Brief Articles

Synthesis of New 2,6-Prolylxylidide Analogues of Tocainide as Stereoselective Blockers of Voltage-Gated Na^+ Channels with Increased Potency and Improved Use-Dependent Activity

Carlo Franchini,*,† Filomena Corbo,† Giovanni Lentini,† Gemma Bruno,† Antonio Scilimati,† Vincenzo Tortorella,† Diana Conte Camerino,‡ and Annamaria De Luca‡

Dipartimento Farmaco-Chimico and Farmaco-Biologico, Università di Bari, Via Orabona n.4, 70125 Bari, Italy

Received June 6, 2000

A series of tocainide chiral analogues were designed, synthesized, and evaluated in vitro, in pure enantiomeric form, as use-dependent blockers of skeletal muscle sodium channels to better understand the structural requirements responsible for the antimyotonic activity. The voltage clamp recordings showed a remarkable increase of both potency and use-dependent behavior with the analogue *N*-(2,6-dimethylphenyl)-2-pyrrolidinecarboxamide **(1a).** In fact (*R*)-**1a** was 5-fold more potent than (*R*)-tocainide in producing the tonic block, i.e., the reduction of peak sodium current in resting conditions after application of the compound, but it was 21-fold more potent in condition of high frequency of stimulation (phasic block). Furthermore, as opposite to tocainide, this compound was also stereoselective, (*S*)-**1a** being 2−3-fold less potent than (*R*)-**1a**. The introduction in **1a** of a methyl group in place of the hydrogen bonded to either the aminic nitrogen atom [*N*-(2,6-dimethylphenyl)-1-methyl-2-pyrrolidinecarboxamide **(2a)**] or the amidic nitrogen atom [*N*-(2,6-dimethylphenyl)-*N*-methyl-2-pyrrolidinecarboxamide **(3a)**] led unexpectedly to an inversion of stereoselectivity, the (*S*)-enantiomers being 3-fold more potent than the (*R*)-ones. The comparison between eutomers showed that (*S*)-**2a** and (*S*)-**3a** are almost equieffective to (*R*)-**1a** in producing a tonic block, the half-maximal concentrations being about 100 μM; however, the use-dependent behavior was remarkably decreased by the presence of the methyl group: i.e., the gain of potency observed at high frequency of stimulation amounted to 3 and 1.6 times for **2a** and **3a**, respectively. The replacement of both hydrogens bonded to the aminic and amidic nitrogen atoms resulted in *N*-(2,6-dimethylphenyl)-*N*,1-dimethyl-2-pyrrolidinecarboxamide **(4a)** in which the (*S*)-isomer was still twice as potent as the (*R*)-one, but the absolute potency and mostly the use-dependent behavior were strongly reduced, showing therefore no clear advantages with respect to tocainide. The use-dependent behavior, which plays a pivotal role for antimyotonic activity, is strongly reduced by the presence of methyl groups on the nitrogen atoms, likely for modification of pK_a and/or for constraint of molecular conformation.

Introduction

The antiarrhythmic action of the type I antiarrhythmic drugs is thought to be mediated via blockade of the fast voltage-gated sodium channel.[1] Models based on electrophysiological data have been developed to explain the interaction between antiarrhythmic drugs and the cardiac sodium channel.[2,3] According to these models, type I antiarrhythmic drugs bind to specific site(s) or receptor(s) on the functional α-subunit of sodium channels.[4]

Tocainide hydrochloride is a class Ib antiarrhythmic drug once used in the treatment of symptomatic life-threatening ventricular arrhythmias. It has also a marked analgesic effect in trigeminal neuralgia in humans[5−7] and antinociceptive effect in rats as well.[8] Finally, by virtue of its ability to block sodium channels in a use-dependent manner, i.e., with an increased potency in condition of high-frequency discharges of action potentials, tocainide has been proposed as a clinically useful antimyotonic drug. In fact, myotonic syndromes are hereditary disorders of skeletal muscle due to genetic defects in sodium or chloride channels whose main result is an abnormal membrane hyperexcitability that triggers muscle stiffness. Due to the wide spectrum of pharmacological activity, the use of tocainide as antimyotonic is hindered by unwanted side effects.

Tocainide is a chiral α-amino acid derivative clinically used as racemate, although most of its biological activities reside mainly in the (*R*)-enantiomer. In fact, the (−)-(*R*)-stereoisomer represents the eutomer in binding cardiac sodium channels[9] and in blocking cardiac-like embryonic sodium channels expressed in cultured muscle cells[10] but fails to show any significant stereoselectivity on sodium channel of adult skeletal muscle.[11,12] The (−)-

* To whom correspondence should be addressed. Tel: +39 080 5442743. Fax: +39 080 5442231. E-mail: cfranc@farmchim.uniba.it.
† Dipartimento Farmaco-Chimico.
‡ Dipartimento Farmaco-Biologico.

10.1021/jm000931l CCC: $19.00
Published on Web 09/19/2000

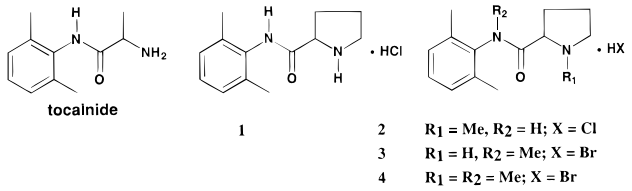


Figure 1. Structures of *N*-2-(2,6-dimethylphenyl)-2-pyrrolidinecarboxamide (1) and derivatives. Free bases of compounds 1−4 will be indicated as 1a−4a.

(*R*)-tocainide is the eutomer also responsible for exerting analgesia. Pharmacological tests have shown that the antinociceptive effect of the (−)-(*R*)-enantiomer, like the analgesia induced by lidocaine, procaine, and mexiletine, is due to a central presynaptic cholinergic mechanism of action.[9]

These observations suggest that stereoselectivity plays an important role for the pharmacological activities of tocainide.[9] The identification of the chemical features specifically responsible for the various biological activities of tocainide can help to identify new compounds with a more selective pharmacological spectrum and therefore with less side effects.

In our previous work,[13] we have synthesized both enantiomers of a rigid analogue of tocainide [e.g., *N*-(2,6-dimethylphenyl)-2-pyrrolidinecarboxamide, **1a**] and started to evaluate their pharmacological activity as well. Such an anilide was still endowed with analgesic activity, but it exerted antinociception without any stereoselectivity.[13] Moreover, it is worthy of note that all structural modifications previously described[13] shifted the antinociceptive activity from central cholinergic to a different, not well characterized mechanism of analgesia. In addition, *N*-methyltocainide (methyl group bonded to the amidic nitrogen) has no analgesic activity, which makes the pharmacological dissociation of tocainide activities conceivable, still being more active than tocainide on the cardiac sodium channel.[14] Concomitantly, voltage clamp experiments on sodium currents of adult muscle fibers have shown that chiral tocainide derivatives with substitutions on the asymmetric carbon atom, i.e., with an isopropyl group in place of the methyl one, are more potent and stereoselective, corroborating the importance of this part of the molecule for the antimyotonic activity as well.[12]

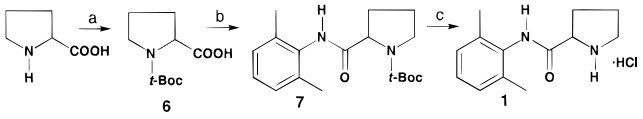
These findings prompted us to synthesize further anilide derivatives, structurally related to tocainide, sterically hindered at the level of the chiral carbon atom, and therefore conformationally restricted, in which only one nitrogen (aminic or amidic) or both bear a methyl group (Figure 1) to evaluate their pharmacological activity and to gain a better insight into the molecular determinants specifically related to the antimyotonic activity. In this paper the results of the above-mentioned studies are reported, and the rationale of the pharmacological data will be discussed.

Chemistry and Biological Results

Assuming tocainide as a "lead compound", we synthesized new analogues. The proline derivatives (object of this study) are reported in Figure 1 and were synthesized as described in Schemes 1 and 2.

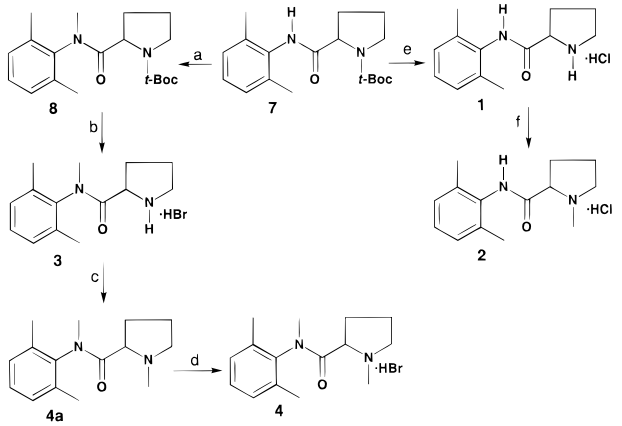
The synthesis of *N*-(2,6-dimethylphenyl)-2-pyrrolidinecarboxamide was carried out as indicated in Scheme 1: α-proline was converted into its N-Boc derivative **6** by reaction with 2-*tert*-butoxycarbonylimino-2-phenylacetonitrile (Boc-ON)/triethylamine (Et_3N) in dioxane/water. Then, *N*-Boc-α-proline **(6)** was condensed with 2,6-dimethylaniline in the presence of EEDQ affording the desired 1-(*tert*-butoxycarbonyl)-*N*-(2,6-dimethylphenyl)-2-pyrrolidinecarboxamide **(7)** that in turn was converted into *N*-(2,6-dimethylphenyl)-2-pyrrolidinecarboxamide **(1a)** (free amines will be identified with the same number of the corresponding hydrochloride or hydrobromide followed by a letter, such as 1a, 2a, etc.; see also Figure 1) and its hydrochloride **1**.

Scheme 1[a]



[a] Reagents and conditions: (a) Boc-ON, dioxane/H_2O; (b) 2,6-dimethylaniline, EEDQ/Et_3N, $CHCl_3$; (c) HCl(g).

Scheme 2[a]



[a] Reagents and conditions: (a) CH_3I, $Ba(OH)_2$, DMF/H_2O; (b) CH_3COOH/HBr; (c) HCHO/HCOOH; (d) 48% aq HBr; (e) HCl; (f) i. NaOH, ii. 5% Pd/C, H_2 (10 atm), HCHO, iii. HCl (g), Et_2O.

1-(*tert*-Butoxycarbonyl)-*N*-(2,6-dimethylphenyl)-*N*-methyl-2-pyrrolidinecarboxamide **(8)** was prepared by reacting **7** with CH_3I in DMF/H_2O in the presence of $Ba(OH)_2$ (Scheme 2). *N*-(2,6-Dimethylphenyl)-*N*-methyl-2-pyrrolidinecarboxamide hydrobromide **(3)** was then obtained treating **8** with acetic acid and 48% aqueous hydrobromic acid. *N*-(2,6-Dimethylphenyl)-*N*,1-dimethyl-2-pyrrolidinecarboxamide **(4a)** was obtained from **3,** through the classical route of reductive formylation HCHO/HCOOH. The corresponding hydrobromide **4** was prepared by treating **4a** with 48% aqueous hydrobromic acid.

N-(2,6-Dimethylphenyl)-1-methyl-2-pyrrolidinecarboxamide **(2)** was obtained from **1** (previously converted into its free base **1a** by treatment with 2 M NaOH) by reaction with HCHO followed by reduction (H_2/5% Pd−C). **2a** was converted into its hydrochloride by gaseous HCl.

The structural modifications of tocainide were designed (Figure 1) to evaluate in vitro potential antimyotonic activity. We evaluated the capability of the new compounds to exert both tonic and phasic block of Na^+ current (I_{Na}) of skeletal muscle fibers, by using the pulse protocols described in the Experimental Section. Single

Table 1. Concentrations for Half-Maximal Block of Sodium Currents of Skeletal Muscle Fibers by Tocainide and Its Chiral Proline-like Derivatives

compd	absolute config	IC_{50} (μM)[a] tonic block	IC_{50} (μM)[a] phasic block	tonic/phasic block	antimyotonic activity[b]
tocainide	(*R*)	580 ± 11	270 ± 5	2.1	1
	(*S*)	520 ± 8	220 ± 5	2.4	1
1	(*R*)	110 ± 12	13 ± 1	8.5	21
	(*S*)	230 ± 5	44 ± 1	5.2	5
2	(*R*)	300 ± 35	100 ± 15	3	2.7
	(*S*)	106 ± 10	28 ± 3.5	3.4	8
3	(*R*)	340 ± 3.6	217 ± 18	1.6	1
	(*S*)	104 ± 5.3	67 ± 1.6	1.5	3
4	(*R*)	>500	440 ± 2 0	1.1	0.6
	(*S*)	286 ± 11	198 ± 2.5	1.4	1.1

[a] The half-maximal concentrations (IC_{50}) of each compound for producing a tonic block (block of sodium channel at resting conditions evaluated during infrequent depolarizing pulses) and a phasic block (cumulative sodium current reduction by the drug at 10-Hz stimulation frequency) have been obtained by concentration−response curves (see the Experimental Section). [b] Antimyotonic activity: potency in phasic block in relation to tocainide (see the Experimental Section); tocainide = 1.

enantiomers were tested with the aim of evaluating the presence of stereoselectivity in blocking I_{Na}.

The tonic block shown by tested compounds is due to their ability to interact with Na^+ channels in the resting state; thus, tonic block reflects block in tissues at normal firing frequency. However, the use-dependent blockers showed a selectivity of action in favor of tissues characterized by higher frequency of firing and higher depolarized membrane potential than normal ones. Thus, at high frequency of stimulation, an additional cumulative block over the tonic block occurs; this cumulative I_{Na} reduction reflects the highest affinity of the drug for open and/or inactivated channels. For their preferential action on hyperexcitable membranes, use-dependent blockers are the first choice drugs in the therapy of a number of muscolar disorders, like myotonic syndromes. All tested compounds are able to produce both tonic and phasic block, although with different potency. From data reported in Table 1, some considerations can be derived.

The introduction of a rigid proline-like cycle, as in *N*-(2,6-dimethylphenyl)-2-pyrrolidinecarboxyamide **(1a)**, remarkably increased the potency in producing both tonic and phasic block, with respect to tocainide. In fact, the calculated IC_{50} value for tonic block by (*R*)-**1a** is 5-fold lower than that of the parent compound (*R*)-tocainide, the IC_{50} being 110 μM vs 580 μM. At a 10-Hz frequency, the protocol used for evaluating phasic block, the IC_{50} value of (*R*)-**1a** was 21 times lower than that calculated for (*R*)-tocainide, demonstrating that this analogue has a notable use-dependent behavior (Table 1, Figure 2). In support of this view, at high frequency of stimulation the IC_{50} value of tocainide was twice lower than that for tonic block, while the IC_{50} of (*R*)-**1a** was 8.5 times lower when passing from tonic to phasic block. As previously shown[12] and also in the experimental conditions reported here, no significant difference between tocainide enantiomers in producing both tonic and use-dependent block was found, the two enantiomers being almost equieffective. In contrast, **1a** produced a stereoselective block of I_{Na} current, with the (*R*)-antipode being the eutomer. In particular, the eudismic ratio (IC_{50} distomer/IC_{50} eutomer) was about

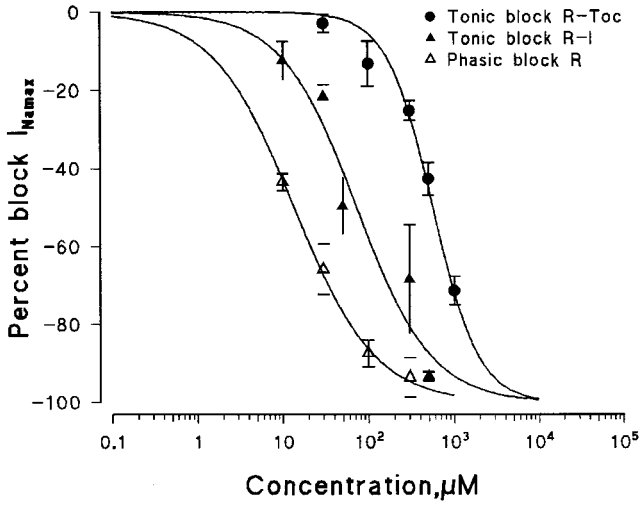


Figure 2. Concentration−response curves for the tonic block exerted by (*R*)-tocainide and the tonic and phasic block at 10 Hz for its derivative (*R*)-**1** obtained by introducing the chiral carbon atom in a rigid proline-like cycle. Each point is the mean ± SEM from 3−7 fibers. The curves have been obtained by fitting the experimental point to the logistic equation described in the Experimental Section.

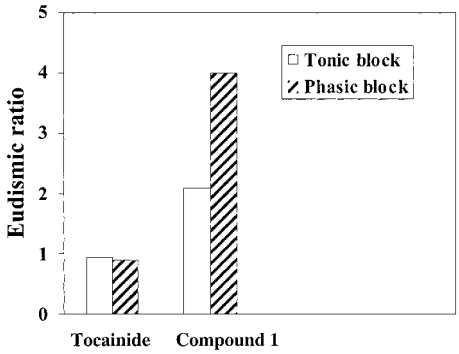


Figure 3. Each bar shows the ratio between the half-maximal concentration (IC_{50}) of the distomer and that of the eutomer, for tonic and phasic block calculated at a 10-Hz frequency. Note that for compound **1** stereoselectivity increases during high stimulation frequency, demonstrating that this analogue has a high-use-dependent behavior.

2 and 4 for tonic and phasic block, respectively (Figure 3). Thus, stereoselectivity was enhanced during phasic block.

The introduction of a methyl moiety on the ionizable amino group as in compound **2a,** as well as on the amidic nitrogen atom as in **3a,** led to an unexpected reversal stereoselectivity, with the (*S*)-isomers being the eutomers, about 3-fold more potent than the opposite (*R*)-enantiomer for tonic block. A comparison between eutomers showed that for tonic block both (*S*)-**2a** and (*S*)-**3a** are equieffective to (*R*)-**1a**, being therefore still more active than tocainide. However, the presence of the methyl group of the nitrogen atoms markedly decreased the use-dependent behavior, with respect to **1a**. In fact, the potency at high frequency of stimulation was increased by a factor of about 3 and 1.5 for **2a** and **3a**, respectively, which was considerably less than that observed with **1a** (Table 1). In this respect, compound **3a** was even less use-dependent than tocainide; indeed the distomer showed an IC_{50} for phasic block similar to that of tocainide (Table 1).

On the other hand, the introduction of a further

methyl group on the amidic nitrogen atom led to **4a**, of which the (*S*)-isomer was the eutomer being about twice as potent as (*S*)-tocainide for tonic block but almost equieffective for phasic block, while the (*R*)-**4a** was much less potent than tocainide.

All the tested compounds behaved as inactivated channel blockers, being able to shift the steady-state inactivation curves toward more negative potentials in a manner correlated to their potency in blocking I_{Na} (data not shown).

Discussion

Previous results, confirmed by the present study, have underlined that tocainide, able to exert various pharmacological actions in a stereoselective manner, is poorly stereoselective in blocking sodium channels of adult skeletal muscle fibers.[12] Nonetheless, the chiral carbon atom of tocainide is an important pharmacophoric part also for the interaction with voltage-gated sodium channel and therefore for antimyotonic activity. In fact, we previously demonstrated that an increase of the steric hindrance at this level increases the potency and stereoselectivity.[12] In the present study we synthesized a series of new tocainide analogues in which the stereogenic center is constrained in a rigid proline-like cycle. All the compounds were effective sodium channel blockers, and in particular **1a**, **2a**, and **3a** were more potent than tocainide and mostly differ for stereoselectivity and use-dependent behavior. The most interesting congener was compound **1a,** which showed a great increase of stereoselectivity with respect to tocainide, the (*R*)-isomer being 2−3 times more potent than (*S*)-**1a** in both tonic and phasic block. Also **1a** was markedly more potent and use-dependent than tocainide; in fact, (*R*)-**1a** was 5-fold more potent than (*R*)-tocainide for tonic block of sodium currents and up to 20-fold more potent than the parent compound at high stimulation frequency: i.e., the IC_{50} of (*R*)-**1a** decreased by 8-fold passing from tonic to phasic blockade. The use-dependent behavior, i.e., the increase of drug potency observed in condition of high frequency of stimulation, plays an important role for the therapeutic activity of sodium channel blockers, use-dependent compounds being very active on pathological tissues discharging at high frequency, such as muscle fibers affected by myotonic syndromes. In fact we previously demonstrated that new derivatives of mexiletine characterized by a remarkable use-dependent behavior were more potent than the parent compound in suppressing in vitro the hyperexcitability of genetically myotonic mouse.[15] For absolute potency and high-use-dependent behavior, the tocainide derivative **1a** seems a compound particularly promising as antimyotonic. The remarkable use-dependent activity of **1a** may be explained assuming that this molecule is provided with more basicity and lipophilicity, both properties being favorable for a ready access to the receptor site supposed to be located in the channel pore. Since the drug reaches its receptor from the intracellular side of the channel[16−18] and we applied the compounds outside the cell, a difference in apparent affinity may come from difference in drug lipophilicity or pK_a, the former facilitating the diffusion through the membrane and the latter through the hydrophilic pathway of the open channel from the cytoplasm[19] and by virtue of the voltage gradient across the membrane.[20,21] Physicochemical aspects such as basicity and lipophilicity can account for the different use-dependent behavior of each compound; however, other factors should be taken into account in order to justify the reversal of stereoselectivity of action observed when passing from **1a** to its methylated derivatives. As may be expected, the introduction of conformational constraints increases the eudismic ratio [ER = (*S*) − IC_{50}/(*R*) − IC_{50}], and a higher stereoselectivity of action was found in all tocainide cyclic analogues. Less predictable was the inversion of the stereoselectivity pattern which assigned the role of eutomer to **2a**, **3a**, and **4a** (*S*)-isomers in opposition to that observed with **1a**. The reason of this finding is far from obvious, even if some hypotheses may be envisaged considering some recent results coming from molecular pharmacology studies.[22,23] The aromatic ring of local anesthetic-like drugs has been proposed to bind to a tyrosine residue at position 1586 in the rat skeletal muscle sodium channel, while the terminal amine group of the drugs may associate with a neighboring phenylalanine residue through π electron interaction (position 1579). Recently, site-specific mutagenesis experiments have added other evidence to the importance of hydrophobicity near or at the local anaesthetic-like drug receptor for drug binding affinity.[24,25] This was corroborated by our studies with a series of mexiletine derivatives, showing that the drug potency for blocking sodium channels is highly correlated to the hydrophobicity of the groups linked to the chiral carbon atom,[26] suggesting the occurrence of a strong $\pi-\pi$ interaction. Very recently an asparagine has also been claimed to be involved in the binding of local anaesthetic-like drugs.[27] These findings led to a hypothesis of a rough receptor model presenting three possible interaction sites, two out of three being represented by aromatic residues. The driving force in binding should be given by groups relatively far from the stereogenic center (xylidide moiety). This model might explain the low ERs observed even in the more potent analogues and the different orientations and/or "three-point" interactions possibly assumed by each ligand.

Conclusion

Given the lack of central analgesic action in both **1a** and *N*-methyl(amidic)tocainide, the introduction of conformational constraints was assumed to be favorable to the dissociation of the main tocainide activities (central analgesic and antimyotonic actions).

1a−**4a** were prepared as depicted in Schemes 1 and 2 in good yields and tested in vitro as possible antimyotonic agents by evaluating the ability to block in a use-dependent manner (phasic block) the sodium current of skeletal muscle fibers recorded by means of voltage clamp experiments. Compounds **1a** and **2a** were markedly more potent than tocainide in blocking sodium currents; however, a reduction of the antimyotonic activity resulted in the order **1a**−**4a** (Table 1). Notably, (*R*)-**1a** was 21 times more active than tocainide in exerting a phasic block, and the IC_{50} value relative to phasic block was at least 8 times lower when compared with the corresponding value derived from tonic block experiments. This highly use-dependent behavior pro-

poses (*R*)-**1a** as a candidate for more complex studies aimed at evaluating its actual usefulness in the treatment of myotonias.

Further studies are in progress by SAR investigation to find a correlation between potency, stereoselectivity, and conformational characteristics of the compounds presented in this paper.

Experimental Section

Pharmacology. 1. Preparation and Solutions. Recordings of Na^+ current were performed on a segment of undamaged single muscle fibers (1−2 cm in length), obtained by microsurgery from the ventral branch of semitendinosus muscle, removed from *Rana Esculenta*. The cut end fiber was superfused with an internal solution, having the following composition (mM): CsF 105; MOPS 5; EGTA 5; $MgSO_4$ 2; Na_2-ATP 0.55 (pH at 7.2 with standard NaOH concentrated solution); then it was transferred in the recording chamber (filled with the same solution) that consisted of three partitions delineating four pools; the width of the gap of the central pool A was set to 70−100 μm. With the fiber in this position, three strips of grease were applied and then carefully sealed to reduce leakage. Four KCl/Agar bridge electrodes, one for each pool, connected the recording chamber and the voltage clamp amplifier, based on methods described by Hille and Campbell[28] and detailed elsewhere.[11] The solution level was adjusted to have the four pools physically and electrically independent from each other. The solution in the central pool A was replaced with an external solution, of the following composition (mM): NaCl 77; choline Cl 38; $CaCl_2$ 1.8; KCl 2.5; Na_2HPO_4 2.15; NaH_2PO_4 0.85. All the tested compounds were solubilized in the external solution; stock solutions of drug were prepared daily and diluted to obtain the required concentration.

2. Recording of Na^+ Current and Pulse Protocols. After an equilibration time of 10 min Na^+ currents recordings were performed at 10 °C. The holding potential used was −100 mV. The voltage clamp amplifier was connected via a 12-bit AD/DA Digidata 1200 interface (Axon Inc.) to a 486 DX2/66 PC. The stimulation protocols and data acquisition were driven by the Clampex Program (pClamp 6 software packge, Axon Inc.). The Na^+ currents flowing in response to depolarizing stimuli were low-pass filtered at 10 kHz, visualized on a oscilloscope, sampled at 20 kHz and stored on the hard disk; when necessary, leak and capacity currents were subtracted using the P/4 method. Data analysis were conducted using the Clampfit program. The voltage dependence of the Na^+ current (*I*/*V* curve) was evaluated by using a single test pulse of 10-ms duration, from the h.p. to −20 mV, able to elicit a nearly maximal Na^+ current (I_{Namax}). Interpulse duration was long enough to allow complete recovery of Na^+ channels from inactivation. Tonic block was calculated as percentage reduction of the peak Na^+ current exerted by each compound. Use-dependent block exerted by each drug was evaluated by using trains of 10-ms test pulses, from the h.p. to −20 mV at 10-Hz frequency for 30 s and then by normalizing the residual current at the end of the stimulation protocol with respect to the current in the absence of drug.

3. Statistical Analysis. The data obtained were expressed as mean ± standard error of the mean (SEM). The molar concentrations of each drug producing a 50% block of I_{Namax} (IC_{50}) were determined by using a nonlinear least-squares fit of the concentration−response curves to the following logistic equation:

$$\text{effect} = -100/1 + (K/[\text{drug}])^n$$

where effect = percentage reduction of I_{Na}, −100 = maximal percent block of I_{Na}, $K = IC_{50}$ of tested drug, n = logistic slope factor, and [drug] = molar concentration of the tested drug. The h_∞ curves were fitted with a single Boltzmann distribution as described in detail elsewere.[12]

Chemistry. Melting points taken on Electrothermal apparatus were uncorrected. ^{1}H NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 on a Varian EM 390 or Bruker 300 MHz spectrometer (ASPECT 3000 model) and chemical shifts are reported in parts per million (δ) from internal Me_4Si. Absolute values of the coupling constant are reported. IR spectra were recorded on a Perkin-Elmer 681 spectrometer. Optical rotations were measured on a Perkin-Elmer digital polarimeter, model 241 MC. Thin-layer chromatography (TLC) was performed on silica gel sheets with fluorescent indicator (Statocrom SIF, Carlo Erba), and the spots on the TLC were observed under ultraviolet light or were visualized with I_2 vapor. Chromatography was conducted by using silica gel with an average particle size of 60 μm, a particle size distribution of 40−63 μm and 230−400 ASTM. GC−MS analyses were performed on an HP 5995C model and microanalyses on a elemental analyzer 1106, Carlo Erba instrument. (−)-(*S*)-2-(4-Chlorophenoxy)-2-phenylacetic acid (CPPA)[29] was used as chiral solvating agent for determining the enantiomeric ratio of **3a** by recording ^{1}H NMR spectra of a mixture of (*R*)-**3a** [or (*S*)-**3a**] (10 mg, 0.043 mmol) and CPPA (25 mg, 0.083 mmol) in C_6D_6. The relative intensities of the singlets, due to the two methyl groups bonded to the phenyl ring, gave the ratio of (*R*)-**3a**·(*S*)-CPPA:(*S*)-**3a**·(*S*)-CPPA [95:5 for **3a** prepared starting from (*R*)-proline and vice versa for **3a** derived from (*S*)-proline].

Chemicals and Reagents. BOC-ON reagent [2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile], (*R*)- and (*S*)-2-pyrrolidinecarboxylic acid, 2,6-dimethylaniline and EEDQ (2-ethoxycarbonyl-1,2-dihydroquinoline) were purchased from Aldrich. Other chemicals were of the highest quality commercially available. Procedure to prepare the compounds shown in Schemes 1 and 2 is as follows.

1-(*tert*-Butoxycarbonyl)-2-pyrrolidinecarboxylic Acid (6). A mixture of (*R*)[or (*S*)]-2-pyrrolidinecarboxylic acid (1 mmol), 2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile[30] (1.1 mmol) and triethylamine (1.5 mmol) in 50 mL of dioxane/water (1:1) was stirred at room temperature for 24 h. The reaction mixture was protected from the light for the entire reaction time. Then, the dioxane layer was evaporated and the aqueous residue extracted twice with ethyl ether. The aqueous phase, acidified with 10% HCl solution (pH = 2), was then extracted six times with ethyl acetate; the organic phases were combined, washed with water and dried over $MgSO_4$, and the solvent evaporated under reduced pressure to give a crude oil, which was purified by crystallization. The product was fully characterized by routine spectroscopic analysis and, all the analytical data were in agreement with those ones reported for the autentical sample.[13]

1-(*tert*-Butoxycarbonyl)-*N*-(2,6-dimethylphenyl)-2-pyrrolidinecarboxamide (7). A mixture of 1-(*tert*-butoxycarbonyl)-2-pyrrolidinecarboxylic acid **(6)** (1 mmol), 2,6-dimethylaniline (1.1 mmol), freshly recrystallized EEDQ (1.2 mmol), and triethylamine (1.5 mmol) in 50 mL of $CHCl_3$ was refluxed for 6 h. The solvent was evaporated and the residue, dissolved in ethyl acetate, was extracted with a 10% HCl solution. The organic layer was then washed with 0.1 M NaOH and H_2O, dried over $MgSO_4$, and then evaporated under reduced pressure to give a crude product, which was purified by recrystallization (86−98% yield): mp 166−167 °C (EtOAc); $[\alpha]^{20}_D$ = −126 (c = 1.0, $CHCl_3$) for (*S*)-enantiomer, $[\alpha]^{20}_D$ = +124 (c = 0.9, $CHCl_3$) for (*R*)-enantiomer; GC−MS (70 eV) *m*/*z* (rel inten.) 318 (M^+, 1), 70 (100), 114 (69), 57 (47).

Full characterization was performed on the deprotected product [*N*-(2,6-dimethylphenyl)-2-pyrrolidinecarboxamide] and its hydrochloride **1** [*N*-(2,6-dimethylphenyl)-2-pyrrolidinecarboxamide hydrochloride] as well (see below).

***N*-(2,6-Dimethylphenyl)-2-pyrrolidinecarboxamide Hydrochloride (1).** A solution of 1-(*tert*-butoxycarbonyl)-*N*-(2,6-dimethylphenyl)-2-pyrrolidinecarboxamide **(7)** (1 mmol) in 3 mL anhydrous ethyl ether was saturated with gaseous HCl and stirred at room temperature for 15 min. After removing the solvent under reduced pressure, the residue was purified by recrystallization from EtOH/Et_2O (70% yield): $[\alpha]^{20}_D$ = −2 (c = 1.0, MeOH) and mp = 240 °C dec (EtOH/Et_2O) for (*S*)-enantiomer, $[\alpha]^{20}_D$ = +1 (c = 2, MeOH) and mp = 240 °C dec (EtOH/Et_2O) for (*R*)-enantiomer; ^{1}H NMR (DMSO-d_6, δ)

10.24−10.05 (bs, 1H, NHCO: exchange with D_2O); 9.50−8.20 (bs, 2 H, $^+NH_2$-ring: exchange with D_2O); 7.09 (s, 3 H, aromatic protons); 4.50−4.45 (t, J = 7.4 Hz, 1 H, CHCO); 3.27−3.18 (m, 2 H, $^+NH_2CH_2$); 2.58−2.37 (m, 1 H, $COCHCH_2$; this multiplet overlaps to the DMSO signals (used as solvent to record the spectrum); by addition of D_2O the two signals appeared at 2.5 ppm for DMSO (as expected) and 2.48−2.30 ppm for $COCHCH_2$, respectively); 2.15 (s, 6 H, CH_3); 2.10−1.87 (m, 3 H, 1H of $COCHCH_2$ and 2 H of $NCHCH_2CH_2$). Anal. ($C_{13}H_{18}N_2O$·HCl·0.5H_2O) C, H, N.

***N*-(2,6-Dimethylphenyl)-2-pyrrolidinecarboxamide (1a).** *N*-(2,6-Dimethylphenyl)-2-pyrrolidinecarboxamide hydrochloride **(1)** (5 mmol) was treated with 10 mL AcOEt and 2 M NaOH (5.5 mmol). The aqueous phase was extracted three times with EtOAc. The organic extracts were combined and dried over Na_2SO_4. The solvent was evaporated under reduced pressure giving the product: ^{1}H NMR ($CDCl_3$, δ) 9.30−9.00 (bs, 1 H, NHCO: exchange with D_2O); 7.09 (s, 3H, aromatic protons); 3.93−3.89 (dd, J = 5.0 Hz and 9.1 Hz, 1 H, CHCO); 3.15−2.97 (m, 2 H, $NHCH_2$); 2.32−2.13 (m, 1 H, $COCHCH_2$); 2.19 (s, 6 H, CH_3); 2.12−2.02 (m, 1 H, $COCHCH_2$); 2.01−1.90 (bs, 1 H, NH-ring: exchange with D_2O); 1.90−1.70 (m, 2 H, $NCHCH_2CH_2$); GC−MS (70 eV) *m*/*z* (rel inten.) 218 (M^+, 2), 70 (100), 41 (7).

1-Methyl-*N*-(2,6-dimethylphenyl)-2-pyrrolidinecarboxamide (2a). *N*-(2,6-Dimethylphenyl)-2-pyrrolidinecarboxamide hydrochloride **(1)** (5 mmol) was treated with 10 mL AcOEt and 2 M NaOH (5.5 mmol). The aqueous phase was extracted three times with EtOAc. The organic extracts were combined and dried over Na_2SO_4. The solvent was evaporated under reduced pressure giving the *N*-(2,6-dimethylphenyl)-2-pyrrolidinecarboxamide, used without any further purification. A 40% aqueous formaldehyde was added to an equimolar ethanolic solution of *N*-(2,6-dimethylphenyl)-2-pyrrolidinecarboxamide. This solution was transferred into the autoclave and treated, in the presence of 5% Pd/C as catalyst, with H_2 at 10 atm for 24 h. The solution was filtered on Celite and the solvent removed under reduced pressure. The residue was purified by chromatography on silica gel (eluent: petroleum ether/ethyl acetate = 8/2). The product obtained was converted into its hydrochloride as previous described: ^{1}H NMR ($CDCl_3$, δ) 9.10−8.55 (bs, 1 H, NHCO: exchange with D_2O); 7.15−6.90 (m, 3H, aromatic protons); 3.22−3.13 (m, 1 H, NCH_2); 3.12−3.02 (dd, J = 4.9 and 10.4 Hz, 1 H, CHCO); 2.52 (s, 3 H, NCH_3); 2.50−2.38 (dd, J = 8.8 and 17.0 Hz, 1 H, NCH_2); 2.37−2.24 (m, 1 H, $COCHCH_2$); 2.21 (s, 6 H, CH_3); 2.07−1.93 (m, 1 H, $COCHCH_2$); 1.92−1.80 (m, 2 H, $NCHCH_2CH_2$); GC−MS (70 eV) *m*/*z* (rel inten.) 232 (M^+, 0.5), 85 (14), 84 (100), 42 (45).

1-Methyl-*N*-(2,6-dimethylphenyl)-2-pyrrolidinecarboxamide hydrochloride (2): mp 215−217 °C ($EtOH/Et_2O$); 63−98% yield; $[\alpha]^{20}_D$ = +14.6 (c = 1.5, MeOH) for (*R*)-enantiomer, $[\alpha]^{20}_D$ = −16 (c = 1.5, MeOH) for (*S*)-enantiomer; ^{1}H NMR (DMSO-d_6, δ) 10.50−10.15 (bs, 1H, NHCO: exchange with D_2O); 8.25−7.80 (bs, 1H, $^+NHCH_3$: exchange with D_2O); 7.15−7.05 (m, 3 H, aromatic protons); 4.32−4.08 (m, 1 H, CHCO); 3.60−3.45 (m, 1 H, NCH_2); 3.20−3.00 (m, 1 H, NCH_2); 2.80 (s, 3 H, NCH_3); 2.70−2.53 (m, 1 H, $COCHCH_2$); 2.15 (s, 6 H, CH_3); 2.13−1.83 (m, 3 H, $COCHCH_2$ (1 H) and $NCHCH_2CH_2$ (2 H)). Anal. ($C_{14}H_{20}N_2O$·HCl) C, H, N.

1-(*tert*-Butoxycarbonyl)-*N*-(2,6-dimethylphenyl)-*N*-methyl-2-pyrrolidinecarboxamide (8). To a mixture of 1-(*tert*-butoxycarbonyl)-*N*-(2,6-dimethylphenyl)-2-pyrrolidinecarboxamide **(7)** (2.2 mmol) and $Ba(OH)_2$ (13.2 mmol) in DMF/H_2O (30 mL/10 mL) was added CH_3I (79.2 mmol). This suspension was stirred at room temperature for 12 h; after this time the yellow reaction mixture was filtered to remove the excess of $Ba(OH)_2$ and then extracted three times with petroleum ether. The organic layer was dried over Na_2SO_4. The solvent was then removed under reduced pressure affording the reaction crude which was purified and fully characterized as hydrobromide (94% yield): GC−MS (70 eV) *m*/*z* (rel inten.) 232 (M^+, 3), 114 (87), 70 (100), 57 (64).

***N*-(2,6-Dimethylphenyl)-*N*-methyl-2-pyrrolidinecarboxamide Hydrobromide (3).** 1-(*tert*-Butoxycarbonyl)-*N*-(2,6-dimethylphenyl)-*N*-methyl-2-pyrrolidinecarboxamide **(8)** (5 mmol) was solubilized in 40 mL of a mixture (1/1) AcOH/48% aqueous HBr. The reaction mixture was stirred at room temperature for 15 min. Then, the excess of AcOH/aqueous HBr mixture was evaporated under reduced pressure and the crude crystalized by $EtOH/Et_2O$ (75% yield): mp 166−167 °C ($EtOH/Et_2O$); $[\alpha]^{20}_D$ = +6.0 (c= 1.1, MeOH) for (*R*)-enantiomer, $[\alpha]^{20}_D$ = −7.6 (c = 1.3, MeOH) for (*S*)-enantiomer; ^{1}H NMR (DMSO-d_6, δ) 9.80−8.20 (bs, 2H, $^+NH_2$-ring: exchange with D_2O); 7.32−7.18 (m, 3H, aromatic protons); 3.67 (t, 1 H, J = 8.5 Hz, CHCO); 3.26−3.15 (m, 1 H, NCH_2); 3.14−3.02 (m, 1 H, NCH_2); 3.11 (s, 3 H, NCH_3); 2.19 (s, 3 H, CH_3); 2.18 (s, 3 H, CH_3); 1.95−1.68 (m, 1 H, $NCHCH_2$); 1.67−1.55 (m, 3 H, $NCHCH_2$ (1 H) and $NCHCH_2CH_2$ (2 H)). Anal. ($C_{14}H_{20}N_2O$·HBr) C, H, N.

***N*-(2,6-Dimethylphenyl)-*N*-methyl-2-pyrrolidinecarboxamide (3a).** N-(2,6-Dimethylphenyl)-*N*-methyl-2-pyrrolidinecarboxamide hydrobromide **(3)** dissolved in 2 M NaOH (20 mL) was extracted with ethyl acetate (4 × 25 mL). The organic layer was dried over Na_2SO_4 and then evaporated under reduced pressure to give the product: ^{1}H NMR ($CDCl_3$, δ) 7.18−7.00 (m, 3 H, aromatic protons); 3.21 (t, J = 8.0 Hz, 1H, COCH); 3.14 (s, 3 H, NCH_3); 3.17−3.05 (m, partially overlapped to the previous singlet, 1 H); 2.70−2.57 (m, 1 H); 2.20 (s, 6 H, CH_3); 1.80−1.44 (m, 4 H, $CHCH_2$ and NCH_2CH_2).

1-Methyl-*N*-(2,6-dimethylphenyl)-*N*-methyl-2-pyrrolidinecarboxamide (4a). *N*-(2,6-Dimethylphenyl)-*N*-methyl-2-pyrrolidinecarboxamide hydrobromide **(3)** (7.3 mmol) was dissolved in 1 mL of 40% aqueous formaldehyde to which 1.3 mL of 70% aqueous formic acid was added. The reaction mixture was stirred at 80 °C for 8 h, then it was concentrated under reduced pressure and the residue, dissolved in 2 M NaOH (20 mL) was extracted with ethyl acetate (4 × 25 mL). The organic layer was dried over Na_2SO_4 and then evaporated under reduced pressure to give a crude oil which was purified as hydrobromide, prepared as previous described: ^{1}H NMR ($CDCl_3$, δ) 7.20−7.02 (m, 3 H, aromatic protons); 3.12 (s, 3 H, $CONCH_3$); 3.11−3.02 (t, J = 8.6 Hz, 1 H, COCH; the upper field signal of the triplet appeared split by a long-range coupling constant, J = 2.2 Hz); 2.47−2.35 (t, J = 7.8 Hz, 1 H, NCH_2; the central signal of the triplet appeared splitted by a long-range coupling constant, J = 3.1 Hz); 2.27 (s, 3 H, NCH_3); 2.20 (s, 3 H, CH_3); 2.17 (s, 3 H, CH_3); 2.04−1.91 (m, 1 H, $NCHCH_2$); 1.90−1.70 (m, 2 H, 1 H of $NCHCH_2$ and 1 H of NCH_2); 1.64−1.63 (m, 2 H, NCH_2CH_2); GC−MS (70 eV) *m*/*z* (rel inten.) 246 (M^+, 0.3), 84(100), 42 (25).

1-Methyl-*N*-(2,6-dimethylphenyl)-*N*-methyl-2-pyrrolidinecarboxamide hydrochloride (4): mp 197 °C dec ($EtOH/Et_2O$); 30% yield; $[\alpha]^{20}_D$ = +30 (c = 1.15, MeOH) for (*R*)-enantiomer, $[\alpha]^{20}_D$ = −33.5 (c = 2.1, MeOH) for (*S*)-enantiomer; ^{1}H NMR (DMSO-d_6, δ) 10.40−8.30 (bs, 1H, $^+NHCH_3$-ring: exchange with D_2O); 7.37−7.18 (m, 3 H, aromatic protons); 3.56 (t, 1 H, J = 8.5 Hz, CHCO); 3.49−3.37 (m, 1 H, NCH_2); 3.11 (s, 3 H, $CONCH_3$); 3.00−2.84 (m, 1 H, $NCHCH_2$); 2.70 (s, 3 H, NCH_3-ring); 2.18 (s, 3 H, CH_3); 2.17 (s, 3 H, CH_3); 1.88−1.55 (m, 4 H, $NCHCH_2$ (1 H), NCH_2 (1 H) and NCH_2CH_2 (2 H)). Anal. ($C_{15}H_{22}N_2O$·HBr·$^2/_3H_2O$) C, H, N.

Acknowledgment. Financial support by the Ministero dell'Università e della Ricerca Scientifica e Tecnologica (Rome) and Telethon-Italy (No. 1208) is acknowledged.

References

(1) Vaughan, W. E. M. Classification of antiarrhythmic drugs. *Pharmacol. Ther.* **1975**, *1*, 115−138.
(2) Hondeghem, L. M.; Katzung, B. G. Time and voltage-dependent interactions of antiarrhythmic drugs with cardiac sodium channels. *Biochim. Biophys. Acta* **1977**, *472*, 373−398.
(3) Grant, A. O.; Starmer, C. F.; Strauss, M. C. Antiarrhythmic drug action: blockade of the inward sodium current. *Circ. Res.* **1984**, *55* 427−429.
(4) Sheldom, R. S.; Cannon, N. J.; Duff, H. J. A receptor for type I antiarrhythmic drugs associated with rat cardiac sodium channels. *Circ. Res.* **1987**, *61*, 492−497.

(5) Lindblom, U.; Lindstrom, P. Analgesic effect of tocainide in neuralgia. *Pain* **1984** (Suppl. 2), S411.
(6) Lindstrom, P.; Lindblom, U. The analgesic effect of tocainide in trigeminal neuralgia. *Pain* **1987**, *28*, 45−50.
(7) Roden, D. M.; Woosley, R. L. Drug therapy: Tocainide. *N. Engl. J. Med.* **1986**, *314*, 41−45.
(8) Woolf, J. C.; Wiesenfeld-Hallin, Z. The Systemic Administration of Local Anaesthetic Produces a Selective Depression of C-Afferent Fibre Evoked Activity in the Spinal Cord. *Pain* **1985**, *23*, 361−374.
(9) Sheldom, R. S.; Cannon, N. J.; Duff, H. J. Stereospecific interactions of tocainide with the cardiac sodium channel. *Mol. Pharmacol.* **1988**, *33*, 327−331.
(10) Tricarico, D., Fakler, B., Spittlemeister, W., Ruppersberg J. P., Stutzel, R., Franchini C., Tortorella V., Conte Camerino D., Rüdel R. Stereoselective interaction of tocainide and its chiral analogues with the sodium channels in human myoballs. *Pflügers Arch.* **1991**, *415*, 234−237.
(11) De Luca, A.; Natuzzi, F.; Lentini, G.; Franchini, C.; Tortorella, V.; Conte Camerino, D. Stereoselective effects of mexiletine enantiomers on sodium currents and excitability characteristics of adult skeletal muscle fibers. *Naunyn-Schmiedberg's Arch. Pharmacol.* **1995**, *352*, 653−661.
(12) De Luca, A.; Natuzzi, F.; Duranti, A.; Lentini, G.; Franchini, C.; Tortorella, V.; Conte Camerino, D. Inhibition of frog skeletal muscle sodium channels by newly synthesized chiral derivatives of mexiletine and tocainide. *Naunyn-Schmiedberg's Arch. Pharmacol.* **1997**, *356*, 777−787.
(13) Franchini, C.; Chiaia Noja, F.; Corbo, F.; Lentini, G.; Tortorella, V.; Bartolini, A.; Ghelardini, C.; Matucci, R.; Giotti, A. Stereoselectivity in Central Analgesic Action of Tocainide and Its Analogues. *Chirality* **1993**, *5*, 135−142.
(14) Tenthorey, P. A.; Di Rubio, R. L.; Feldman, H. S.; Takman, B. H. New antiarrhythmic agents.3.Primary β-amino anilides. *J. Med. Chem.* **1979**, *22*, 1182−1186.
(15) De Luca, A.; Pierno, S.; Natuzzi, F.; Franchini, C.; Duranti, A.; Lentini, G.; Tortorella, V.; Jockush, H.; Conte Camerino, D. Evaluation of the antimyotonic activity of mexiletine and some new analogues on sodium currents of single muscle fibers and on the abnormal excitability of the myotonic adr mouse. *J. Pharmacol. Exp. Ther.* **1997**, *282*, 93−100.
(16) Frazier, D. T.; Narahashi, T.; Yamada, M. The site of action and active form of local anesthetics. *J. Pharmacol. Exp. Ther.* **1970**, *171*, 45−51.
(17) Hille, B. Local anesthetics: hydrophilic and hydrophobic pathways for the drug-receptor reaction. *J. Gen. Physiol.* **1977**, *69*, 497−515.
(18) Postma, S. W.; Catterall, W. A. Inhibition of binding of [^{3}H]-batrachotoxin A 20-alpha-benzoate to sodium channels by local anesthetics. *Mol. Pharmacol.* **1984**, *25*, 219−225.
(19) Schwarz, W.; Palade, P. T.; Hille, B. Local anesthetics: effect of pH on use-dependent block of sodium channels in frog muscle. *Biophys. J.* **1977**, *20*, 343−368.
(20) Wang, G. K.; Simon, R.; Bell, D.; Mok, W. M.; Wang, S.-Y. Structural determinants of quaternary ammonium blockers for batrachotoxin-modified Na^+ channels. *Mol. Pharmacol.* **1993**, *44*, 667−676.
(21) Wang, G. K.; Mok, W. M.; Wang, S.-Y. Charged tetracaine as an inactivation enhancer in batrachotoxin-modified Na^+ channels. *Biophys. J.* **1994,** *51*, 123−135.
(22) Ragsdale, D. S.; McPhee, J. C.; Scheuer, T.; Catterall, W. A. Molecular determinants of state-dependent block of Na^+ channels by local anesthetics. *Science* **1994**, *265*, 1724−1728.
(23) Wright, S. N.; Wang, S.-Y.; Wang, G. K. Lysine point mutations in Na+ channel D4-S6 reduce inactivated channel block by local anesthetics. *Mol. Pharmacol.* **1998**, *54*, 733−739.
(24) Sunami, A.; Dudley, S. C.; Fozzard, H. A. Sodium channel selectivity filter regulates antiarrhymic drug binding. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 14126−14131.
(25) Wang, G. K.; Quan, C.; Wang, S.-Y. A common local anesthetic receptor for benzocaine and etidocaine in voltage-gated μL Na^+ channels. *Pflugers Arch.* **1998,** *435*, 293−302.
(26) De Luca, A.; Natuzzi, F.; Desaphy, J.-F.; Loni, G.; Lentini, G.; Franchini, C.; Tortorella, V.; Conte Camerino, D. Molecular determinants of mexiletine structure for potent and use-dependent block of skeletal muscle sodium channels. *Mol. Pharmacol.* **2000**, *57*, 268−277.
(27) Nau, C.; Wang, S.-Y.; Strichartz, G. R.; Wang, G. K. Point mutations at N434 in D1-S6 of μl Na^+ channels modulate binding affinity and stereoselectivity of local anesthetic enantiomers. *Mol. Pharmacol.* **1999**, *56*, 404−413.
(28) Hille, B.; Campbell, D. T. An improved vaseline gap voltage clamp for skeletal muscle fibers. *J. Gen. Physiol.* **1976**, *67*, 265−293.
(29) Rangwala, S. M.; O'Brien, M. L.; Tortorella, V.; Longo, A.; Loiodice, F.; Noonan, D. J.; Feller, D. R. Stereoselective effects of chiral clofibric acid analogues on rat peroxisome proliferator-activated receptor α (rPPARα) activation and peroxisomal fatty acid β−oxidation. *Chirality* **1997**, *9*, 37−47.
(30) Izumiya, N.; Muraoka, M. A racemization test in peptide chemistry. *J. Am. Chem. Soc.* **1969**, *92*, 2391−2392.

JM000931L