CHIRALITY 22:299–307 (2010)

Synthesis and In Vitro Sodium Channel Blocking Activity Evaluation of Novel Homochiral Mexiletine Analogs

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ABSTRACT New chiral mexiletine analogs were synthesized in their optically active forms and evaluated in vitro as use-dependent blockers of skeletal muscle sodium channels. Tests carried out on sodium currents of single muscle fibers of *Rana esculenta* demonstrated that all of them exerted a higher use-dependent block than mexiletine. The most potent analog, (*S*)-3-(2,6-dimethylphenoxy)-1-phenylpropan-1-amine (*S*)-(**5**), was six-fold more potent than (*R*)-Mex in producing a tonic block. As observed with mexiletine, the newly synthesized compounds exhibit modest enantioselective behavior, that is more evident in 3-(2,6-dimethylphenoxy)butan-1-amine (**3**). *Chirality 22:299–307, 2010.*

KEY WORDS: voltage-gated sodium channels; myotonia; mexiletine; use-dependent block; enantiomeric excess

INTRODUCTION

Myotonic syndromes are hereditary skeletal muscle disorders because of mutations of either sodium or chloride channel genes that result in abnormally prolonged membrane depolarization and/or excessive firing of action potentials.[1–3] Mexiletine, 1-(2,6-dimethylphenoxy)-2-propanamine (Mex, Fig. 1), a Class Ib antiarrhythmic drug, represents the standard therapy for myotonic patients because of its ability to use-dependently block the voltage gated sodium channels, i.e. with higher potency in conditions of high stimulation frequencies (phasic block) than in situations of physiological excitability (tonic block).[4–7] In therapy, mexiletine is used as a racemate even though in vivo[8] and in vitro[9] studies showed the presence of stereoselective active sites on the cardiac sodium channel which preferentially bind the (–)-(*R*)-enantiomer. (–)-(*R*)-Mexiletine is more active than its enantiomer also on native skeletal muscular fibers.[10,11] Nevertheless, the use of mexiletine in the therapy of myotonic syndromes is restricted by its side effects on cardiac function, central nervous system (CNS), and hematopoietic system.[9] Conceivably, mexiletine analogs with higher use-dependency of action should present a wider therapeutic ratio, being more selectively active on hyperexcited tissues, i.e. myotonic muscle fibers. The structural requirements for molecules able to exert a potent and use-dependent block on sodium channels have not yet been completely defined. Different evidences point to lipophilicity as the property that modulates blocking potency and basicity as the property able to affect the use-dependent blocking potency of compounds active on such channels.[7,10,12] However, basicity and lipophilicity cannot explain all aspects of local anaesthetic action; further stereoelectronic aspects should be considered.[13–15]

In the past decade our efforts were focused on the development of analogs of mexiletine and tocainide in order to clarify the structural determinants for blocking voltage-gated sodium channels of skeletal muscle.[7,10–12] We reported that the insertion of a methylene moiety between the asymmetric centre and the amino terminal group of Mex, as in **1** (Fig. 1), led to an increase in the use-dependent behavior for blocking sodium current (I_{Na}) but to a concomitant decrease of drug potency for producing a tonic block with respect to Mex.[16] Furthermore, we established a main pharmacophoric role for the asymmetric carbon atom near to the terminal amino group of Mex. Substitution here enhanced potency, use-dependent behaviour or both.[10,12] In particular, we observed that the replacement of the methyl group on the asymmetric carbon of Mex with a lipophilic aromatic phenyl group, as in **4** (Fig. 1), markedly enhanced drug potency.[17] The sum of

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Contract grant sponsor: Ministero dell'Istruzione, dell'Università e della Ricerca; Contract grant number: MIUR 2005033023
Received for publication 25 September 2008; Accepted 26 March 2009
DOI: 10.1002/chir.20741
Published online 18 June 2009 in Wiley InterScience (www.interscience.wiley.com).

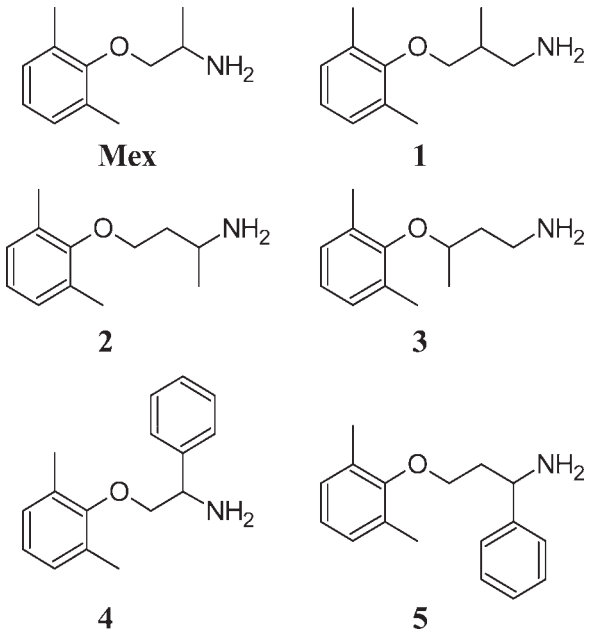


Fig. 1. Structures of Mex and its analogs.

these findings prompted us to synthesize two new compounds (**2** and **3**, Fig. 1) in order to better explore the importance of the position of the stereogenic center relative to the terminal amino group of Mex on the homologated alkyl chain of **1**. Then the phenyl analog **5** was designed in order to evaluate the steric requirements for potent and use-dependent block of skeletal muscle sodium channels. Given that studies on adult skeletal muscle fibers evidenced the stereoselective behavior of Mex and its analogs toward the binding sites[18,19] we prepared compounds **2**, **3**, and **5** in their homochiral forms. The effects of the newly synthesized compounds were evaluated on single fibers of frog skeletal muscle, under both tonic and phasic conditions.

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals were purchased from Sigma-Aldrich or Lancaster in the highest quality commercially available. Solvents were RP grade unless otherwise indicated. Compounds **7–9** were prepared as reported in the literature.[20] (−)-(*R*)-Phenyl(quinolin-2-yloxy)acetic acid, used as chiral solvating agent (CSA), was home made; experimental details will be reported elsewhere.

Instrumentation

Yields refer to purified products and were not optimized. The structures of the compounds were confirmed by routine spectrometric analyses. Only spectra for compounds not previously described are given. Melting points were determined on a Gallenkamp melting point apparatus in open glass capillary tubes and are uncorrected. The infrared spectra were recorded on a Perkin-Elmer (Norwalk, CT) Spectrum One FT spectrophotometer and band positions are given in reciprocal centimeters (cm^{-1}). ^1H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury-VX spectrometer operating at 300 and 75 MHz for ^1H and ^{13}C, respectively, using $CDCl_3$ or CD_3OD (where indicated) as solvents. Chemical shifts are reported in parts per million (ppm) relative to solvent resonance: $CDCl_3$, δ 7.26 (^1H NMR) and δ 77.3 (^{13}C NMR); CD_3OD, δ 3.30 (^1H NMR) and δ 47.8 (^{13}C NMR), unless otherwise indicated. *J* values are given in Hz. Electrophoretic runs were performed on a BioFocus 3000 CE system (Bio-Rad, USA). A fused silica capillary of 69.7 cm (effective length 65.2 cm) and 0.05 mm i.d. (Quadrex Corporation) thermostated at 15°C was used as a separation tube. The samples (0.1 mg/ml) were pressure injected and detected at 214 nm. When determining ee values by ^1H NMR, (*R*)- and (*S*)-**3** were recovered by extraction of a sample of the corresponding hydrochloride salts and dissolved with 2 equiv. of CSA in toluene-d_8. Spectra were registered at 25°C and the splitting of the α-methyl group doublet was observed ($\Delta\delta$ = 0.082 ppm ca). EIMS spectra were recorded on a Hewlett-Packard 6890-5973 MSD gas chromatograph/mass spectrometer at low resolution. Elemental analyses were performed on a Eurovector Euro EA 3000 analyzer. Optical rotations were measured on a Perkin Elmer (Norwalk, CT) Mod 341 spectropolarimeter; concentrations are expressed in g/100 ml, and the cell length was 1 dm, thus $[\alpha]_D^{20}$ values are given in units of 10^{-1} deg cm^2 g^{-1}. Chromatographic separations were performed on silica gel columns by flash chromatography (Kieselgel 60, 0.040–0.063 mm, Merck, Darmstadt, Germany) as described by Still et al.[21] TLC analyses were performed on precoated silica gel on aluminum sheets (Kieselgel 60 F_{254}, Merck).

(–)-(*R*)-2-[3-(2,6-Dimethylphenoxy)-1-methylpropyl]-1*H*-isoindole-1,3(2*H*)-dione (*R*)-10. To a stirred solution of (*R*)-**9** (0.80 g, 3.65 mmol), 2,6-dimethylphenol (0.67 g, 5.48 mmol) and triphenylphosphine (1.44 g, 5.48 mmol) in dry THF (35 ml), under N_2 atmosphere, a solution of DIAD (1.11 g, 1.08 ml, 5.48 mmmol) in dry THF (25 ml) was added dropwise. The mixture was stirred at room temperature for 24 h. The solvent was then evaporated under reduced pressure, ether was added and the precipitate formed was filtered off. The filtrate was evaporated in vacuo and the residue was purified by flash chromatography using silica gel (eluent EtOAc/petroleum ether 1:99, then 5:95) to give 0.96 g of a colorless oil (81%): $[\alpha]_D^{20}$ = −2.9 (c 2.1, $CHCl_3$); IR (neat): 1775, 1713 (C=O) cm^{-1}; ^1H NMR: δ 1.56 (d, 3H, *J* = 6.9 Hz, CH_3CH), 2.19 (s overlapping m at 2.15–2.34, 6H, CH_3Ar), 2.15–2.34 (m overlapping s at 2.19, 1H, C*H*HCH), 2.60–2.75 (m, 1H, CH*H*CH), 3.76 (t, 2H, *J* = 6.2 Hz, CH_2O), 4.65–4.70 (m, 1H, CH), 6.81−6.85 (m, 1H, ArO *H*C-4), 6.94 (d, 2H, *J* = 7.1 Hz, ArO *H*C-3,5), 7.66–7.74 (m, 2H, Ar *H*C-5,6), 7.78–7.86 (m, 2H, Ar *H*C-4,7); ^{13}C NMR: δ 16.5 (2C), 19.3 (1C), 34.4 (1C), 45.0 (1C), 69.6 (1C), 123.3 (1C), 123.9 (2C), 129.0 (2C), 131.1 (2C), 132.3 (2C), 134.1 (2C), 156.2 (1C), 168.7 (2C); MS (70 eV) m/z (%) 323 (M^+, 1), 202 (100).

(+)-(*S*)-2-[3-(2,6-Dimethylphenoxy)-1-methylpropyl]-1*H*-isoindole-1,3(2*H*)-dione (*S*)-10. Prepared as reported above for (*R*)-**10** starting from (*S*)-**9**. Colorless oil (78% yield); $[\alpha]_D^{20} = +2.0$ (*c* 2, $CHCl_3$). Spectrometric data were in agreement with those reported for the (*R*)-isomer.

(–)-(*R*)-4-(2,6-Dimethylphenoxy)butan-2-amine (*R*)-2. To a stirred solution of (*R*)-**10** (0.91 g, 2.82 mmol) in MeOH (10 ml), glacial AcOH (5.64 mmol) and $N_2H_4{\cdot}H_2O$ (11.3 mmol) were added and the mixture was kept under reflux for 4 h. The solid residue was filtered off. After evaporation of the filtrate, the residue was taken up with EtOAc and extracted with 2 N HCl; then the aqueous phase was made alkaline and extracted twice with EtOAc. The combined organic layers were dried (Na_2SO_4) and concentrated under vacuum. The final product was a slightly yellowish oil (0.45 g, 83%): $[\alpha]_D^{20} = -2.8$ (*c* 1.9, $CHCl_3$); IR (neat): 3366 (NH_2) cm^{-1}; ^1H NMR: δ 1.17 (d, 3H, *J* = 6.3 Hz, C*H*$_3$CH), 1.71 (br s overlapping m at 1.75–1.85, 2H, N*H*$_2$), 1.75–1.85 (m overlapping br s at 1.71, 2H, C*H*$_2$CH), 2.28 (s, 6H, C*H*$_3$Ar), 3.20–3.35 (m, 1H, C*H*), 3.75–3.95 (m, 2H, C*H*$_2$O), 6.91 (t, *J* = 8.3 Hz, 1H, Ar *H*C-4), 7.00 (d, 2H, *J* = 7.4 Hz, Ar *H*C-3,5); ^{13}C NMR: δ 16.5 (2C), 24.6 (1C), 40.5 (1C), 45.0 (1C), 70.3 (1C), 124.0 (1C), 129.0 (2C), 131.1 (2C), 156.2 (1C); MS (70 eV) *m/z* (%) 193 (M^+, 25), 44 (100).

(+)-(*R*)-4-(2,6-Dimethylphenoxy)butan-2-amine hydrochloride (*R*)-2·HCl. Into a solution of (*R*)-**2** in dry Et_2O, gaseous HCl was bubbled until saturation was reached. The organic phase was concentrated. Purification of the crude residue solid by recrystallization from abs EtOH/Et_2O gave (*R*)-**2.HCl** as white crystals in 38% yield: mp 164–165°C (abs EtOH/Et_2O); ≥ 99% ee (CE) (injection: 10 psi/s; BGE: phosphate buffer 0.035 M at pH = 3.0; chiral selector: β-cyclodextrin sulfated sodium salt 2 mg/ml; voltage: 20 kV); $[\alpha]_D^{20} = +6.1$ (*c* 2, MeOH); IR (KBr): 2945 (NH_3^+) cm^{-1}; ^1H NMR (CD_3OD): δ 1.44 (d, 3H, *J* = 6.6 Hz, C*H*$_3$CH), 1.94–2.08 (m, 2H, C*H*$_2$CH), 2.27 (s, 6H, C*H*$_3$Ar), 3.60–3.74 (m, 1H, C*H*), 3.84–4.00 (m, 2H, OC*H*$_2$), 6.86–6.95 (m, 1H, Ar *H*C-4), 6.96–7.05 (m, 2H, Ar *H*C-3,5); ^{13}C NMR (CD_3OD): δ 15.3 (2C), 17.7 (1C), 34.9 (1C), 46.3 (1C), 68.5 (1C), 124.1 (1C), 128.8 (2C), 130.5 (2C), 155.6 (1C). Anal. calcd for $C_{12}H_{19}NO.HCl$: C, 62.73; H, 8.77; N, 6.10; Found: C, 62.44; H, 8.81; N 6.12.

(+)-(*S*)-4-(2,6-Dimethylphenoxy)butan-2-amine (*S*)-2. Prepared as reported above for (*R*)-**2** starting from (*S*)-**10**. Slightly yellowish oil; yield: 69%; $[\alpha]_D^{20} = +2.5$ (*c* 2, $CHCl_3$). Spectrometric data were in agreement with those reported for the (*R*)-isomer.

(–)-(*S*)-4-(2,6-Dimethylphenoxy)butan-2-amine hydrochloride (*S*)-2·HCl. Prepared as reported above for (*R*)-**2·HCl** starting from (*S*)-**2**. White crystals (62% yield): mp 165–166°C (abs EtOH/Et_2O); ≥ 99% ee (CE) (injection: 10 psi/s; BGE: phosphate buffer 0.035 M at pH = 3.0; chiral selector: β-cyclodextrin sulfated sodium salt 2 mg/ml; voltage: 20 kV); $[\alpha]_D^{20} = -6.0$ (*c* 2.1, MeOH). Anal. calcd for $C_{12}H_{19}NO.HCl$: C, 62.73; H, 8.77; N, 6.10. Found: C, 62.70; H, 8.89; N, 6.15.

(–)-(*R*)-1-(*tert*-Butyldimethylsilyloxy)-3-(2,6-dimethylphenoxy)butane (*R*)-11. Prepared as reported above for (*R*)-**10** starting from (*S*)-**7**. The residue was purified by column chromatography using silica gel (eluent EtOAc/petroleum ether 0.5:9.5) giving a slightly yellowish oil; yield: 66%; $[\alpha]_D^{20} = -1.6$ (*c* 2, $CHCl_3$); IR (neat): 836 (Si–CH_3) cm^{-1}; ^1H NMR: δ 0.047 (s overlapping s at 0.053, 3H, C*H*$_3$SiCH$_3$), 0.053 (s overlapping s at 0.047, 3H, CH$_3$SiC*H*$_3$), 0.89 (s, 9H, *t*-Bu), 1.20 (d, *J* = 6.3 Hz, 3H, C*H*$_3$CH), 1.76 (apparent dq, six lines, *J* = 14.3, 6.0 Hz, 1H, C*H*HCH), 2.05 (apparent dq, six lines, *J* = 14.3, 6.0 Hz, 1H, CH*H*CH), 2.27 (s, 6H, C*H*$_3$Ar), 3.74 (dt, *J* = 10.3, 6.2 Hz, 1H C*H*HO), 3.85 (dt, *J* = 10.3, 6.3 Hz, 1H CH*H*O), 4.29 (apparent sextet, *J* = 6.2 Hz, 1H, C*H*), 6.85–6.95 (m, 1H, Ar *H*C-4), 7.00 (d, *J* = 7.4 Hz, 2H, Ar *H*C-3,5); ^{13}C NMR: δ −5.2 (2C), 17.5 (2C), 18.5 (3C), 20.2 (1C), 26.1 (1C), 40.4 (1C), 59.9 (1C), 74.9 (1C), 123.3 (1C), 129.0 (2C), 131.6 (2C), 154.6 (1C); MS (70 eV) *m/z* (%) 308 (M^+, <1), 209 (100).

(+)-(*S*)-1-(*tert*-Butyldimethylsilyloxy)-3-(2,6-dimethylphenoxy)butane (*S*)-11. Prepared as reported above for (*R*)-**10** starting from (*R*)-**7**. Slightly yellowish oil; yield: 66%; $[\alpha]_D^{20} = +1.5$ (*c* 2, $CHCl_3$). Spectroscopic data were in agreement with those reported for the (*R*)-isomer.

(–)-(*R*)-3-(2,6-Dimethylphenoxy)butan-1-ol (*R*)-12. A solution of (*R*)-**11** (0.73 g, 2.36 mmol) in a mixture of AcOH/water/THF, 3/1/1 (7.5 ml) was stirred overnight at room temperature. The mixture was concentrated under vacuum and the water and acetic acid traces were removed by codistillation with toluene/abs EtOH under reduced pressure. The oil obtained was purified by flash chromatography using silica gel (eluent EtOAc/petroleum ether 0.5:9.5) to give 0.27 g (60%) of a colorless oil: $[\alpha]_D^{20} = -22.0$ (*c* 1.8, $CHCl_3$); IR (neat): 3379 (OH) cm^{-1}; ^1H NMR: δ 1.17 (d, *J* = 6.1 Hz, 3H, C*H*$_3$CH), 1.88–2.10 (m, 2H, C*H*$_2$CH), 2.28 (s, 6H, C*H*$_3$Ar), 2.48 (br s, 1H, O*H*), 3.84–4.02 (m, 2H, C*H*$_2$O), 4.39 (apparent sextet, *J* = 6.3 Hz, 1H, C*H*), 6.86–6.96 (m, 1H, Ar *H*C-4), 7.00 (d, *J* = 7.4 Hz, 2H, Ar *H*C-3,5); ^{13}C NMR: δ 17.5 (2C), 19.9 (1C), 39.5 (1C), 61.0 (1C), 77.2 (1C), 123.8 (1C), 129.2 (2C), 131.6 (2C), 153.8 (1C); MS (70 eV) *m/z* (%) 194 (M^+, 13), 122 (100).

(+)-(*S*)-3-(2,6-Dimethylphenoxy)butan-1-ol (*S*)-12. Prepared as reported above for (*R*)-**12** starting from (*S*)-**11**. Yield: 83%; $[\alpha]_D^{20} = +23.0$ (*c* 1.4, $CHCl_3$). Spectrometric data were in agreement with those reported for the (*R*)-isomer.

(+)-(*R*)-2-[3-(2,6-Dimethylphenoxy)butyl]-1*H*-isoindole-1,3(2*H*)-dione (*R*)-13. To a stirred solution of (*R*)-**12** (0.47 g, 2.42 mmol), phthalimide (0.53 g, 3.63 mmol) and triphenylphosphine (0.95 g, 3.63 mmol) in dry THF (30 ml), under N_2 atmosphere, a solution of DIAD (0.73 g, 0.7 ml, 3.63 mmol) in dry THF (30 ml) was added dropwise. The mixture was stirred at room temperature for 24 h. The solvent was then evaporated under reduced pressure, ether was added and the precipitate formed was filtered off. The filtrate was evaporated in vacuo and the residue was purified by column chromatography using

silica gel (eluent EtOAc/petroleum ether 0.5:9.5) to give 0.69 g of a white solid (88% yield): mp 97–98°C; $[\alpha]_D^{20}$ = +22.0 (*c* 2, $CHCl_3$); IR (KBr): 1771, 1720 (C=O) cm^{-1}; ^1H NMR: δ 1.24 (d, *J* = 6.0 Hz, 3H, C*H*$_3$CH), 1.96–2.22 (m overlapping s at 2.24, 2H, C*H*$_2$CH), 2.24 (s overlapping m at 1.96–2.22, 6H, C*H*$_3$Ar), 3.78–4.02 (m, 2H, C*H*$_2$N), 4.21 (apparent sextet, *J* = 6.0 Hz, 1H, C*H*), 6.84–6.92 (m, 1H, ArO, *H*C-4), 6.96 (d, *J* = 7.4 Hz, 2H, ArO *H*C-3,5), 7.68–7.76 (m, 2H, Ar *H*C-5,6), 7.80–7.88 (m, 2H, Ar *H*C-4,7); ^{13}C NMR: δ 17.5 (2C), 19.9 (1C), 35.3 (1C), 36.0 (1C), 75.4 (1C), 123.4 (2C), 123.5 (1C), 129.1 (2C), 131.5 (2C), 132.4 (2C), 134.2 (2C), 154.3 (1C), 168.6 (2C); MS (70 eV) *m/z* (%) 323 (M^+, 1), 160 (100).

(–)-(*S*)-2-[3-(2,6-Dimethylphenoxy)butyl]-1*H*-isoindole-1,3(2*H*)-dione (*S*)-13. Prepared as reported above for (*R*)-**13** starting from (*S*)-**12**. White solid (60% yield): mp 95–97°C; $[\alpha]_D^{20}$ = −21.0 (*c* 1.1, $CHCl_3$). Spectrometric data were in agreement with those reported for the (*R*)-isomer.

(+)-(*S*)-3-(2,6-Dimethylphenoxy)butan-1-amine (*S*)-3. Prepared as reported above for (*R*)-**2** starting from (*S*)-**13**. Slightly yellowish oil (72% yield); $[\alpha]_D^{20}$ = +0.4 (*c* 2, $CHCl_3$). IR (neat): 3367 (NH_2) cm^{-1}; ^1H NMR: δ 1.18 (d overlapping br s at 1.25, *J* = 6.3 Hz, 3H, C*H*$_3$CH), 1.25 (br s overlapping d at 1.18, 2H, N*H*$_2$), 1.78 (apparent dq, six lines, *J* = 15.5, 6.9 Hz, 1H, C*H*HCH), 1.92 (apparent dq, six lines, *J* = 13.5, 6.9 Hz, 1H, CH*H*CH), 2.26 (s, 6H, C*H*$_3$Ar), 2.90 (apparent br s, 2H, C*H*$_2$), 4.20 (apparent sextet, *J* = 6.2 Hz, 1H, C*H*), 6.85–6.95 (m, Ar *H*C-4), 6.99 (d, *J* = 7.2 Hz, 2H, Ar *H*C-3,5); ^{13}C NMR: δ 17.5 (2C), 20.1 (1C), 39.2 (1C), 41.1 (1C), 76.1 (1C), 123.4 (1C), 129.1 (2C), 131.5 (2C), 154.4 (1C); MS (70 eV) *m/z* (%) 193 (M^+, 6), 122 (100).

(+)-(*S*)-3-(2,6-Dimethylphenoxy)butan-1-amine hydrochloride (*S*)-3·HCl. Prepared as reported above for (*R*)-**2·HCl** starting from (*S*)-**3**. White solid (53% yield): mp 117–118 °C (abs EtOH/Et_2O); 98% ee (^1H NMR); $[\alpha]_D^{20}$ = +5.7 (*c* 2, MeOH); IR (KBr): 2970 (NH_3^+) cm^{-1}; ^1H NMR (CD_3OD, δ 3.11): δ 0.97 (d, 3H, *J* = 6.0 Hz, C*H*$_3$CH), 1.80–1.95 (m, 2H, C*H*$_2$CH), 2.06 (s, 6H, C*H*$_3$Ar), 2.92–3.08 (m, 2H, C*H*$_2$N), 4.15 (apparent sextet, *J* = 6.0 Hz, 1H, C*H*), 6.66–6.74 (m, 1H, Ar *H*C-4), 6.80 (d, *J* = 7.4 Hz, 2H, Ar *H*C-3,5); ^{13}C NMR (CD_3OD, δ 47.7): δ 16.2 (2C), 18.3 (1C), 34.5 (1C), 36.9 (1C), 75.0 (1C), 123.5 (1C), 128.8 (2C), 131.0 (2C), 153.4 (1C). Anal. calcd for $C_{12}H_{19}NO$·HCl: C, 62.73; H, 8.77; N, 6.10. Found: C, 62.67; H, 8.63; N, 6.16.

(–)-(*R*)-3-(2,6-Dimethylphenoxy)butan-1-amine (*R*)-3. Prepared as reported above for (*R*)-**2** starting from (*R*)-**13**. Slightly yellowish oil (78% yield); $[\alpha]_D^{20}$ = −0.4 (*c* 2, $CHCl_3$). Spectrometric data were in agreement with those reported for the (*S*)-isomer.

(–)-(*R*)-3-(2,6-Dimethylphenoxy)butan-1-amine hydrochloride (*R*)-3·HCl. Prepared as reported above for (*R*)-**2·HCl** starting from (*R*)-**3**. White solid (60% yield): mp 117–118°C (abs EtOH/Et_2O); 98% ee (^1H NMR); $[\alpha]_D^{20}$ = −5.2 (*c* 2, MeOH). Anal. calcd for $C_{12}H_{19}NO$·HCl: C, 62.73; H, 8.77; N, 6.10. Found: C, 62.65; H, 8.76; N, 6.25.

(+)-(*R*)-*tert*-Butyl (3-hydroxy-1-phenylpropyl)carbamate (*R*)-15. To a solution of (*R*)-**14** (0.42 g, 1.60 mmol) in dry THF (20 ml), $LiAlH_4$ (0.12 g, 3.20 mmol) was added under N_2 atmosphere at 0°C and the mixture stirred for 16 h. The reaction was quenched by the careful addition of cold water until the end of gas evolution. After evaporation of the solvent, the residue was taken up with EtOAc, washed with 2 N NaOH and dried over Na_2SO_4. The filtrate was concentrated under vacuum to give 0.35 g (88%) of a colorless oil: $[\alpha]_D^{20}$ = +55.5 (*c* 2.2, $CHCl_3$); IR (neat): 3348 (OH), 1693 (C=O) cm^{-1}; ^1H NMR: δ 1.44 (s, 9H, *t*-Bu), 1.74–1.90 (m, 1H, C*H*HCH), 1.98–2.16 (m, 1H, CH*H*CH), 3.18 (br s, 1H, O*H*), 3.69 (dd, 2H, *J* = 7.6, 3.7 Hz, C*H*$_2$O), 4.80–4.96 (m, 1H, C*H*), 5.0 (br s, 1H, N*H*), 7.17 (d, *J* = 6.9 Hz, 1H, Ar *H*C-4), 7.20–7.40 (m, 4H, Ar *H*C-2,3,5,6); ^{13}C NMR: δ 28.5 (3C), 39.7 (1C), 51.8 (1C), 59.3 (1C), 80.3 (1C), 126.6 (2C), 127.7 (2C), 129.0 (1C), 142.2 (1C), 156.7 (1C); MS (70 eV) *m/z* (%) 206 (M^+ – 45, 33), 150 (100).

(–)-(*S*)-*tert*-Butyl (3-hydroxy-1-phenylpropyl)carbamate (*S*)-15. Prepared as reported above for (*R*)-**15** starting from (*S*)-**14**. Colorless oil (73% yield); $[\alpha]_D^{20}$ = −59.2 (*c* 2.2, $CHCl_3$). ^1H NMR data were in agreement with those reported for the (*R*)-isomer.

(+)-(*R*)-*tert*-Butyl [3-(2,6-dimethylphenoxy)-1-phenylpropyl]carbamate (*R*)-16. Prepared as reported above for (*R*)-**10** starting from (*R*)-**15**. White solid (79% yield): mp 96–97°C; $[\alpha]_D^{20}$ = +25.3 (*c* 2, $CHCl_3$); IR (KBr): 3370 (NH), 1683 (C=O) cm^{-1}; ^1H NMR: δ 1.42 (s, 9H, *t*-Bu), 2.24 (s overlapping m at 2.16–2.36, 6H, C*H*$_3$Ar), 2.16–2.36 (m overlapping s at 2.24, 2H, C*H*$_2$CH), 3.70–3.85 (m, 2H, C*H*$_2$O), 4.95 (br s, 1H, C*H*), 5.43 (br s, 1H, N*H*), 6.85–6.95 (m, 1H, ArO *H*C-4), 6.99 (d, *J* = 7.1 Hz, 2H, ArO *H*C-3,5), 7.25–7.35 (m, 1H, Ar *H*C-4), 7.35 (m, 4H, Ar *H*C-2,3,5,6); ^{13}C NMR: δ 16.6 (2C), 28.6 (3C), 37.3 (1C), 53.2 (1C), 69.3 (1C), 79.6 (1C), 124.1 (2C), 126.4 (1C), 127.4 (2C), 128.8 (2C), 129.0 (1C), 131.1 (2C), 142.8 (1C), 155.5 (1C), 156.0 (1C); MS (70 eV) *m/z* (%) 281 (M^+ – 74, 6), 178 (100).

(–)-(*S*)-*tert*-Butyl [3-(2,6-dimethylphenoxy)-1-phenylpropyl]carbamate (*S*)-16. Prepared as reported above for (*R*)-**10** starting from (*S*)-**15**. White solid (72% yield): mp 96–97°C; $[\alpha]_D^{20}$ = −25.9 (*c* 1.9, $CHCl_3$). Spectroscopic data were in agreement with those reported for the (*R*)-isomer.

(–)-(*R*)-3-(2,6-dimethylphenoxy)-1-phenylprpan-1-amine hydrochloride (*R*)-5·HCl. To a solution of (*R*)-**16** (0.21 g, 0.6 mmol) in 5 ml of EtOAc, 3 N HCl (3 ml) was added at 0°C. The reaction mixture was stirred at room temperature for 15 h. The solvent was evaporated under reduced pressure azeotropically removing water. Crystallization from abs EtOH/Et_2O gave 0.11 g (65% yield) of (*R*)-**5·HCl** as white crystals: mp 185–186°C; ≥99% ee (CE) (injection: 15 psi/s; BGE: phosphate buffer 0.035 M at pH = 4.3; chiral selector: 2-hydroxypropyl-β-cyclodextrin 40 mg/ml;

voltage: 10 kV); $[\alpha]_D^{20} = -11.2$ (*c* 2, MeOH); IR (KBr): 2884 (NH_3^+) cm^{-1}; ^1H NMR (CD_3OD): δ 2.19 (s, 6H, C*H*$_3$), 2.40–2.54 (m, 1H, C*H*HCH), 2.64–2.78 (m, 1H, CH*H*CH), 3.66–3.82 (m, 2H, C*H*$_2$O), 4.70 (dd, *J* = 9.9, 5.2, 1H, C*H*), 5.0 (br s, 3H, N*H*$_3$), 6.85−6.95 (m, 1H, ArO *H*C-4), 6.95–7.05 (m, 2H, ArO *H*C-3,5), 7.45–7.65 (m, 5H, Ar); ^{13}C NMR (CD_3OD): δ 15.3 (2C), 34.5 (1C), 53.3 (1C), 67.7 (1C), 124.0 (1C), 127.7 (2C), 128.7 (2C), 129.3 (2C), 129.5 (1C), 130.5 (2C), 136.1 (1C), 155.7 (1C). Anal. calcd for $C_{17}H_{21}NO$.HCl: C, 69.97; H, 7.60; N, 4.80. Found: C, 70.39; H, 7.64; N, 4.96.

(+)-(*S*)-2-(2,6-dimethylphenoxy)-1-phenylpropan-1-amine hydrochloride (*S*)-5·HCl. Prepared as reported above for (*R*)-**5·HCl** starting from (*S*)-**16**. Yield: 72%; mp 187−188 °C (abs EtOH/Et_2O); ≥99% ee (CE) (injection: 15 psi/s; BGE: phosphate buffer 0.035 M at pH = 4.3; chiral selector: 2-hydroxypropyl-β-cyclodextrin 40 mg/ml; voltage: 10 kV); $[\alpha]_D^{20} = +11.1$ (*c* 2.1, MeOH). Spectroscopic data were in agreement with those reported for the (*R*)-isomer. Anal. calcd for $C_{17}H_{21}NO$·HCl: C, 69.97; H, 7.60; N, 4.80. Found: C, 69.85; H, 7.58; N, 4.90.

Pharmacology

Recording of Na^+ current and pulse protocols. The actions of the mexiletine analogs (**2**, **3**, and **5**) were tested in vitro on sodium currents (I_{Na}) of single fibers of frog semitendinosus muscle by vaseline-gap voltage clamp method, as described in detail elsewhere.[12] The tonic block (TB) exerted by each compound was calculated as percentage reduction of the maximal peak sodium transient ($I_{Na\ max}$) elicited by infrequent depolarizing steps to −20 mV from the holding potential (h.p.) of −100 mV at a frequency of 0.3 Hz. The use-dependent block (UDB) 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 normalizing the residual current at the end of the stimulation protocol with respect to the current in the absence of drug.

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 $I_{Na\ max}$ (IC_{50}) were determined by using a nonlinear least-squares fit of the concentration-response curves to the following logistic equation:

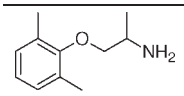
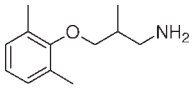
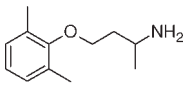
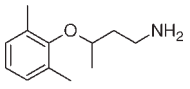
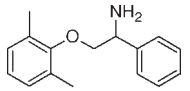
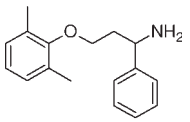
$$\text{Effect} = -100/[1 + (K/[\text{drug}])^n]$$

Where effect = percentage change of I_{Na}, −100 = maximal percentage block of I_{Na}, $K = IC_{50}$ of tested compound, n = logistic slope factor, and [drug] = molar concentration of the tested compound.[12]

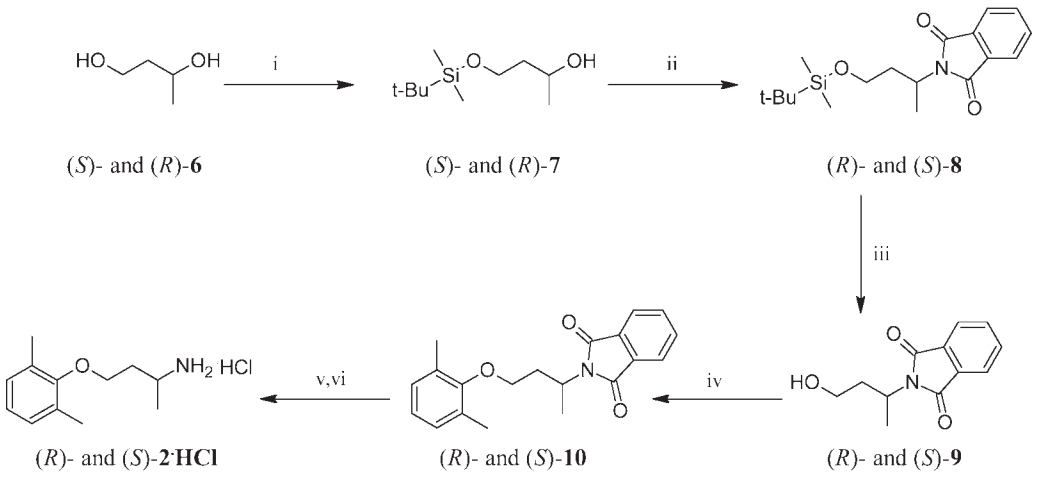
Physicochemical data

Physicochemical data of compounds shown in Table 1 were obtained by a pH metric technique using a GlpK_a apparatus (Sirius Analytical Instruments Ltd., Forrest Row, East Sussex, UK).[22–25] Because of the low solubility of the investigated compounds in aqueous medium, methanol was used as a cosolvent for pK_a measurements. Three separate solutions of ~10^{-5} M, in 10–30% w/w (MeOH/H_2O), were prepared. They were then acidified with 0.5 M HCl to pH 4. These solutions were then titrated with 0.5 M KOH to pH 12. Initial pK_a values, which are the apparent ionization constants relative to the mixture of the solvents, were obtained by Bjerrum Plot, i.e. the curve obtained by the difference between the curve of titration of the ionizable substance and that of the blank solution. These values were then optimized by a weighted nonlinear least-squares procedure (Refinement Pro 1.0 software) to obtain pK_a values in the absence of cosolvent, by extrapolation using the Yasuda-Shedlovsky equation.[26] To obtain logP data, at least three separate titrations were performed on each compound. The concentration of the analite was ~10^{-5} M, in mixtures of H_2O (7.5 ml) and *n*-octanol (0.1–10 ml). The biphasic solutions were acidified to pH 4 with 0.5 M HCl and then titrated with 0.5 M KOH until pH 12. The obtained data were optimized as described above and the average of these data gave the logP value for each

TABLE 1. Physicochemical properties of mexiletine and its analogs

Compound	LogP[a]	pK_a[a]
 **Mex**	2.21 ± 0.01	9.28 ± 0.01
 **1**	2.40 ± 0.01	9.76 ± 0.01
 **2**	2.34 ± 0.01	9.86 ± 0.01
 **3**	2.32 ± 0.03	9.97 ± 0.01
 **4**	3.20 ± 0.01	7.89 ± 0.03
 **5**	3.40 ± 0.01	8.60 ± 0.01

[a]LogP and pK_a values were determined by the pH metric technique using a GlpK_a apparatus, as detailed in the experimental section.



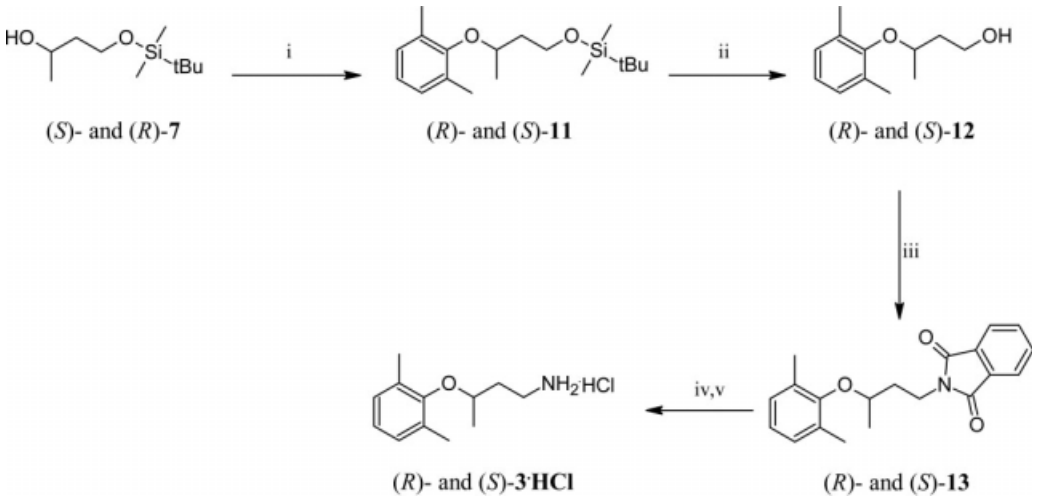
Scheme 1. Reagents and conditions: (i) *tert*-butyldimethylsilyl chloride, imidazole, THF, rt, 2h; (ii) phthalimide, PPh_3, DIAD, THF, rt, 24 h; (iii) glacial AcOH, H_2O, THF, rt, 12 h; (iv) 2,6-dimethylphenol, PPh_3, DIAD, THF, rt, 24 h; (v) aq N_2H_4, glacial AcOH, MeOH, 70 °C, 4 h; (vi) gaseous HCl, rt.

compound.[23,24] All titrations were carried out at 25 ± 0.1°C under nitrogen gas atmosphere to exclude CO_2.

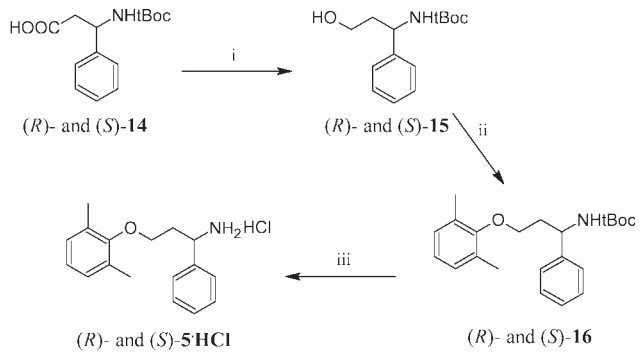
RESULTS

The stereospecific route to highly optically enriched 4-(2,6-dimethylphenoxy)butan-2-amine hydrochlorides [(*R*)- and (*S*)-**2**·**HCl**] is shown in Scheme 1. (*R*)- and (*S*)-**7**–**9** were obtained following the procedure reported in the literature.[20] Thus, the primary alcohol function of the optically active butane-1,3-diols [(*S*)- and (*R*)-**6**] was selectively protected giving the corresponding *tert*-butyldimethylsilyl derivatives (*S*)- and (*R*)-**7** which were in turn transformed into the corresponding phthalimides (*R*)- and (*S*)-**8** through the Mitsunobu reaction.[27] The alcohol functions were then deprotected to give (*R*)- and (*S*)-**9**, which were in turn transformed into the xylyloxy derivatives (*R*)- and (*S*)-**10**, following the Mitsunobu procedure. The deprotection of the amino function by hydrazinolysis[28] gave the amines (*R*)- and (*S*)-**2**, which were purified as the corresponding hydrochlorides obtained by treatment with gaseous HCl.

(*R*)- and (*S*)-**3**·**HCl** were obtained following the route shown in Scheme 2. (*S*)- and (*R*)-**7** were submitted to condensation with 2,6-dimethylphenol under Mitsunobu conditions to give (*R*)- and (*S*)-**11**, which were deprotected to give (*R*)- and (*S*)-**12**. The alcohols obtained underwent the Mitsunobu reaction with phthalimide to give (*R*)- and (*S*)-**13**. Hydrazinolysis of these compounds gave the desired amines (*R*)- and (*S*)-**3** which were purified as the corresponding hydrochlorides by treatment with gaseous HCl.



Scheme 2. Reagents and conditions: (i) 2,6-dimethylphenol, PPh_3, DIAD, THF, rt, 24 h; (ii) glacial AcOH, H_2O, THF, rt, 12 h; (iii) phthalimide, PPh_3, DIAD, THF, rt, 24 h; (iv) aq N_2H_4, glacial AcOH, MeOH, 70 °C, 4 h; (v) gaseous HCl, rt.



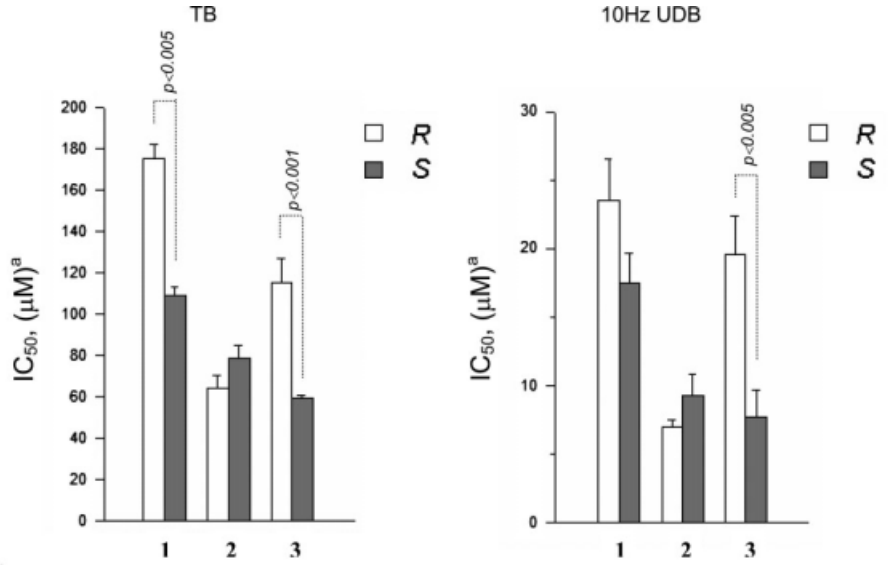
Scheme 3. Reagents and conditions: (i) $LiAlH_4$, THF, 0 °C, 16 h; (ii) 2,6-dimethylphenol, PPh_3, DIAD, THF, rt, 24 h; (iii) 3 N HCl, EtOAc, rt, 4 h.

The synthetic route to 3-(2,6-dimethylphenoxy)-1-phenylpropan-1-amine hydrochlorides [(*R*)- and (*S*)-**5**·**HCl**] is shown in Scheme 3. This starts from the reduction of commercially available optically active 3-[(*tert*-butoxycarbonyl)amino]-3-phenylpropanoic acids [(*R*)- and (*S*)-**14**] with $LiAlH_4$. The following two steps consisted of condensation of the alcohols (*R*)- and (*S*)-**15** with 2,6-dimethylphenol, through the Mitsunobu reaction, followed by deprotection of the amino function with aqueous HCl obtaining directly (*R*)- and (*S*)-**5**·**HCl**. All the newly synthesized compounds were easily obtained in few steps (ranging from 3 to 5) and in acceptable overall yields. Ee values for **2**, **3**, and **5** enantiomers were evaluated by both chiral ^{1}H NMR analysis and capillary electrophoresis. The former was performed on free amine samples, recovered by extraction from the corresponding hydrochloride salts, using (−)-(*R*)–phenyl(quinolin-2-yloxy)acetic acid, a home-made CSA.[29] Capillary electrophoretic analyses were performed directly on the hydrochloride salts, using β-cyclodextrin sulfated sodium salt and 2-hydroxypropyl-b-cyclodextrin as chiral selectors. In both cases, ee values ranged from 98% to ≥99%. Physicochemical properties (Table 1) were obtained by a pH metric technique using a Glp*K*a apparatus (Sirius Analytical Instruments Ltd., Forrest Row, East Sussex, UK).

Homochiral **2**, **3**, and **5** were tested on sodium currents (I_{Na}) of single frog skeletal muscle fibers by means of voltage-clamp recordings.[10,18] Depolarizing steps to −20 mV from the holding potential of −100 mV, at two different stimulation frequencies (0.3 and 10 Hz), were applied in order to evaluate tonic and use-dependent blocks (TB and UDB, respectively) exerted by drugs. Positional isomers **2** and **3** showed about 1.5−2.5 fold increased potency for both TB and UDB with respect to compound **1** (Fig. 2). The highest use-dependent behavior was observed for compound **2** with selectivity ratio (TB IC_{50}/10 Hz-UDB IC_{50}) about 9. It is noteworthy that compound **2** showed almost no stereoselectivity while compound **3** acted as a stereoselective blocker of sodium channels, the eudismic ratio being 2 and 2.5 for TB and UDB, respectively. Phenyl analog **5** showed no improvement in drug potency for tonic block, with respect to **4**: 10.5 ± 0.5 μM IC_{50} value for the eutomer (*S*)-**5** vs. 10.1 ± 0.8 μM for the eutomer (*S*)-**4**. The profile of compound **5**, as observed for **4**,[17] showed low stereoselectivity, the eudismic ratio being <1.5 for both tonic ($P < 0.02$) and use-dependent block ($P < 0.001$). However, the phenyl substituted analog **5** showed improved use-dependent behavior, with an IC_{50} value of 1.9 ± 0.1 μM for 10 Hz UDB vs. 3.1 ± 0.5 μM for (*S*)-**4**. Tested compounds behaved as inactivating channel blockers (data will be given elsewhere).

DISCUSSION

Newly synthesized analogs of Mex, in their homochiral forms, were tested on sodium channels of native skeletal



[a]Half-maximal concentration of each compound for producing a tonic block (TB) and a phasic block (UDB). The IC_{50} values were obtained with the nonlinear least square fit of the concentration-response data.[18]

Fig. 2. Effects of mexiletine analog enantiomers [(*R*)- and (*S*)-**1**, (*R*)- and (*S*)-**2**, (*R*)- and (*S*)-**3**] on sodium currents of frog skeletal muscle fibers.

muscles fibers to clarify the structural requirements necessary to get potent and use-dependent channel blockers, potentially useful for a safer treatment of the hyperexcitability of myotonic muscles. The choice of synthesizing and testing Mex analogs in their optically active forms derives from our previous studies on a series of mexiletine and tocainide analogs which showed the existence of a stereoselective site on sodium channels of skeletal muscle fibers.[10–12,30] These findings are in agreement with what had been reported by Yeh who measured stereoselectivity of phasic and tonic block in some chiral local anesthetics.[31] In our previous work, we found that the activity of Mex-like sodium channel blockers is strongly modulated by the part of the molecule near the asymmetric carbon atom.[7,10] The insertion of a methylene spacer in the alkyl chain of Mex, as in **1** (S enantiomer being the eutomer), led to a noticeable increase in the use-dependent behavior for blocking sodium current but to a concomitant decrease in drug potency, producing a lower tonic block with respect to (*R*)-Mex. On the other hand, replacing the methyl group with a phenyl one, as in **4**, caused a marked increase in potency for producing a tonic block of sodium current. On the basis of these findings, starting from Mex homolog **1**, we decided to explore the importance of the position of the methyl group on the homologated alkyl chain. Thus, following the approach of methylene shuffle, we prepared the optical isomers of **2** and **3** (Fig. 1), which bear the asymmetric centre either near or two methylene groups apart from the amino group, respectively. The eutomers of **2** and **3**, R and S enantiomers respectively, showed a more relevant use-dependent behavior, being the ratio IC_{50} tonic block/IC_{50} use-dependent block higher than that of the related (*S*)-**1** (9 and 8 for **2** and **3**, respectively, versus 6 for **1**). Furthermore, (*R*)-**2** and (*S*)-**3** were 1.7- and 1.9-fold more potent than (*S*)-**1** in producing tonic block, respectively (Fig. 2). Even though **2** and **3** do not substantially differ in activity, to go on with our research, we focused our attention on compound **2** because it should be less liable to metabolic oxidative deamination than compound **3**. Thus, considering that the group on the asymmetric carbon atom is pivotal for drug potency,[12] we decided to replace the methyl group on the homologated alkyl chain with a phenyl moiety. Biological results confirmed our expectations; (*S*)-**5**, the eutomer, showed the highest activity in tonic block experiments: compared to (*R*)-**2** and (*R*)-Mex, it was about 6- and 4-fold more potent, respectively. Nevertheless the relevant increase of potency of **5** was accompanied by a partial loss of use-dependent activity with respect to **2** (about 5 vs. 9), even though it retained a higher use-dependent behavior than Mex and **4** (about 5 vs. 2 and 3, respectively). Conceivably, the increase in potency for producing a tonic block obtained with compound **5** could be rationalized on the basis of increased lipophilicity, caused by the insertion of the phenyl group, which does favor the access to the cytoplasmic side of the membrane where the binding site is thought to be located. Tested drugs are normally applied outside the cell so increased lipophilicity would facilitate the diffusion through the membrane, increasing the amount of drug that has access to the binding site (the determined logP values for **5**, **2** and Mex were 3.4, 2.3, and 2.2 respectively, Table 1). On the other hand, an optimal basicity (pK_a values) of the amino group of these three compounds could explain their high use-dependent behavior (phasic block). As regards the stereospecificity index (SSI) in the tonic block experiments, this was low for compound **5** (1.3); this result might suggest that phenyl and amine groups are viewed as interchangeable by the receptor, and this is in agreement with what had been previously observed for **4**.[12] It is noteworthy that, among the newly synthesized compounds, **3** presented the highest pattern of stereoselectivity both in phasic and tonic block, with the eudismic ratios of 2.5 and 1.9, respectively. Furthermore, the eutomer of **3**, in spite of its stereochemical descriptor, does share the same configuration of the eutomers of Mex and analogs in muscle fiber sodium channel studies.[12] In conclusion, facile stereospecific synthetic routes to new homochiral mexiletine analogs, **2**, **3**, and **5**, have been proposed. All of the tested compounds show a use-dependent behavior higher than that of Mex. The biological results obtained with compound **5**, the most potent of the series, provide some new evidence in favour of the fact that the presence of the lipophilic phenyl group on the asymmetric centre of Mex analogs is pivotal to reinforce hydrophobic interactions with the receptor, thus confirming what was suggested before.[12]

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