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Brief Article

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Synthesis and Toxicopharmacological Evaluation of *m*-Hydroxymexiletine, the First Metabolite of Mexiletine More Potent Than the Parent Compound on Voltage-Gated Sodium Channels

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Supporting Information

ABSTRACT: The first synthesis of *m*-hydroxymexiletine (MHM) has been accomplished. MHM displayed hNav1.5 sodium channel blocking activity, and tests indicate it to be ~2-fold more potent than the parent mexiletine and to have more favorable toxicological properties than mexiletine. Thus, MHM and possible related prodrugs might be studied as agents for the treatment of arrhythmias, neuropathic pain, and myotonias in substitution of mexiletine (metabolite switch), which has turned out to be tainted with common toxicity.

INTRODUCTION

The past decade has witnessed a dramatic reduction in the number of new drugs (new molecular entities) approved for therapy.[1] Correspondingly, research costs for a new drug[2] have compelled researchers to look for new strategies to cope with the current, relative shortage of new lead compounds. A relatively less explored approach is the so-called metabolite switch, i.e., the selection of an active metabolite as the substitute for the parent compound, provided that the former has more favorable properties when compared with the latter.[3] In the past, this strategy has scored only a few anecdotic successes, such as the well-known history of paracetamol, an active metabolite of phenacetine and safer analgesic than the retired parent compound,[4] or the replacement of terfenadine, a nonsedative H_1 antagonist possibly cardiotoxic when coadministered with several xenobiotics, by its active metabolite fexofenadine.[3] Further examples of successful application of the metabolite switch strategy have been recently reviewed.[5] Mexiletine, 1-(2,6-dimethylphenoxy)-2-propanamine (Figure 1), is a class IB antiarrhythmic drug.[6,7] Although it is primarily used in treating ventricular arrhythmias, its recent uses include chronic pain[8−11] and myotonia.[12−15] Mexiletine is administered in doses of 150−200 mg two to three times a day and is generally well tolerated. At high doses, mexiletine causes drowsiness, confusion, nausea, hypotension, sinus bradycardia, paresthesia, seizures, bundle branch block, atrioventricular heart block, ventricular arrhythmias, asystole, cardiovascular collapse, and coma.[16] In Europe, the use and commerce of mexiletine hydrochloride (Mexitil, Boehringer Ingelheim) were discontinued in 2008 but it has been reintroduced in some European countries owing to specific national pharmaceutical productions. The treatment of high-dose mexiletine intoxication, for example, in the case of suicide attempt, includes decontamination and supportive therapy and recently hemodialysis.[16] Mexiletine is eliminated slowly in humans with a half-life of 10−12 h, although the drug undergoes extensive metabolism.[17] Indeed, mexiletine is metabolized in the liver by oxidation, deamination, reduction, and conjugation.[18,19] Unchanged mexiletine in urine accounts for only 10% of the administered dose.[20] Eleven metabolites of mexiletine, most of which are eliminated as glucuronide conjugates, were identified, but none of them were found to possess any pharmacological activity.[17,21,22] Indeed, we recently demonstrated that two metabolites, hydroxymethylmexiletine (HMM, Figure 1) and *p*-hydroxymexiletine (PHM), conserve only residual blocking activity on sodium currents recorded in skeletal muscle fibers, when compared to mexiletine.[23−25] In this paper we

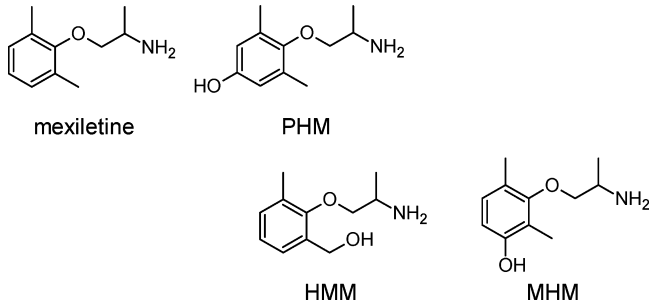

Figure 1. Structures of mexiletine and its hydroxylated metabolites (PHM, HMM, MHM).

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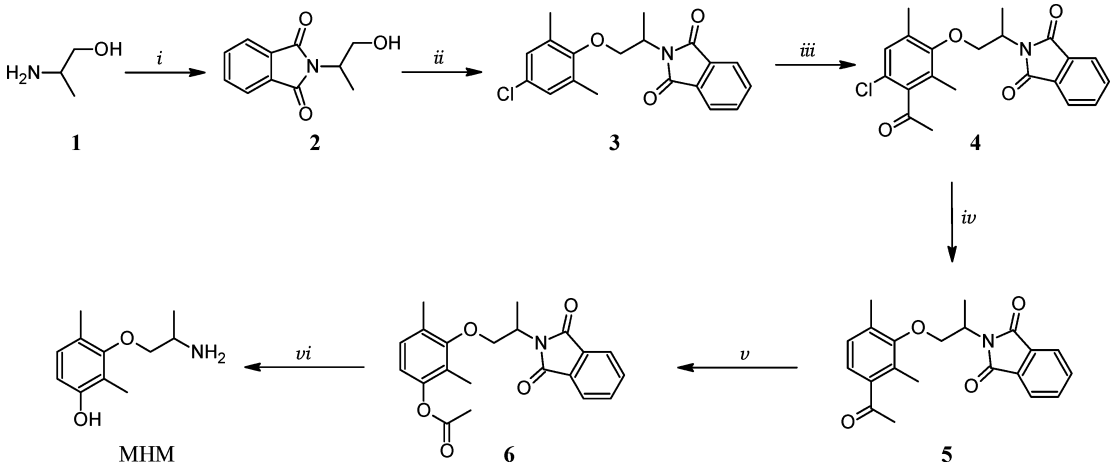
Scheme 1. Synthesis of *m*-Hydroxymexiletine MHM[a]



[a]Reagents and conditions: (i) phthalic anhydride, Et_3N, toluene, reflux; (ii) 4-chloro-2,6-dimethylphenol, PPh_3, DIAD, anhyd THF, room temp.; (iii) CH_3COCl, $AlCl_3$, anhyd CH_2Cl_2, reflux; (iv) HCOONa, $Pd(OAc)_2$, 2-(di-*tert*-butylphosphino)biphenyl, CH_3OH, reflux; (v) MCPBA (77%), anhyd CH_2Cl_2, reflux; (vi) aq N_2H_4, AcOH, MeOH, reflux.

report the synthesis and biological activity of a minor metabolite of mexiletine, *m*-hydroxymexiletine (MHM), which in humans accounts for approximately 2% of an administered oral dose of mexiletine. Since mexiletine is clinically used as a racemic mixture, MHM and the parent compound were studied in this form.

RESULTS AND DISCUSSION

Chemistry. The synthetic sequence to MHM (Scheme 1) is similar to the one previously reported for PHM.[23] The amino propanol **1** was protected with phthalic anhydride in quantitative yield to give the phthalimido alcohol **2** which was reacted with 4-chloro-2,6-dimethylphenol under Mitsunobu conditions.[26,27] Friedel–Crafts acylation, run on alkyl aryl ether **3**, gave the acetyl derivative **4**.[28] Then **4** was dehalogenated to give **5**[29] which underwent Bayer–Villiger oxidation[28] to give the desired acetoxy derivative **6**. Hydrazinolysis on **6** allowed the removal of the acetyl and phthaloyl groups, furnishing MHM.[30] All compounds were characterized by routine spectrometric and spectroscopic analyses. The ^1H NMR spectrum of MHM was fully assigned and compared to that of the literature.[31] The correct assignment of aromatic protons was obtained on the basis of NOESY experiments, partially disproving what was reported in the literature.[31]

Biological Results. Drugs were tested in vitro on voltage-gated sodium currents recorded in HEK293 cells transiently transfected with the human cardiac sodium channel, hNav1.5, using the whole-cell patch-clamp method. Sodium currents were elicited by 25 ms long depolarizing test pulses at −30 mV from the holding potential of −120 mV at two stimulation frequencies, 0.1 Hz for determination of tonic block and 10 Hz for use-dependent block determination. The IC_{50} values were calculated by fitting the concentration/effect relationships with a first-order binding function and are reported with the SE of the fit (Table 1, Figure 2).[32]

Surprisingly, MHM was about 2-fold more potent than mexiletine in tonic and phasic block. These positive results led us to evaluate MHM activity on isolated cardiac tissue. Mexiletine and MHM were tested for antiarrhythmic activity on guinea pig isolated left atria driven at 1 Hz. MHM increased the threshold of ac arrhythmia more than mexiletine did. The efficacy of MHM was not significantly different from that of mexiletine (Table 2).

Table 1. Concentrations for Half-Maximal Tonic (0.1 Hz) and Use-Dependent (10 Hz) Block of Sodium Currents (IC_{50}) and Slope Factor (nH) of Mexiletine and MHM on Heterologously Expressed hNav1.5 Channels

	0.1 Hz		10 Hz	
compd	IC_{50} (μM)	nH	IC_{50} (μM)	nH
mexiletine	420 ± 70	1.0 ± 0.2	58 ± 13	1.0 ± 0.2
MHM	174 ± 6	1.0 ± 0.3	30 ± 3	0.8 ± 0.1

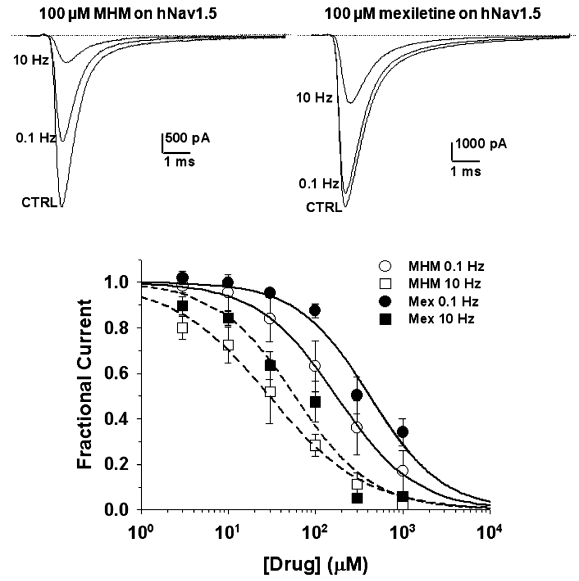


Figure 2. Effects of mexiletine and MHM on hNav1.5 channels: representative sodium current traces elicited from a holding potential of −120 mV to a test potential of −30 mV in the absence of drug (CTRL) and in the presence of 100 μM drug at 0.1 or 10 Hz frequency stimulation. Concentration–response relationships were constructed at 0.1 and 10 Hz stimulation and fitted with binding equation described in Supporting Information. Each point is the mean ± SEM from at least three cells.

To better define the cardiovascular profile of mexiletine metabolite, its inotropic effect was measured in guinea pig left atrium stimulated at 1 Hz, its chronotropism was evaluated in

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Table 2. Antiarrhythmic Activity of Compounds

compd	max % increase[a] (mean ± SEM)	EC_{50}[b] (μM)	95% confidence limit ($\times 10^{-6}$)
amiodarone	10 ± 0.5[c]		
lidocaine	34 ± 2.6		
procainamide	11 ± 0.4		
quinidine	69 ± 0.4	10.26	8.44−12.46
mexiletine	64 ± 1.4[c]	11.61	8.71−13.47
MHM	72 ± 2.7[c]	10.18	8.19−12.67

[a]Max % increase of threshold of ac arrhythmia after pretreatment with compounds. Increase of threshold of ac arrhythmia is the increase in the intensity of 50 Hz alternating current required to produce arrhythmia in guinea pig left atria driven at 1 Hz in the presence of each tested compounds at 5×10^{-5} M. For all data $P < 0.05$. [b]Calculated from log concentration−response curves (Probit analysis according to Litchfield and Wilcoxon[33] with $n = 6-8$). When the maximum effect was <50%, the EC_{50} values were not calculated. [c]At 10^{-4} M.

spontaneously beating right atrium, and its vasorelaxant activity was assessed in K^+-depolarized (80 mM) guinea pig aortic strips. Data are collected in Table 3 with those of mexiletine and other well-known antiarrhythmic drugs.

MHM exhibited a similar negative inotropism, an increased vasorelaxant activity, and a very limited negative chronotropism. Since mexiletine clinical use is often associated with CNS toxicity, a preliminary toxicological evaluation of MHM was performed by assessing the motor coordination of treated animals using the rotarod test. MHM, even injected at a dose of 50 mg/kg, did not increase the number of falls in comparison with control animals treated with saline. By contrast, mexiletine at the same dosage caused an increase of falls from rotating rod in comparison with saline treated animals (Table 4).

Since mexiletine undergoes extensive, first-pass oxidative metabolism and since oxidative metabolism may in turn cause cellular oxidative stress and/or generate reactive metabolites, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay on human hepatocellular liver carcinoma cells (HepG2) was performed. Mexiletine and MHM displayed low toxicity, presenting EC_{50} of 2.0 ± 0.1 and >2 mM, respectively (Figure S1, Supporting Information). Considering that mexiletine plasma concentrations range between 4 and 11 μM in clinical use,[20] both compounds are safe drugs as far as cytotoxicity is concerned. Altogether these results suggest that MHM may display a more favorable pharmacological/toxicological profile than mexiletine. In addition we verified if these ligands may display different ability to cross the blood−brain barrier (BBB) by defining their P-glycoprotein (P-gp) interacting activity. For this purpose, the apparent permeability (P_{app}) of both compounds across epithelial colorectal adenocarcinoma (Caco-2) cell monolayer overexpressing human P-gp was evaluated in the basolateral−apical (BA) and apical−basolateral (AB) fluxes. The Caco-2 cells overexpressing P-gp grown on permeable filters are commonly employed to characterize P-gp substrates. In this assay, ligands that behave as P-gp substrates are effluxed and therefore unable to cross the BBB. Generally, ligands displaying BA/AB > 2 are defined as P-gp substrates, whereas nonsubstrates or P-gp inhibitors display BA/AB < 2.[34] The results shown in Table 5 suggest that mexiletine and MHM may not be substrates for P-gp, since the BA/AB values were approximately 2.

CONCLUSION

In this paper, we report the first synthesis and complete characterization of MHM, a minor metabolite of mexiletine, and its biological evaluation. The synthetic route was designed on the basis of that of PHM.[23] It was easily performed and brought to the final compound in moderate overall yield. MHM antiarrhythmic activity was evaluated and compared to that of mexiletine. Interestingly, in vitro assays revealed that MHM does possess about twice the blocking activity of mexiletine on cardiac sodium channels, which may account for its increases of antiarrhythmic and vasorelaxant activities on isolated tissues. In addition, MHM at the highest tested doses, did not impair motor coordination, in contrast to mexiletine, and showed no cytotoxicity. Our preliminary in vitro studies on a BBB permeation model indicate that none of the two compounds are effluxed by transporters present in Caco-2 cells overexpressing P-gp. This suggests that differences in CNS toxicity may not be a result of selective BBB permeability. Nevertheless, keeping in mind the complexity of the BBB that expresses a large number of transporters, we cannot definitely exclude a role for the BBB in the observed different toxicological profiles. Since new drugs for the treatment of arrhythmias are needed, MHM may be viewed as a promising starting point toward clinical candidates for the treatment of these diseases. The metabolite switch[3] from mexiletine to MHM might be possible,

Table 3. Influence of Tested Compounds on Cardiovascular Parameters

	% decrease (mean ± SEM)		EC_{50} of inotropic negative activity		EC_{30} of chronotropic negative activity		
compd	negative inotropic activity[a,m]	negative chronotropic activity[b,m]	EC_{50}[c] (μM)	95% conf lim ($\times 10^{-6}$)	EC_{30}[c] (μM)	95% conf lim ($\times 10^{-6}$)	vasorelaxant activity[d,m] (mean ± SEM)
amiodarone	30 ± 2.6*[e]	72 ± 4.5*			5.57	4.93−6.02	3 ± 0.1
lidocaine	88 ± 3.0*[f]	29 ± 0.9*[j,k]	0.017	0.012−0.024			14 ± 0.9*
procaine	92 ± 1.4*[g]	9 ± 0.6[k]	0.014	0.011−0.017			3 ± 0.2
quinidine	71 ± 3.6*	86 ± 0.5*[h]	3.38	2.69−4.25	3.99	3.81−4.06	30 ± 1.6*
mexiletine	90 ± 1.3*[i]	85 ± 2.6*[l]	0.045	0.035−0.058	0.014	0.009−0.023	5 ± 0.3
MHM	96 ± 1.9*	39 ± 2.2*	0.098	0.066−0.14			19 ± 0.7*

[a]Activity: decrease in developed tension on isolated guinea pig left atrium at 5×10^{-5} M, expressed as percentage change from the control ($n = 4-6$). The left atria were driven at 1 Hz. [b]Activity: decrease in atrial rate on guinea pig spontaneously beating isolated right atria at 10^{-4} M, expressed as the percentage change from the control ($n = 6-8$). The pretreatment heart rate ranged from 170 to 195 beats/min. [c]Calculated from log concentration−response curve (Probit analysis according to Litchfield and Wilcoxon[33] with $n = 6-8$). When the maximum effect was <50%, the EC_{50} inotropic, EC_{30} chronotropic, and IC_{50} vasorelaxant values were not calculated. [d]Activity: percent inhibition of calcium-induced contraction on K^+-depolarized guinea pig aortic strip at 10^{-4} M. The 10^{-4} M concentration gave the maximum effect for most compounds. [e]At 10^{-4} M. [f]At 10^{-6} M. [g]At 5×10^{-7} M. [h]At 5×10^{-5} M. [i]At 10^{-5} M. [j]At 5×10^{-6} M. [k]Positive chronotropic effect. [l]At 10^{-7} M. [m]An asterisk indicates $P < 0.05$.

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Table 4. Effect of Mexiletine and MHM on Motor Coordination Expressed as Number of Falls from Rotarod[a]

		falls from the rota-rod			
			after treatment		
treatment	dose, mg kg^{-1}po	before treatment	15 min	30 min	45 min
saline		2.5 ± 0.4	1.9 ± 0.3	1.2 ± 0.3	0.9 ± 0.2
mexiletine	25	2.8 ± 0.5	2.1 ± 0.4	1.3 ± 0.4	0.8 ± 0.3
mexiletine	50	2.4 ± 0.3	4.6 ± 0.2*	3.8 ± 0.3*	3.5 ± 0.2*
MHM	25	3.1 ± 0.5	1.8 ± 0.3	1.0 ± 0.2	0.7 ± 0.2
MHM	50	3.0 ± 0.5	2.0 ± 0.4	1.2 ± 0.3	0.9 ± 0.3

[a]Each value represents the mean of six to eight mice. *, $P < 0.01$ in comparison with saline controls.

Table 5. Apparent Permeability of Mexiletine and MHM across Caco-2 Cell Monolayers Overexpressing Human P-gp from the Basolateral to Apical (BA) Sides and from the Apical to Basolateral (AB) Compartments

	P_{app},[a] nm/s				
compd	BA	AB	P_{app}(BA)/P_{app}(AB)[a]	λ (nm)	ε
mexiletine	2818	1214	2.3	210	9530
MHM	3006	1425	2.1	220	7670

[a]Data are the mean of three independent determinations (samples in triplicate) each with SEM < 10%.

assuming that MHM presents a more favorable profile than mexiletine. Indeed, the use of prodrugs of mexiletine and its active metabolites has been recently suggested for the treatment of neuropathic pain and arrhythmias.[35] Finally, to verify possible distinct biological activities for MHM enantiomers paralleling the light stereoselectivity observed in mexiletine pharmacodynamics and pharmacokinetics,[36] the preparation of MHM enantiomers has been undertaken in view of a possible switch from the use of (*RS*)-MHM to that of one of the enantiomers (chiral switch).[3]

EXPERIMENTAL SECTION

Materials and Methods. Chemicals were purchased from Sigma-Aldrich or Lancaster. Yields refer to purified products and were not optimized. The structures of the compounds were confirmed by routine spectrometric and spectroscopic analyses. Compounds **2**, **3**, and mexiletine hydrochloride were prepared as previously described.[26] For the synthesis and characterization of **4**, **5**, and **6**, see Supporting Information. Only spectra for compounds not previously described are given. Melting points were determined on a Gallenkamp apparatus in open glass capillary tubes and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer (Norwalk, CT) Spectrum One FT spectrophotometer, and band positions are given in reciprocal centimeters (cm^{-1}). ^{1}H and ^{13}C NMR spectra were recorded on a Varian VX Mercury spectrometer operating at 300 and 75 MHz for ^{1}H and ^{13}C, respectively, using $CDCl_3$ as solvent unless otherwise indicated. Chemical shifts are reported in parts per million (ppm) relative to the residual nondeuterated solvent resonance: $CDCl_3$, δ 7.26 (^{1}H NMR) and δ 77.3 (^{13}C NMR). *J* values are given in Hz. EI mass spectra were recorded on a Hewlett-Packard 6890-5973 MSD gas chromatograph/mass spectrometer at low resolution. Elemental analyses (C, H, N) were used to confirm the purity of all new compounds (>95%) and were performed on a Eurovector Euro EA 3000 analyzer (results within ±0.4% of the theoretical values; see Supporting Information). 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.[37] TLC analyses were performed on precoated silica gel on aluminum sheets (Kieselgel 60 F_{254}, Merck).

Synthesis of (*RS*)-3-(2-Aminopropoxy)-2,4-dimethylphenol (MHM). To a stirred solution of (*RS*)-**6** (0.13 g, 0.35 mmol) in MeOH (5 mL), glacial AcOH (2.1 mmol) and aqueous hydrazine (2.1 mmol) were added. The mixture was kept under reflux for 5 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 (3 × 10 mL). Then the aqueous phase was brought to 9 < pH < 11 with 2 N NaOH (20 mL) and 2 N Na_2CO_3 (20 mL) and extracted twice with EtOAc. The combined organic layers were dried (Na_2SO_4) and concentrated under vacuum. The final product was a reddish solid (44 mg, 64%) which was recrystallized from Et_2O: mp 109−110 °C; IR (KBr) 3346, 3279 (NH_2, OH). ^{1}H NMR (CD_3OD, δ 3.30) chemical shifts were attributed on the basis of NOESY experiment: δ 1.18 (d, J = 6.3 Hz, 3H, CH_3CH), 2.10 (s, 3H, ArO CH_3C-2), 2.15 (s, 3H, ArO CH_3C-6), 3.23−3.34 (m, 1H, C*H*), 3.46−3.65 (m, 2H, CH_2), 6.45 (d, J = 8.1 Hz, 1H, Ar *H*C-4), 6.77 (d, J = 8.1 Hz, 1H, Ar *H*C-5); ^{13}C NMR (CD_3OD, δ 47.8) δ 8.32 (1C), 14.9 (1C), 18.0 (1C), 47.0 (1C), 77.3 (1C), 110.4 (1C), 117.5 (1C), 121.1 (1C), 127.7 (1C), 154.5 (1C), 156.1 (1C); MS (70 eV) *m*/*z* (%) 195 (M^+, 6), 58 (99), 44 (100). Anal. ($C_{11}H_{17}NO_2$·0.50H_2O) C, H, N.

Additional Information. For details on patch clamp, functional, motor coordination, cytotoxicity, and permeability experiments, see Supporting Information sections S5, S6, S8, S9, and S10, respectively.

ASSOCIATED CONTENT

Supporting Information

Elemental analysis results, procedures for the synthesis of intermediates, procedure for biological studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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ABBREVIATIONS USED

AB, apical−basolateral; BA, basolateral−apical; BBB, blood−brain barrier; HMM, hydroxymethylmexiletine; MHM, *m*-hydroxymexiletine; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; P_{app}, apparent permeability; P-gp, P-glycoprotein; PHM, *p*-hydroxymexiletine

REFERENCES

(1) Hughes, B. 2008 FDA drug approvals. *Nat. Rev. Drug Discovery* **2009**, *8*, 93−96.

(2) Di Masi, J. A.; Hansen, R. W.; Grabowski, H. G. The price of innovation: new estimates of drug development costs. *J. Health. Econ.* **2003**, *22*, 151−185.

(3) Tucker, G. T. Chiral switches. *Lancet* **2000**, *355*, 1085−1087.
(4) Brodie, B. B.; Axelrod, J. The fate of acetophenetidin (phenacetin) in man and methods for the estimation of acetophenetidin and its metabolites in biological materials. *J. Pharmacol. Exp. Ther.* **1949**, *97*, 58−67.
(5) Teague, S. J. Learning lessons from drugs that have recently entered the market. *Drug Discovery Today* **2011**, *16*, 398−411.
(6) Yamauchi, M.; Watanabe, E.; Yasui, K.; Takeuchi, H.; Terasawa, T.; Sawada, K.; Hishida, H.; Kodama, I. Prevention of ventricular extrasystole by mexiletine in patients with normal QT intervals is associated with a reduction of transmural dispersion of repolarization. *Int. J. Cardiol.* **2005**, *103*, 92−97.
(7) Fenster, P. E.; Comess, K. A. Evaluations of new drugs. *Pharmacotherapy* **1986**, *6*, 1−9.
(8) Sloan, P.; Basta, M.; Storey, P.; von Gunten, C. Mexiletine as an adjuvant analgesic for the management of neuropathic cancer pain. *Anesth. Analg.* **1999**, *89*, 760−761.
(9) Jarvis, B.; Coukell, A. J. Mexiletine. A review of its therapeutic use in painful diabetic neuropathy. *Drugs* **1998**, *56*, 691−707.
(10) Oskarsson, P.; Ljiunggren, J.-G.; Lins, P.-E. Efficacy and safety of mexiletine in the treatment of painful diabetic neuropathy. *Diabetes Care* **1997**, *20*, 1594−1597.
(11) Tremont-Lukats, I. W.; Challapalli, V.; McNicol, E. D.; Lau, J.; Carr, D. B. Systemic administration of local anesthetics to relieve neuropathic pain: a systematic review and meta-analysis. *Anesth. Analg.* **2005**, *101*, 1738−1749.
(12) Rüdel, R.; Lehmann-Horn, F. Membrane changes in cells from myotonia patiens. *Physiol. Rev.* **1985**, *65*, 310−356.
(13) Lehmann-Horn, F.; Jurkat-Rott, K. Voltage-gated ion channels and hereditary disease. *Physiol. Rev.* **1999**, *79*, 1317−1372.
(14) Chrestian, R.; Puymirat, J.; Bouchard, J.-P.; Dupré, N. Myotonia congenital—a cause of muscle weakness and stiffness. *Nat. Clin. Pract. Neurol.* **2006**, *2*, 393−399.
(15) Gay, S.; Dupuis, D.; Faivre, L.; Masurel-Paulet, A.; Labenne, M.; Colombani, M.; Soichot, P.; Huet, F.; Hainque, B.; Sternberg, D.; Fontaine, B.; Gouyon, J.-B.; Thuvin-Robinet, C. Severe neonatal non-distrophic myotonic secondary to a novel mutation of the voltage-gated sodium channel (SCN4A) gene. *Am. J. Med. Genet., Part A* **2008**, *146*, 380−383.
(16) Akinci, E.; Yüzbaşioglu, Y.; Coşkun, F. Hemodialysis as an alternative treatment of mexiletine intoxication. *Am. J. Emerg. Med.* **2011**, *29*, 1235−1236.
(17) Labbè, L.; Turgeon, J. Clinical pharmacokinetics of mexiletine. *Clin. Pharmacokinet.* **1999**, *37*, 361−384.
(18) Beckett, A. H.; Chidomere, E. C. The distribution, metabolism and excretion of mexiletine in man. *Postgrad. Med. J.* **1977**, *53* (Suppl. 1), 60−66.
(19) Beckett, A. H.; Chidomere, E. C. The identification and analysis of mexiletine and its metabolic products in man. *J. Pharm. Pharmacol.* **1977**, *29*, 281−285.
(20) Campbell, N. P.; Kelly, J. G.; Adgey, A. A.; Shanks, R. G. The clinical pharmacology of mexiletine. *Br. J. Clin. Pharmacol.* **1978**, *6*, 103−108.
(21) Hashimoto, K. Mexiletine. *New Cardiovasc. Drugs* **1986**, 141−161.
(22) Kates, R. E. Metabolites of cardiac antiarrhythmic drugs: their clinical role. *Ann. N.Y. Acad. Sci.* **1984**, *432*, 75−89.
(23) Catalano, A.; Carocci, A.; Fracchiolla, G.; Franchini, C.; Lentini, G.; Tortorella, V.; De Luca, A.; De Bellis, M.; Desaphy, J.-F.; Conte Camerino, D. Stereospecific synthesis of para-hydroxymexiletine and sodium channel blocking activity evaluation. *Chirality* **2004**, *16*, 72−78.
(24) Cavalluzzi, M. M.; Catalano, A.; Bruno, C.; Lovece, A.; Carocci, A.; Corbo, F.; Franchini, C.; Lentini, G.; Tortorella, V. Synthesis of (*R*)-, (*S*)-, and (*RS*)-hydroxymethylmexiletine, one of the major metabolites of mexiletine. *Tetrahedron: Asymmetry* **2007**, *18*, 2409−2417.
(25) De Bellis, M.; De Luca, A.; Rana, F.; Cavalluzzi, M. M.; Catalano, A.; Lentini, G.; Franchini, C.; Tortorella, V.; Conte Camerino, D. Evaluation of the pharmacological activity of the major mexiletine metabolites on skeletal muscle sodium currents. *Br. J. Pharmacol.* **2006**, *149*, 300−310.
(26) Carocci, A.; Catalano, A.; Corbo, F.; Duranti, A.; Amoroso, R.; Franchini, C.; Lentini, G.; Tortorella, V. Stereospecific synthesis of mexiletine and related compounds: Mitsunobu versus Williamson reaction. *Tetrahedron: Asymmetry* **2000**, *11*, 3619−3634.
(27) Mitsunobu, O. The use of diethyl azodicarboxylate and triphenylphosphine in synthesis and transformation of natural products. *Synthesis* **1981**, 1−28.
(28) Adejare, A.; Sciberras, S. S. Synthesis and β-adrenergic activities of *R*-fluoronaphtyloxypropanolamine. *Pharm. Res.* **1997**, *14*, 533−536.
(29) Logan, M. E.; Oinen, M. E. Dechlorination of aryl chlorides with sodium formate using a homogeneous palladium catalyst. *Organometallics* **2006**, *25*, 1052−1054.
(30) Roubini, E.; Laufer, R.; Gilon, C.; Selinger, Z.; Roques, B. P.; Chorev, M. Pseudopeptide analogues of substance P and leucine enkephalinamide containing the Ψ (CH_2O) modification: synthesis and biological activity. *J. Med. Chem.* **1991**, *34*, 2430−2438.
(31) Grech-Bélanger, O.; Turgeon, J.; Lalande, M.; Bélanger, P. M. Meta-hydroxymexiletine, a new metabolite of mexiletine. *Drug. Metab. Dispos.* **1991**, *19*, 458−461.
(32) Desaphy, J.-F.; Dipalma, A.; Costanza, T.; Bruno, C.; Lentini, G.; Franchini, C.; George, A. L. Jr.; Conte Camerino, D. Molecular determinants of state-dependent block of voltage-gated sodium channels by pilsicainide. *Br. J. Pharmacol.* **2010**, *160*, 1521−1533.
(33) Tallarida, R. J.; Murray, R. B. *Manual of Pharmacologic Calculations with Computer Programs*, 2nd ed.; Springer-Verlag: New York, 1987.
(34) Colabufo, N. A.; Berardi, F.; Cantore, M.; Perrone, M. G.; Contino, M.; Inglese, C.; Niso, M.; Perrone, R.; Azzariti, A.; Simone, G. M.; Porcelli, L.; Paradiso, A. Small P-gp modulating molecules: SAR studies on tetrahydroisoquinoline derivatives. *Bioorg. Med. Chem.* **2008**, *16*, 362−373.
(35) Franklin, R.; Golding, B. T.; Tyson, R. G. Mexiletine Amino Acid and Peptide Prodrugs and Uses Thereof. Patent WO 2010/149760, 2010.
(36) Carocci, A.; Franchini, C.; Lentini, G.; Loiodice, F.; Tortorella, V. Facile entry to (−)-(*R*)- and (+)-(*S*)-mexiletine. *Chirality* **2000**, *12*, 103−106.
(37) Still, W. C.; Kahn, M.; Mitra, A. Rapid chromatographic technique for preparative separations with moderate resolution. *J. Org. Chem.* **1978**, *43*, 2923−2925.