPS) binding to rat whole brain membranes. Introduction of halogen (Cl, Br, and I) and benzoyl substituents in the para position of the phenyl group resulted in ligands with higher potency at inhibiting [^{35}S]TBPS binding. A quantitative structure−affinity relationship (QSAR) study demonstrated that affinity is enhanced by increases in lipophilicity of the ligand whereas affinity is adversely affected by increases in size of the substituent para to the phenolic hydroxyl group. Consistent with the displacement of [^{35}S]TBPS and with the activation of $GABA_A$ receptors, we demonstrate that ligands displaying high affinity (i.e., **2**−**4**, and **8**) are able to increase GABA-stimulated chloride currents in oocytes expressing human $GABA_A$ receptors and to directly activate chloride currents in an electrophysiological assay. Among them, compound **4** showed a rather peculiar profile in the electrophysiological examination with cloned $\alpha_1\beta_2\gamma_2$ $GABA_A$ receptors. Indeed, compared to propofol, it displayed a much greater efficacy at potentiating GABA-elicited chloride currents, but a much lower efficacy at producing a direct activation of the chloride channel in the absence of GABA. This behavior may give to compound **4** pharmacological properties that are more similar to anxiolytic and anticonvulsant drugs than to those of general anesthetics.

Introduction

Propofol (2,6-diisopropylphenol, **1**) is a short-acting hypnotic agent, effective for induction and maintenance of anesthesia when administered intravenously either as repeated bolus injections or as a continuous infusion.[1] It has the desirable properties of rapid onset and offset of action and minimal accumulation on long-term administration.[2] Despite these favorable clinical properties which account for its popularity in anesthesia, its precise neurochemical mechanism of action still remains to be clarified. In this regard, recent electrophysiological and biochemical studies[3,4] have shown a possible interaction of propofol with the GABA receptor complex similar to that observed for other general anesthetics. Indeed, a great body of experimental evidence supports the view that the function of $GABA_A$ receptors is influenced by a number of chemically unrelated volatile and nonvolatile compounds, such as enflurane, isoflurane, alphaxalone, etomidate, and pentobarbital, showing that $GABA_A$ receptors play an important role in general anesthesia.[5] Accordingly, it has been shown that propofol produces an increase in [^{3}H]GABA binding and a decrease in [^{35}S]-*tert*-butylbicyclophosphorothionate ([^{35}S]TBPS) binding to the picrotoxin site on $GABA_A$ receptors in a concentration-dependent manner, similar to that of the general anesthetics pentobarbital and alphaxalone.[4] Supporting this hypothesis, Concas et al.[6] used radiolabeled propofol to detect a specific propofol recognition site in rat brain, suggesting that this anesthetic agent could bind directly to a site associated with ion channel proteins. Furthermore, recent molecular biology studies demonstrated that the $GABA_A$ receptor is a heterooligomeric ligand-gated chloride channel consisting of α-, β-, γ-, and δ-subunits.[7] In this regard, data from recombinant receptor expression experiments indicate that while the allosteric potentiation of GABA action does not seem to be dependent on any specific receptor subunit, the β1 subunit of the GABA receptor forms a functional chloride channel which contains sites for directly mediating the effects of GABA, propofol, and pentobarbital.[8] These data strongly support the idea that the action of propofol and pentobarbital is exerted through a specific interaction with some hydrophobic pocket or domain of the receptor protein rather than on membrane lipid components.

From a clinical viewpoint, several adverse effects have been found in patients undergoing propofol treatment. These include pain on injection, apnoea, reduction in blood pressure, bradycardia,[1] and excitatory events including convulsions.[9] It is possible that the emulsion-based formulation of **1** currently used (Diprivan) is responsible for many of these side effects.[10] Hence, there is a clinical need for aqueous formulations of **1**,

* Address correspondence to: Giuseppe Trapani, Dipartimento Farmaco-Chimico, Facoltà di Farmacia, Università degli Studi di Bari, Via Orabona 4, 70125 Bari, Italy.
† Università degli Studi di Bari.
‡ Università di Cagliari.

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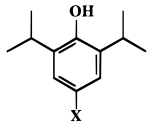
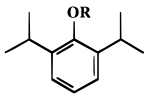
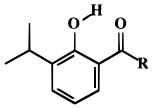
Chart 1



1 -15



16 - 24



25 - 26

1 X = H
2 X = Cl
3 X = Br
4 X = I
5 X = NO_2
6 X = SO_3CH_3
7 X = CHO
8 X = COC_6H_5
9 X = $CH_2N(CH_3)_2$
10 X = $N(CH_3)_2$
11 X = NH_2
12 X = $NHCOCH_3$
13 X = $NHCOCF_3$
14 X = $NHSO_2CH_3$
15 X = $^+N(CH_3)_3\ I^-$

16 R = CH_3
17 R = $COCH_3$
18 R = COC_6H_5
19 R = $COCH_2Br$
20 R = $COCH_2NHCH_2CH(OCH_3)_2$
21 R = $COCH_2N(C_2H_5)_2$
22 R = $COCH_2N(C_3H_7)_2$
23 R = $COCH_2N$-$(CH_2)_4$-
24 R = $COCH_2N$-$(CH_2)_2$-O-$(CH_2)_2$-

25 R = OH
26 R = OCH_3

which might improve its delivery, biodistribution, and therapeutic index.

Despite the popularity of **1**, to the best of our knowledge, with the exception of the early work by James and Glen[11] confined exclusively to evaluating the changes in the ortho alkyl groups of **1**, no structure−activity relationship (SAR) studies aimed at identifying novel compounds with better pharmacodynamic properties and reduced side effects have so far been carried out. In this paper, we report the results of our SAR studies on a new series of 2,6-diisopropylphenol compounds designed by structural modifications of the lead compound **1**. Specifically, we wanted to explore the effects both of the substitution at the para position of the aromatic ring leading to compunds **2**−**15** (Chart 1), and the etherification or esterification of the phenolic hydroxyl group affording compounds **16**−**18** and **20**−**24** as potential prodrugs of **1**. Furthermore, the effect of the change of one isopropyl group of **1** with a carboxy or carboxymethyl group (i.e., compounds **25** and **26**) was also investigated. The lead compound **1** was chemically modified in this study with the aim of examining the influence on $GABA_A$ receptor affinity of lipophilicity and bulkiness of substituents at para position, as well as of assessing the importance of hydrogen bond (HB) donor/acceptor properties of the phenolic hydroxyl group. Indeed, early studies on propofol metabolism had demonstrated that some hydrophilic derivatives, such as the 1- and 4-glucuronide as well as the 4-sulfate conjugates of 2,6-diisopropyl-1,4-quinol, found as the main metabolites of **1**, are lacking in pharmacological activity.[12] Also the importance of the HB donor/acceptor phenolic hydroxyl in determining the anesthetic potency had been clearly pointed out by James and Glen.[11] Compounds **2**−**15**, **25** and **26** synthesized by us offer an opportunity to systematically examine the variation in physicochemical properties and possibly to derive quantitative models of structure−activity relationships.

The synthesis of glycinates **20**−**22** and acetates **23** and **24** was carried out in an attempt to circumvent the potential drawback of the very limited aqueous solubility of **1**. The choice of the promoiety followed from the fact that esters containing an ionizable group, such as α-amino acid esters, have been used to increase the aqueous solubility of many hydroxyl-containing drugs.[13]

One of the most important molecular actions underlying the anesthetic properties of propofol is exerted at the level of $GABA_A$ receptors.[4,14] We therefore compared and contrasted the activity of propofol against various propofol analogues by testing their activity in both neurochemical and functional assays associated with the $GABA_A$ receptors.

Chemistry

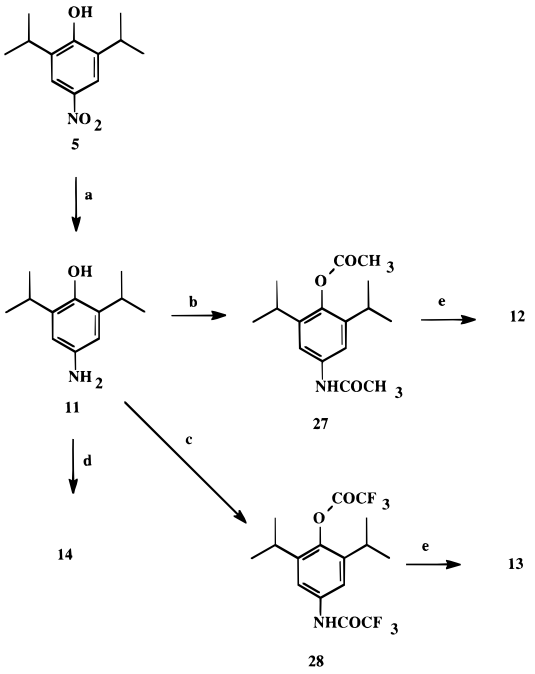
Standard methods were used to prepare compounds **2**−**15**. In particular, compounds **2** and **3** were prepared according to Wheatley and Holdrege's procedure[15] while aromatic halogenation of **1** with iodonium chloride gave compound **4** in moderate yield. The nitro compound **5** was obtained according to literature[16] with modifications (HNO_3/H_2SO_4 mixture as nitrating agent and lower reaction temperature) that improved the overall yield. In all of these reactions considerable amounts of dimerization product arising from the well-known oxidative-coupling reaction of the phenol **1** occur. Catalytic hydrogenation of **5** by using 10% Pd/C in ethanol and in the presence of formaldehyde yielded **10** which, in turn, was converted to the corresponding quaternary salt **15** by treatment with methyl iodide. The methyl benzenesulfonate **6** was prepared by treatment of **1** with dimethyl sulfate. In contrast, when the reaction with dimethyl sulfate was carried out on the corresponding phenoxide of **1**, formation of **16** in good yield was obtained. Reaction of **1** with formaldehyde, carried out in the presence of dimethylamine, afforded the expected Mannich-type compound **9** in moderate yield while by using ammonia instead of dimethylamine gave the formyl derivative **7** in 35% yield. Compounds **8** and **18** were obtained by reaction of **1** with benzoyl chloride in the presence of $AlCl_3$. The syntheses of the amides **12** and **13** as well as of the sulfonamide **14** are outlined in Scheme 1. Thus, reduction of the nitro compound **5** with Sn/HCl gave the corresponding amine **11** which, in turn, when treated with methanesulfonyl chloride or acetic or trifluoroacetic anhydride afforded the sulfonamide **14** or the amido ester **27** or **28**, respectively. Treatment of **27** and **28** with NaOH in ethanol at room temperature gave the desired amides **12** and **13**, respectively.

The synthetic route for obtaining the amino esters **20**−**24** is outlined in Scheme 2. Compound **1** was esterified with bromoacetic acid by using dicyclohexylcarbodiimide as dehydrating agent. A long reaction time (30 h reflux in dry CH_2Cl_2) was required to obtain the expected bromoacetate **19**[17] in satisfactory yield (75%). Reaction of **19** with the appropriate mono- or dialkylamine gave the corresponding amino acid esters **20**−**24**. Finally, compound **26**[18] was obtained by esterification of the carboxylic acid **25**. All compounds were fully characterized by IR, ^1H NMR, mass spectral, and microanalytical data.

Biological Results

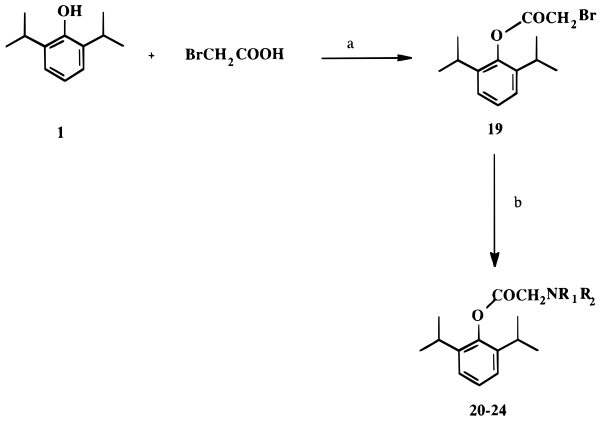
Binding Studies. The binding of [^{35}S]TBPS, a cage convulsant which binds in close proximity to the chloride channel portion of the $GABA_A$ receptor at the level of the picrotoxin binding site, constitutes a very sensitive tool for studying the function of the $GABA_A$/ionophore

Scheme 1[a]



[a] Reagents: (a) Sn/HCl, EtOH; (b) acetic anhydride, pyridine; (c) trifluoroacetic anhydride, pyridine; (d) CH_3SO_2Cl, pyridine; (e) NaOH, EtOH.

Scheme 2[a]



[a] Reagents: (a) DCC; (b)R_1R_2NH.

receptor complex.[19] Propofol, mimicking the action of other general anesthetics such as alphaxalone and pentobarbital, reduces [^{35}S]TBPS binding in a concentration-dependent manner.[5] In this binding assay, the rank order potency of these anesthetic agents was as follows: alphaxalone > propofol > pentobarbital. Also the effects of compounds **2**−**18** and **20**−**26** (3×10^{-7} to 10^{-4} M) on [^{35}S]TBPS binding to cortical unwashed membranes were determined. Almost all the compounds tested had the ability to dose-dependently and completely inhibit [^{35}S]TBPS binding. Figure 1 shows the competitive inhibition curves for the most active compounds **2**−**4** and **8**. The IC_{50} values of all compounds are shown in Table 1.

Electrophysiology in *Xenopus* Oocytes Expressing Human $GABA_A$ Receptors. The profile of the most active compounds, **2**−**4** and **8**, respectively, was further investigated in order to better characterize their biochemical and molecular effects. Expression of specific $GABA_A$ receptor subunit constructs in *Xenopus*

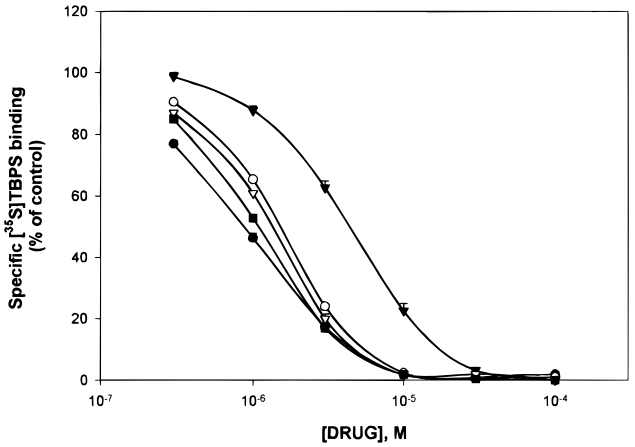


Figure 1. Effect of propofol and its analogues on [^{35}S]TBPS binding to unwashed rat cortical membranes. Rat cortical membranes were incubated with 2 nM [^{35}S]TBPS for 90 min in the presence of different concentrations of propofol (▼), or compounds **2** (○), **3** (■), **4** (▽), and **8** (●). Data are expressed as a percentage of binding measured in the presence of solvent and are means ± standard error of four to six experiments.

Table 1. Effect of Compounds **1**−**18** and **20**−**26** on the [^{35}S]TBPS Binding in the Rat Cerebral Cortex

compd	[^{35}S]TBPS binding (IC_{50}, μM)[a]	compd	[^{35}STBPS binding (IC_{50}, μM)[a]
1	3.95	**15**	ND[b]
2	1.44	**16**	37.0
3	1.07	**17**	8.5
4	1.20 (3.10)	**18**	15.2
5	1.95	**20**	15.6
6	9.40	**21**	21.8
7	5.80	**22**	ND[b]
8	0.85	**23**	6.52
9	739	**24**	ND[b]
10	5.70	**25**	ND[b]
11	23.6 (5.9)	**26**	39.5
12	ND[b]	alphaxalone	0.3[c]
13	14.0 (5.9)	pentobarbital	40[c]
14	131		

[a] Concentration necessary for 50% inhibition (IC_{50}); data are means of two determinations which differed by less than 10%. The IC_{50} value of the parallel determination of propofol on the same tissue preparation is reported in the table unless otherwise stated in parentheses. [b] ND = no displacement. [c] From ref 4.

laevis oocytes was utilized to evaluate electrophysiologically the efficacy of the positive modulation of GABA-evoked chloride currents by propofol derivatives in comparison with that of propofol. In addition, taking into consideration a number of studies reporting that propofol produces a direct activation of $GABA_A$ receptors in the absence of GABA,[8b,14c,20] we wanted to evaluate whether propofol derivatives could mimic this action of propofol.

Direct Activation of Chloride Currents. As shown in Figure 2, at concentrations ranging from 10^{-6} to 5×10^{-5} M, all drugs directly evoked chloride currents in oocytes expressing $\alpha1\beta2\gamma2$ receptors. Compounds **2**, **3**, and **8** had similar efficacy to propofol, while **4** was much weaker at producing such activation. Similar results were obtained with receptor constructs formed by $\alpha1$, $\beta1$, and $\gamma2$ subunits. However, at variance with $\beta2$-containing receptors, **4** showed efficacy and potency similar to that of propofol in direct chloride current activation (Table 2).

Modulation of GABA-Evoked Chloride Currents. The effects of all compounds (10^{-6} M to 5×10^{-5} M) at

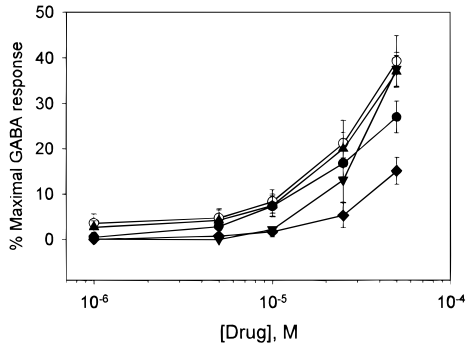


Figure 2. Direct effects of propofol and its analogues at human $\alpha_1\beta_2\gamma_2$ $GABA_A$ receptors expressed in Xenopus laevis oocytes. Evoked chloride currents were in response to various concentrations of propofol (○) or compounds **2** (▲), **3** (●), **4** (◆), and **8** (▼). Values are expressed as mean (from 8 to 12 different oocytes) ± standard error percentage of the control response obtained with 10 mM GABA.

Table 2. Direct Activation of Chloride Currents by Propofol (**1**) and Its Derivatives **2**−**4** and **8** in *Xenopus laevis* Oocytes Expressing Human $\alpha_1\beta_1\gamma_2$ $GABA_A$ Receptors[a]

compd	*n*	maximal activation (%)	EC_{50} (μM)
propofol	8	38 ± 6	44
2	6	37 ± 6	38
3	6	34 ± 4	38
4	7	36 ± 4	51
8	6	41 ± 6	47

[a] Maximal direct activation of chloride currents is expressed as a percentage of the control response obtained with 10 mM GABA; values are mean ± SEM for the indicated number (*n*) of oocytes.

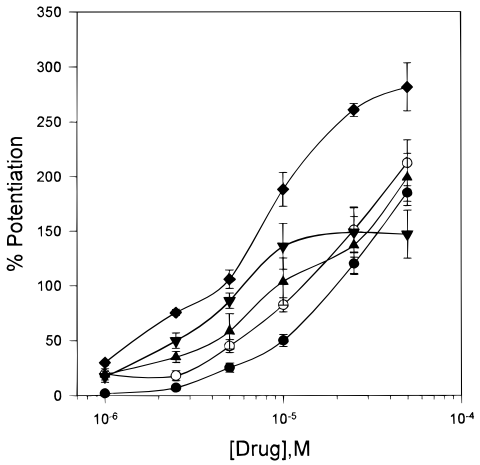


Figure 3. Modulatory action of propofol and its analogues at human $\alpha_1\beta_2\gamma_2$ $GABA_A$ receptors expressed in *Xenopus laevis* oocytes. Values are expressed as mean (from 6 to 13 different oocytes) ± standard error percentage of the potentiation of the control response to GABA (EC_{20}, 2−10 μM) by various concentrations of propofol (○), or compounds **2** (▲), **3** (●), **4** (◆), and **8** (▼).

receptors composed of α1, β2, and γ2 are shown in Figure 3. GABA-evoked chloride currents (GABA, 5−15 μM, 20% of maximal GABA response) were potentiated in the presence of all these compounds. Potentiation was concentration-dependent and reversible following washout. Compared to propofol, compounds **2, 3,** and **8** showed similar efficacy in modulating GABA responses, whereas **4** produced a much greater potentiation. In addition, **4** and **8** showed a greater potency compared to propofol. Similar results were obtained with $GABA_A$ receptors in which the β2 subunit was substituted with the β1 subunit (Table 3).

Table 3. Modulation by Propofol (**1**) and Its Derivatives **2**−**4** and **8** of GABA-Induced Chloride Currents in *Xenopus Laevis* Oocytes Expressing Human $\alpha_1\beta_1\gamma_2$ $GABA_A$ Receptors[a]

compd	*n*	maximal potentiation (%)	EC_{50} (μM)
propofol	10	296 ± 32	8
2	4	205 ± 21	10
3	5	223 ± 25	12
4	10	311 ± 28	12
8	5	220 ± 30	9

[a] Maximal potentiation of chloride currents is expressed as a percentage of the control response obtained with GABA at EC_{20}; values are mean ± SEM for the indicated number (*n*) of oocytes.

Discussion

Structure−Affinity Relationships. Evaluation of the data in Table 1 allowed us to derive structure-affinity relations which gave insights into the pharmacophore for the propofol binding site. In an attempt to look for properties which modulate the binding affinity we divided the compounds into two subsets, namely the para-X-substituted congeners, **1**−**15**, and derivatives, **16**−**18** and **20**−**24**. Within the series of para-X-substituted congeners, compound **8** (X = benzoyl) was the most active one, showing a binding affinity at submicromolar level. In contrast, the hydrophilic and electron-withdrawing trimethylammonium group in the para position led to the disappearance of any detectable affinity. Halogens (**2**−**4**), as well as the nitro group (**5**), increased affinity, whereas other electron-withdrawing groups, such as sulfonate ester (**6**) and formyl (**7**), disfavored binding to the receptor site. Introduction of the lipophilic and electron-donating $N(CH_3)_2$ group (**10**) resulted in no significant increase of radioligand displacement, whereas dimethylaminomethyl group (**9**) led to a marked decrease in pIC_{50} value. Even though electronic and steric properties seemed to be involved in the modulation of affinity, hydrophobicity appeared as the main driving force which promotes the binding, as demonstrated by the rank order of the IC_{50} values observed, i.e., **8** > **2**−**5** > **6** ≫ **9**.

To place the above observations on a more quantitative basis, we carried out a regression analysis using as the dependent variable an index of binding affinity, AI (i.e., pIC_{50} (p-X-congeners) minus pIC_{50} (propofol)), that should more correctly express biological activity of a given congener, due to interday variations of tissue preparations. As molecular descriptors, parameters of lipophilicity (octanol/water partition coefficients, log *P*), bulk (molar refractivities, MR, McGowan Volume, V_x[21]) and polar binding (Hammett σ constants) were examined (Table 4).

Lipophilicity emerged as the physicochemical property which mainly correlates with binding affinity, as demonstrated by the following linear equation

$$AI = 0.74(\pm 0.21) \log P - 3.48(\pm 0.95) \qquad (1)$$

$$n = 13 \qquad r^2 = 0.8462 \qquad s = 0.3511$$

where *n* represents the numbers of data points, r^2 the squared correlation coefficient, and *s* the standard deviation; the 95% confidence intervals are indicated in

Table 4. $Gaba_A$ Receptor Affinity Data and Parameters Used in QSAR of p-X-Substituted Congeners[a] of Propofol

						AI[f]		
compd	X	log P[b]	MR[c]	V_x[d]	σ[e]	exp	calc by eq 3	res
1	H	4.33	0.10	0.02	0	0.00	0.03	−0.03
2	Cl	5.34	0.60	0.14	0.23	0.44	0.63	−0.19
3	Br	5.49	0.89	0.20	0.23	0.57	0.68	−0.11
4	I	5.75	1.39	0.28	0.18	0.52	0.79	−0.27
5	NO_2	4.71	0.74	0.20	0.78	0.31	0.13	0.18
6	SO_3CH_3	3.70	1.70	0.50	0.36	−0.37	−0.87	0.50
7	CHO	4.30	0.70	0.18	0.42	−0.16	−0.14	−0.02
8	COC_6H_5	5.91	3.03	0.79	0.43	0.67	0.42	0.25
9	$CH_2N(CH_3)_2$	2.50[g]	1.87	0.65	0.01	−2.27	−1.87	−0.40
10	$N(CH_3)_2$	4.52	1.56	0.40	−0.83	−0.16	−0.20	0.04
11	NH_2	3.10	0.54	0.12	−0.66	−0.60	−0.94	0.34
13	$NHCOCF_3$	4.46	1.43	0.47	0.12	−0.38	−0.31	−0.07
14	$NHSO_2CH_3$	3.14	1.82	0.54	0.03	−1.52	−1.31	−0.21

[a] Only congeners having measurable IC_{50} values were examined for QSARs. [b] Estimated by CLOG P program.[25] [c] Molar refractivities taken from standard compilation[22] and scaled by a factor of 10. [d] McGowan volume of substituents calculated according to ref 21 and scaled by a factor of 100. [e] Hammett constant taken from standard compilation.[24] [f] Affinity index (see definition in the text). [g] log P corrected for ionization at pH = 7.40; pK_a of the tertiary amine group, estimated according to equations reported in ref 26, equals 9.0 ± 0.2.

parentheses. The above equation rationalizes ca. 85% of the variance in the affinity data. A cross-validated multiple linear regression (MLR) analysis yielded the following best two-variable equation

$$\mathrm{AI} = 0.71(\pm 0.17)\log P - 0.95(\pm 0.79)V_x - 3.02(\pm 0.86) \quad (2)$$

$$n = 13 \qquad r^2 = 0.9098 \qquad s = 0.2820$$

which explains ca. 91% of the variance in the biological data and has a good predictive ability, as demonstrated by the cv-explained variance (80.82%). In Table 4 experimental and calculated by eq 2 affinity data and residuals are reported. Compound **6**, which may be hydrolyzed under the assay conditions, was the strongest outlier in this relationship. To assess the relative contribution of each independent variable to the affinity index, MLR was repeated on the matrix of autoscaled data[22] and eq 3 was formulated

$$(\mathrm{AI})' = 0.88(\log P)' - 0.26(V_x)' \quad (3)$$

which shows that lipophilicity is the major contributor to affinity, whereas a minor but significant (ca. 20%) contribution has to be ascribed to detrimental steric effects exerted by the substituents in the para position.

MLR showed that the acidity of the propofol p-X-substituted congeners, accounted for by the Hammett σ constant, does not significantly contribute to rationalizing the variance in binding affinity data, whereas the subset of propofol derivatives (**16**−**18**, **20**−**24**) and compounds **25** and **26** were useful in assessing what role is played by the HB properties of the phenolic hydroxyl in determining the binding to the [^{35}S]TPBS receptor site. The importance of the HB-donating hydroxyl group was demonstrated by the 9.4-fold loss in binding affinity which occurs in the methyl ether **16**. Consistent with this finding, the replacement of one isopropyl group of **1** with a carboxy or carboxymethyl group to give compounds **25** and **26** resulted either in abolishing or in an almost 10-fold decrease in receptor affinity, respectively. Intramolecular HB between the phenolic hydroxyl group and the adjacent acid or ester carbonyl should limit the HB donor capacity of compounds **25** and **26** in their interaction with the receptor. While the IC_{50} value of compound **26** is close to that of the HB acceptor compound **16**, the difference in affinity of compounds **25** and **26** could depend on the lipophilicity of the ortho substituents, since the $COOCH_3$ substituent is more lipophilic than COOH.

Acetylation of **1** (compound **17**) resulted in a less pronounced decrease in affinity. The potential prodrugs **20**−**24**, are less potent than **1** or almost completely devoid of affinity, demonstrating once again the importance of the hydroxyl functional group for receptor binding. However, our stability and solubility data (not shown) led us to conclude that compounds **20**−**24** cannot be considered promising prodrug candidates of **1** since they showed high chemical and enzymatic stability. This high stability could most likely depend on the steric protection of the C(O)−O bond by bulky flanking diisopropyl groups on the aromatic ring. In light of the observed stabilities, it is reasonable to ascribe the activity of compounds **20**−**24** to their intrinsic properties.

Pharmacology. The electrophysiological results provide evidence that the most active compounds **2**−**4** and **8** increase GABA-evoked chloride currents in oocytes expressing human $GABA_A$ receptors. This effect varied with different degrees of potency and efficacy, and among the derivatives tested, compound **4** showed a rather peculiar profile, with respect to compounds **2**, **3**, and **8**, in the electrophysiological examination with cloned $GABA_A$ receptors. In fact, at β2-containing receptors, compound **4** displayed a much greater efficacy at potentiating GABA-elicited chloride currents than propofol, but it had a much lower efficacy at producing a direct activation of the chloride channel in the absence of GABA. It is noteworthy that such differences between **4** and propofol, particularly in their direct current activation, were not detected at receptors containing the β1 subunit, suggesting that this compound can discriminate between the different β-subunit isoforms. Although the precise molecular mechanism by which propofol produces its anesthetic effects is not well established, it is commonly accepted that general anesthesia induced by this drug may be the result of the contribution of both potentiation of GABA action and direct activation of the chloride channel. In this view, compound **4** differs markedly from propofol and may represent a drug in which the allosteric modulatory action is enhanced, while at the same time, the direct action has been greatly reduced. Thus, because $\alpha 1\beta 2\gamma 2$ receptors are those with the highest expression among different subpopulations of $GABA_A$ receptors in the mammalian brain,[7] these differences shown by compound **4** with respect to propofol may also be important for potential differences in its pharmacological actions in vivo. Indeed, our preliminary pharmacological results indicate that, at variance with propofol, compound **4** induces anticonvulsant and anticonflict effects in rodents at doses which do not produce sedation or loss of righting reflex.[23] In this respect, a more detailed evaluation of the in vitro and in vivo effects of **4**, in

comparison with those of the parent compound, may be very important and prove useful in further understanding the molecular mechanisms involved in general anesthesia.

Conclusions

The present work describes the synthesis of a series of 2,6-diisopropylphenol analogues and their potency and efficacy in inhibiting [^{35}S]TBPS binding in rat brain membranes, in potentiating GABA-evoked chloride currents, and in directly activating chloride currents in *Xenopus* oocytes expressing human $GABA_A$ receptors. Appropriate substitution of the phenyl group resulted in ligands with higher affinity for the propofol site on $GABA_A$ receptors. A QSAR study demonstrated that lipophilicity, modeled by the calculated octanol−water partition coefficient, is the property which mainly favors the affinity of propofol congeners to the receptor site. Steric effects negatively influenced the binding as demonstrated by a minor but significant contribution to QSAR models (eqs 2 and 3) of volume descriptors. The capability of the compounds examined to inhibit [^{35}S]TBPS binding, similar to the effect of propofol, is consistent with a positive modulation of $GABA_A$ receptors.[4] As a further support to this mechanism we found that the most active compounds **2**−**4** and **8** increase GABA-evoked chloride currents in oocytes expressing human $GABA_A$ receptors. Interestingly, unlike compounds **2**, **3**, and **8**, *p*-iodo-2,6-diisopropylphenol **4** displayed electrophysiological properties different from those of propofol and general anesthetics (i.e., much greater efficacy at potentiating GABA-elicited chloride currents and much lower efficacy at producing a direct activation of the chloride channel in the absence of GABA), but more similar to those of anxiolytic and anticonvulsant drugs.

Experimental Section

Melting points were determined in open capillary tubes with a Büchi apparatus and are uncorrected. Propofol, 2-hydroxy-3-isopropyl-benzoic acid (**25**), and other reagents were used as supplied from commercial source (Aldrich). IR spectra were obtained on a Perkin-Elmer 283 spectrophotometer (KBr disks or Nujol mulls for solids or as liquid films). NMR spectra were determined on a Varian 390 or Bruker 300 MHz instrument. Chemical shifts are given in δ values downfield from Me_4Si as internal standard. Mass spectra were recorded on a Hewlett-Packard 5995c GC−MS low-resolution spectrometer. All compounds showed appropriate IR, ^{1}H NMR, and mass spectra. Elemental analyses were carried out with a Carlo Erba model 1106 analyzer and results were within ±0.40% of the theoretical values. Silica gel 60 (Merck 70−230 mesh) was used for column chromatography. All the following reactions were carried out under a nitrogen atmosphere.

4-Chloro-2,6-diisopropylphenol (2). This compound was prepared following a published procedure.[15] Full characterization of this compound is reported here: IR 3580 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.28 (d, J = 6.5 Hz, 12H, CH_3), 3.18 (septet, J = 6.5 Hz, 2H, C-H(CH_3)$_2$), 4.3 (br s, 1H, OH), 7.08 (s, 2H, arom); MS m/z 212 (30, M^+), 197 (base). Anal. ($C_{12}H_{17}ClO$) C, H.

4-Bromo-2,6-diisopropylphenol (3). This compound was prepared following a published procedure.[15] Full characterization of this compound is reported here: IR 3575 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.23 (d, J = 6.5 Hz, 12H, CH_3), 3.10 (septet, J = 6.5 Hz, 2H, C-H(CH_3)$_2$), 4.70 (s, 1H, OH), 7.16 (s, 2H, arom); MS m/z 256 (43, M^+), 241 (base). Anal. ($C_{12}H_{17}BrO$) C, H.

4-Iodo-2,6-diisopropylphenol (4). To a stirred solution of 2,6-diisopropylphenol (**1**) (1.78 g, 10 mmol) in 30 mL of acetic acid at room temperature was added dropwise a solution of iodonium chloride (0.81 g, 5 mmol) in acetic acid (30 mL). The solution was stirred for 3 h at room temperature and then evaporated under reduced pressure to give a residue which was purified by silica gel column chromatography [petroleum ether/ethyl acetate 95:5 (v/v) as eluent)] to give 1.2 g (40%yield) of **4** as a yellow oil (bp 90−92 °C, 1 mmHg): IR 3575 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.20 (d, J = 6.5 Hz, 12H, CH_3), 3.05 (septet, J = 6.5 Hz, 2H, C-H(CH_3)$_2$), 4.70 (s, 1H, OH), 7.20 (s, 2H, arom); MS m/z 304 (83, M^+), 289 (base). Anal. ($C_{12}H_{17}IO$) C, H.

4-Nitro-2,6-diisopropylphenol (5). To 5.35 g (30 mmol) of 2,6-diisopropylphenol (**1**) was added dropwise a mixture of concentrated sulfuric acid (4 mL) and concentrated nitric acid (3.3 mL) at such a rate as to keep the temperature below 5 °C. After 0.5 h of stirring, the color of the mixture changed from pale yellow to orange and a precipitate formed. Stirring was continued at room temperature for another 1.5 h, and then the mixture was washed with water, the organic phase was extracted with $CHCl_3$ (20 mL) and dried (Na_2SO_4), and the solvent was removed by rotary evaporation. The residue was purified by silica gel column chromatography [methanol/ethyl acetate 95:5 (v/v) as eluent)] to give 2.30 g (35%yield) of **5**: IR 3480 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.31 (d, J = 6.5 Hz, 12H, CH_3), 3.18 (septet, J = 6.5 Hz, 2H, C-H(CH_3)$_2$), 5.50 (s, 1H, OH), 8.00 (s, 2H, arom); MS m/z 223 (23, M^+), 208 (base). Anal. ($C_{12}H_{17}NO_3$) C, H, N.

Methyl (3,5-diisopropyl-4-hydroxy)benzenesulfonate (6). To 2.90 g (16.2 mmol) of 2,6-diisopropylphenol (**1**) dimethyl sulfate (8 g, 63.4 mmol) was added dropwise maintaining the temperature at 20−30 °C. The solution was refluxed under stirring for 1 h and, after cooling, was diluted with cold water. Stirring was continued overnight at room temperature, and then the organic phase was extracted with $CHCl_3$ (20 mL) and dried (Na_2SO_4) and the solvent removed by rotary evaporation. The residue was purified by silica gel column chromatography [petroleum ether/ethyl acetate 8/2 (v/v) as eluent)] to give 2 g (45%yield) of **6**: mp 68−70 °C; IR 3540 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.29 (d, J = 6.5 Hz, 12H, CH_3), 3.18 (septet, J = 6.5 Hz, 2H, C-H(CH_3)$_2$), 3.76 (s, 3H, OCH_3), 5.20 (s, 1H, OH), 7.66 (s, 2H, arom); MS m/z 272 (20, M^+), 257 (base). Anal. ($C_{13}H_{20}O_4S$) C, H.

3,5-Diisopropyl-4-hydroxybenzaldehyde (7). To a solution of 2,6-diisopropylphenol (**1**) (1.5 g, 8.4 mmol) in glacial acetic acid (80 mL) were added 40% formaldehyde (1.5 mL) and 30% NH_4OH (1.12 mL). After 24 h on the steam bath, the solvent was evaporated under reduced pressure and the residue dissolved in $CHCl_3$ (20 mL), washed with 5% $NaHCO_3$, and dried (Na_2SO_4). Evaporation of the solvent gave a residue which was purified by silica gel column chromatography [petroleum ether/ethyl acetate 9/1 (v/v) as eluent)] to give 0.60 g (35%yield) of **7**: mp 103−105 °C; IR 3250 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.31 (d, J = 6.5 Hz, 12H, CH_3), 3.23 (septet, J = 6.5 Hz, 2H, C-H(CH_3)$_2$), 5.90 (s, 1H, OH), 7.68 (s, 2H, arom), 9.93 (s, 1H, HCO); MS m/z 206 (30, M^+), 191 (base). Anal. ($C_{13}H_{18}O_2$) C, H.

3,5-Diisopropyl-4-hydroxybenzophenone (8). To a solution of 2,6-diisopropylphenol (**1**) (1 g, 5.6 mmol) in 30 mL of toluene were added dropwise benzoyl chloride (10 mmol) and $AlCl_3$ (0.2 g, 2.2 mmol), while the temperature was maintained at 20−30 °C. Stirring was continued for 7 h, and then the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography [petroleum ether/ethyl acetate 95:5 (v/v) as eluent)] to give two fractions. The first one corresponding to the compound having the higher R_f on TLC was identified as compound **18**: yield 0.55 g (35%); white solid; mp 68−70 °C; IR 1730 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.20 (d, J = 6.5 Hz, 12H, CH_3), 3.00 (septet, J = 6.5 Hz, 2H, C-H(CH_3)$_2$), 7.2−7.7 (m, 6H, arom), 8.2−8.4 (m, 2H, arom); MS m/z 282 (6.5, M^+), 105 (base). Anal. ($C_{19}H_{22}O_2$) C, H. The fraction corresponding to the compound having the lower R_f was identified as compound **8**: yield 0.72 g (45%); yellow-orange solid; mp 103−106 °C; IR 3300, 1625 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.30 (d, J = 6.5 Hz, 12H, CH_3), 3.20 (septet, J = 6.5

Hz, 2H, C-*H*(CH_3)$_2$), 5.70 (s, 1H, OH), 7.2−7.9 (m, 7H, arom); MS *m*/*z* 282 (56, M^+), 267 (base). Anal. ($C_{19}H_{22}O_2$) C, H.

***N*,*N*-Dimethyl-(3,5-diisopropyl-4-hydroxy)benzylamine (9).** To a solution of 2,6-diisopropylphenol **1**(2 g, 11.2 mmol) in glacial acetic acid (30 mL) were added 40% formaldehyde (3.0 mL) and 40% dimethylamine (4.0 mL). After 12 h on the steam bath, the solvent was evaporated under reduced pressure and the residue dissolved in $CHCl_3$ (20 mL), washed with 5% $NaHCO_3$, and dried (Na_2SO_4). Evaporation of the solvent gave a residue which was purified by silica gel column chromatography [petroleum ether/ethyl acetate 7/3 (v/v) as eluent)] to give 0.8 g (30%yield) of **9**: IR 3350 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.18 (d, J = 6.5 Hz, 12H, CH_3), 2.43 (s, 6H, NCH_3), 3.26 (septet, J = 6.5 Hz, 2H, C-*H*(CH_3)$_2$), 3.73 (s, 2H, CH_2N), 7.10 (s, 2H, arom), 7.20 (s, 1H, OH); MS *m*/*z* 235 (29, M^+), 191 (base). Anal. ($C_{15}H_{25}NO$) C, H, N.

***N*,*N*-Dimethyl-3,5-diisopropyl-4-hydroxyaniline (10).** A solution of **5** (0.5 g, 2.2 mmol) in EtOH (250 mL) containing 40% formaldehyde (0.75 mL) was hydrogenated at 3 atm of hydrogen in the presence of 10% Pd/C (0.1 g) for 48 h. The reaction mixture was filtered, and the catalyst was washed with ethanol. The combined filtrate was evaporated under reduced pressure to give a residue which was purified by silica gel column chromatography [petroleum ether/ethyl acetate 8/2 (v/v) as eluent)] to give 0.33 g (68%yield) of **10**: IR 3350 cm^{-1}; ^1H NMR (DMSO) δ 1.28 (d, J = 6.5 Hz, 12H, CH_3), 2.88 (s, 6H, NCH_3), 3.45 (septet, J = 6.5 Hz, 2H, C-*H*(CH_3)$_2$), 6.51 (s, 2H, arom), 7.35 (s, 1H, OH); MS *m*/*z* 221 (27, M^+), 207 (base). Anal. ($C_{14}H_{23}NO$) C, H, N.

3,5-Diisopropyl-4-hydroxyaniline (11). To a solution of **5** (1.4 g, 6.3 mmol) in EtOH (10 mL) and concentrated HCl (25 mL) was added granular Sn (5 g), and the mixture under stirring was refluxed for 1 h. Then, the yellow reaction mixture was filtered, and on cooling the HCl salt of the desired compound precipitated (1.2 g, yield 85%): mp 121−124 °C; IR 3380, 2900 cm^{-1}; ^1H NMR (D_2O) δ 1.50 (d, J = 6.5 Hz,12H, CH_3), 3.60 (septet, J = 6.5 Hz, 2H, C-*H*(CH_3)$_2$), 7.48 (s, 2H, arom). Anal. ($C_{12}H_{20}ClNO$) C, H, N.

***N*-(3,5-Diisopropyl-4-hydroxy)phenylacetamide (12).** A solution of **11**·HCl (0.2 g, 0.9 mmol) in acetic anhydride (10 mL) and dry pyridine (2 mL) was stirred at room temperature for 0.5 h. Then, the reaction mixture was acidified with dilute HCl and washed with water and the organic phase was extracted with ethyl ether (3 × 30 mL) and dried (Na_2SO_4). Evaporation of the solvent gave a yellow solid (0.15 g, 60% yield) identified as compound **27**: IR 3280, 1750, 1650 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.60 (d, J = 6.5 Hz, 12H, CH_3), 2.60 (s, 6H, $COCH_3$), 3.26 (septet, J = 6.5 Hz, 2H, C-*H*(CH_3)$_2$), 7.73 (s, 2H, arom), 8.40 (s, 1H, NH); MS *m*/*z* 277 (11, M^+), 235 (base). Anal. ($C_{16}H_{23}NO_3$) C, H, N.

A solution of **27** (0.5 g, 1.8 mmol) in EtOH (10 mL) was treated with 2 N NaOH (2 mL) at room temperature. The progress of the reaction was monitored on TLC [light petroleum ether/ethyl acetate 1/2 (v/v) as eluent)]. When no trace of starting material was observed, the reaction mixture was diluted with ice cooled water, extracted with ethyl ether (3 × 30 mL), and dried (Na_2SO_4). Evaporation of the solvent gave the crude product which was purified by silica gel column chromatography [petroleum ether/ethyl acetate 1/2 (v/v) as eluent)] to give 0. 4 g (94%yield) of **12**: mp 165−166 °C; IR 3420, 3280, 1630 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.40 (d, J = 6.5 Hz, 12H, CH_3), 2.30 (s, 3H, $COCH_3$), 3.26 (septet, J = 6.5 Hz, 2H, C-*H*(CH_3)$_2$), 5.0 (br s,1H,OH), 7.33(s, 2H, arom), 7.40 (s, 1H, NH); MS *m*/*z* 235 (92, M^+), 193 (base). Anal. ($C_{14}H_{21}NO_2$) C, H, N.

***N*-(3,5-Diisopropyl-4-hydroxy)phenyltrifluoroacetamide (13).** A solution of **11**·HCl (0.5 g, 2.2 mmol) in trifluoroacetic anhydride (10 mL) and dry pyridine (2 mL) was stirred at room temperature for 1 h. Then, the reaction mixture was acidified with dilute HCl and washed with water and the organic phase was extracted with ethyl ether (3 × 30 mL) and dried (Na_2SO_4). Evaporation of the solvent gave a white solid (0.6 g, 72% yield) identified as compound **28**: IR 3270, 1790, 1700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.29 (d, J = 6.5 Hz, 12H, CH_3), 2.90 (septet, J = 6.5 Hz, 2H, C-*H*(CH_3)$_2$), 7.50(s, 2H, arom), 8.0 (br s, 1H, NH); MS *m*/*z* 385 (base). Anal. ($C_{16}H_{17}F_6NO_3$) C, H, N.

A solution of **28** (0.15 g, 0.4 mmol) in EtOH (10 mL) was treated with 2 N NaOH (1 mL) at room temperature. The progress of the reaction was monitored on TLC [petroleum ether/ethyl acetate 1/2 (v/v) as eluent)]. When no trace of starting material was observed, the reaction mixture was diluted with ice-cooled water, extracted with ethyl ether (3 × 30 mL), and dried (Na_2SO_4). Evaporation of the solvent gave the crude product **13** which was purified by crystallization from CCl_4 (0.10 g, 91% yield): mp 194−196 °C; IR 3460, 3300 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.24 (d, J = 6.5 Hz, 12H, CH_3), 3.14 (septet, J = 6.5 Hz, 2H, C-*H*(CH_3)$_2$), 4.8 (br s, 1H, OH), 7.22 (s, 2H, arom), 7.77 (s, 1H, NH); MS *m*/*z* 289 (68, M^+), 274 (base). Anal. ($C_{14}H_{18}F_3NO_2$) C, H, N.

***N*-(3,5-Diisopropyl-4-hydroxy)phenylmethanesulfonamide (14).** To an ice-cooled solution of **11**·HCl (0.4 g, 1.7 mmol) in dioxane (15 mL) containing dry pyridine (3 mL) was dropwise added methanesulfonyl chloride (0.24 g, 2.0 mmol). The stirring was continued for 10 min and then for 1 h at room temperature. The reaction mixture was acidified with dilute HCl and washed with water and the organic phase was extracted with ethyl ether (3 × 30 mL) and dried (Na_2SO_4). Evaporation of the solvent gave the crude product **14** which was purified by recrystallization from CCl_4 (0.33 g, 72% yield): mp 145−148 °C; IR 3480, 3200 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.33 (d, J = 6.5 Hz, 12H, CH_3), 3.00 (s, 3H, SO_2CH_3), 3.20 (septet, J = 6.5 Hz, 2H, C-*H*(CH_3)$_2$), 3.5 (br s, 1H, OH), 6.4 (br s, 1H, NH), 7.00 (s, 2H, arom); MS *m*/*z* 271 (14, M^+), 192 (base). Anal. ($C_{13}H_{21}NO_3S$) C, H, N.

***N*-(3,5-Diisopropyl-4-hydroxyphenyl)-*N*,*N*,*N*-trimethylammonium Iodide (15).** A solution of **10** (0.8 g, 3.6 mmol) in $CHCl_3$/MeOH (3:1 v:v) (20 mL), CH_3I (6 mL) was stirred at room temperature overnight. Evaporation of the solvent gave a residue which was purified by recrystallization from petroleum ether/ethanol. **15** (yield 0.6 g, 46%): mp 235−238 °C; IR 3240 cm^{-1}; ^1H NMR (D_2O) δ 1.06 (d, J = 6.5 Hz, 12H, CH_3), 3.17(septet, J = 6.5 Hz, 2H, C-*H*(CH_3)$_2$), 3.44 (s, 9H, CH_3), 7.27(s, 2H, Ar−H). Anal. ($C_{15}H_{26}INO$) C, H, N.

2,6-Diisopropylanisole (16). To a solution of 2,6-diisopropylphenol (**1**) (1.78 g, 10 mmol) in methanol (40 mL) containing sodium (0.5 g) dimethyl sulfate (2 mL, 2.67 g, 21.1 mmol) was added dropwise, while the temperature was maintained at 20−30 °C. The solution was refluxed under stirring for 1 h, and after cooling, was diluted with cold water. Stirring was continued overnight at room temperature, the organic phase was extracted with $CHCl_3$ (20 mL) and dried (Na_2SO_4), and the solvent was removed by rotary evaporation. The residue was purified by silica gel column chromatography [petroleum ether/ethyl acetate 9/1 (v/v) as eluent)] to give 1.05 g (55%yield) of **16**: ^1H NMR ($CDCl_3$) δ 1.23 (d, J = 6.5 Hz, 12H, CH_3), 3.33 (septet, J = 6.5 Hz, 2H, C-*H*(CH_3)$_2$), 3.73 (s, 3H, OCH_3), 7.1−7.3 (m, 3H, arom); MS *m*/*z* 192 (38, M^+), 177 (base). Anal. ($C_{13}H_{20}O$) C, H.

2,6-Diisopropylphenyl Acetate (17). A solution of 2,6-diisopropylphenol (**1**) (1 g, 5.6 mmol) in acetic anhydride (0.63 mL) in a sealed tube was heated at 160 °C for 20 h. Evaporation of the solvent gave a residue which was the essentially pure compound **17** (1.17 g, 95% yield): IR 1760 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.20 (d, J = 6.5 Hz, 12H, CH_3), 2.33 (s, 3H, $COCH_3$), 2.90 (septet, J = 6.5 Hz, 2H, C-*H*(CH_3)$_2$), 7.1−7.3 (m, 3H, arom); MS *m*/*z* 220 (8, M^+), 163 (base). Anal. ($C_{14}H_{20}O_2$) C, H.

2,6-Diisopropylphenyl Bromoacetate (19). To a solution of 2,6-diisopropylphenol (**1**) (1.78 g, 10 mmol), monobromoacetic acid (1.53 g, 11 mmol), and (dimethylamino)pyridine (0.1 g) in dry dichloromethane (15 mL) was added dropwise a solution of dicyclohexylcarbodiimide (3.09 g, 15 mmol) in dry dichloromethane (10 mL) for 10 min. Stirring was continued at room temperature for 30 h, and then the resulting precipitate was removed. The solution was evaporated under reduced pressure to give a residue which was purified by column chromatography on silica gel (petroleum ether−ethyl acetate,

98:2 v/v, as eluent) to give 2.15 g (72%yield) of **19**: IR 1740 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.23 (d, J = 6.5 Hz, 12H, CH_3), 3.00 (septet, J = 6.5 Hz, 2H, C-$H(CH_3)_2$), 4.10 (s, 2H, CH_2), 7.1−7.3 (m, 3H, arom); MS m/z 298 (20, M^+), 163 (base). Anal. ($C_{14}H_{19}BrO_2$) C, H.

Reaction of 2,6-Diisopropylphenyl Bromoacetate with Amines. A mixture of **19** (0.5 mmol) and the appropriate dialkyl- or cycloalkylamine (0.8 mmol) in THF (25 mL) was stirred at room temperature (in the case of the pirrolidine) for 24 h or heated at 150 °C in a sealed tube (in the other cases) for 20 h. Evaporation of the solvent under reduced pressure gave a residue which was purified by column chromatography on silica gel (petroleum ether−ethyl acetate, 98:2 v/v, as eluent) to give compounds **20**−**24**.

***N*-(2,2-Dimethoxyethyl) (2,6-diisopropylphenyl)glycinate (20)** (75% yield): oil; IR 3330, 1755 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.13 (d, 12H, CH_3), 1.9 (br s, 1H, NH), 2.7−3.1 (m, 4H, CH_2N + C-$H(CH_3)_2$), 3.40 (s, 6H, OCH_3), 3.73 (s, 2H, $COCH_2$), 4.50 (t, 1H, C$H(OCH_3)_2$), 7.1−7.3 (m, 3H, arom); MS m/z 323 (2, M^+), 75 (base). Anal. ($C_{18}H_{29}NO_4$) C, H, N.

***N*,*N*-Diethyl(2,6-Diisopropylphenyl)glycinate (21)** (61% yield): oil; IR 1755 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.13 (t, 6H, CH_3), 1.23 (d, J = 6.5 Hz, 12H, CH_3), 2.77 (q, 4H, CH_2), 2.90 (septet, J = 6.5 Hz, 2H, C-$H(CH_3)_2$), 3.67 (s, 2H, CH_2), 7.1−7.3 (m, 3H, arom); MS m/z 291 (2, M^+), 86 (base). Anal. ($C_{18}H_{29}NO_2$) C, H, N.

***N*,*N*-Di-*n*-propyl(2,6-diisopropylphenyl)glycinate (22)** (55% yield): oil; IR 1750 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.90 (t, 6H, CH_3), 1.20 (d, J = 6.5 Hz, 12H, CH_3), 1.4−1.8 (m, 4H, CH_2), 2.90 (septet, J = 6.5 Hz, 2H, C-$H(CH_3)_2$), 2.6−2.8 (m, 4H, CH_2), 3.66 (s, 2H, CH_2), 7.1−7.3 (m, 3H, arom); MS m/z 319 (2, M^+), 114 (base). Anal. ($C_{20}H_{33}NO_2$) C, H, N.

(2,6-Diisopropylphenyl)(pyrrolidin-1-yl)acetate (23) (71% yield): oil; IR 1770 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.20 (d, J = 6.5 Hz, 12H, CH_3), 1.7−1.9 (m, 4H, CH_2), 2.90 (septet, J = 6.5 Hz, 2H, C-$H(CH_3)_2$), 2.6−2.8 (m, 4H, CH_2), 3.65 (s, 2H, CH_2), 7.1−7.3 (m, 3H, arom); MS m/z 289 (3.4, M^+), 84 (base). Anal. ($C_{18}H_{27}NO_2$) C, H, N.

(2,6-Diisopropylphenyl)(morpholin-1-yl)acetate (24) (70% yield): mp 53−55 °C; IR 1770 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.20 (d, J = 6.5 Hz,12H, CH_3), 2.6−2.8 (m, 4H, CH_2), 2.90 (septet, J = 6.5 Hz, 2H, C-$H(CH_3)_2$), 3.55 (s, 2H, CH_2), 3.7−3.9 (m, 4H, CH_2), 7.1−7.3 (m, 3H, arom); MS m/z 305 (M^+, base). Anal. ($C_{18}H_{27}NO_3$) C, H, N.

Methyl 2-Hydroxy-3-isopropylbenzoate (26).[18] A solution of **25** (0.35 g, 1.9 mmol) in methanol (10 mL) containing a drop of sulfuric acid was refluxed under stirring for 48 h. Evaporation of the solvent under reduced pressure gave a residue which was purified by column chromatography on silica gel (petroleum ether−ethyl acetate, 8:2 v/v, as eluent) to give 0.31 g of compound **26** (85% yield): ^1H NMR ($CDCl_3$) δ 1.30 (d, J = 6.5 Hz, 6H, CH_3), 3.42 (septet, J = 6.5 Hz, 1H, C-$H(CH_3)_2$), 3.96 (s, 3H, OCH_3), 6.7−7.8 (m, 3H, arom), 11.5 (br s, 1H OH); MS m/z 194 (48, M^+), 147 (base). Anal. ($C_{11}H_{14}O_3$) C, H.

QSAR Analysis. For each para-X-substituted congener we compiled the following descriptors: (i) octanol/water partition coefficient, log P, calculated by the fragmental method of Hansch and Leo,[24] using the computer program CLOG P;[25] (ii) bulk descriptors (i.e., molar refractivities, MR, and McGowan volume, V_x[21]); (iii) acidity and polar binding parameters (Hammett σ constants). For compound **9**, having a basic moiety with a calculated pK_a[26] of 9.0 ± 0.2, the log P value for the neutral species was transformed into a distribution coefficient (log D) taking into account the correction term for ionization.[27]

Multiple linear regression (MLR) analysis was performed using the commercially available statistical package PARVUS 1.2[28] running on a IBM-compatible personal computer. To find meaningful QSAR equations, MLR analysis was combined with a "*leave-one-out*" cross-validation (cv) procedure.[29] All combinations and permutations of the above molecular descriptors, and their square terms, were tried, and the statistically best two-parameter equation was obtained following the unambiguous criterium to maximize the percent of cv-explained variance. MLR analysis was finally carried out on the matrix of autoscaled data,[22] resulting from a column (variable) centering followed by column standardization, obtained by dividing each matrix element by the standard deviation of the corresponding column.

Biological Methods. [^{35}S]TBPS Binding to Unwashed Rat Cortical Membranes. Male Sprague−Dawley CD rats (Charles River, Como, Italy) weighing 180−200 g were used. The animals were kept on a controlled light−dark cycle (light period between 8:00 a.m. and 8:00 p.m.) in a room with constant temperature (22 ± 2 °C) and humidity (65%). Upon arrival at the animal facilities there was a minimum of 7 days of acclimation during which the animals had free access to food and water. Rats were killed by decapitation, and their brains were rapidly removed. The cerebral cortex was dissected out and homogenized in 50 volumes of ice-cold 50 mM Tris-citrate buffer (pH 7.4 at 25 °C) containing 100 mM $CaCl_2$ using a Polytron PT 10 (setting 5, for 20 s) and centrifuged at 20000*g* for 20 min. The resulting pellet was resuspended in 50 volumes of 50 mM Tris-citrate buffer (pH 7.4 at 25 °C) and used for the assay. [^{35}S]TBPS binding was determined in a final volume of 500 μL consisting of 200 μL of tissue homogenate (0.20−0.25 mg protein), 50 μL of [^{35}S]TBPS (final assay concentration, 1 nM), 50 μL of 2 M NaCl, 50 μL of drugs or solvent and buffer to volume. Incubations (25 °C) were initiated by addition of tissue and terminated 90 min later by rapid filtration through glass-fiber filter strips (Whatman GF/B, Clifton, NJ), which were rinsed twice with a 4 mL portion of ice-cold Tris-citrate buffer using a Cell Harvester filtration manifold (model M-24m Brandel, Gaithersburg, MD). Filter bound radioactivity was quantitated by liquid scintillation spectrometry. Nonspecific binding was defined as binding in the presence of l00 μM picrotoxin and represented about 10% of total binding. Proteins were assayed with the method of Lowry et al.[30] using bovine serum albumin as standard.

Electrophysiological Studies Using *Xenopus* Oocytes. cDNAs encoding the human α1, β2, and γ2 $GABA_A$ receptor subunits were subcloned into the pCDM8 expression vector (Invitrogen, San Diego, CA).[31] 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* female frogs were obtained from Dipl. Biol.-Dipl Ing. Horst Kähler (Hamburg, Germany). Oocyte isolation and cDNA microinjection were performed essentially as previously described.[32] In brief, stage V and VI oocytes were manually isolated, 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 $Ca(N0_3)_2$ and treated with 0.5 mg/mL of collagenase Type IA (Sigma) in collagenase buffer (83 mM NaCl, 2 mM KCl, 1 mM$MgCl_2$, 5 mM Hepes-NaOH pH 7.5) for 10 min at room temperature, to remove the follicular layer. A mixture of $GABA_A$ receptor α1, β2, and γ2, or α1, β1, and γ2 subunit cDNAs (1.5 ng/30 nL) were injected into the oocyte nucleus using a 10 μL glass micropipet (10−15 μm tip diameter). The injected oocytes were cultured at 19 °C in sterile MBS supplemented with streptomycin (10 μg/mL), penicillin (10 units/mL), gentamicin (50 μg/mL), 0.5 mM theophylline, and 2 mM sodium pyruvate. Electrophysiological recordings began approximately 24 h following cDNA injection. Oocytes were placed in a 100 μL rectangular chamber and continuously perfused with MBS solution at a flow rate of 2 mL/min at room temperature. The animal pole of oocytes was impaled with two glass electrodes (0.5−3 MΩ) filled with filtered 3 M KCl, and the voltage was clamped at −70 mV with an Axoclamp 2-B amplifier (Axon Instruments, Burlingame, CA). Currents were continuously recorded on a strip-chart recorder. Resting membrane potential usually varied between −30 and −50 mV. Drugs were perfused for 20 s (7−10 s were required to reach equilibrium in the recording chamber). Intervals of 5−10 min were allowed between drug applications.

Propofol and derivatives were dissolved in dimethyl sulfoxide (DMSO) at the concentration of 10 mM and diluted at the appropriate concentration with MBS.

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