Stereospecific Synthesis of \textit{m}-Hydroxymexiletine Enantiomers

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Abstract: \textit{m}-Hydroxymexiletine (MHM) is a metabolite of mexiletine, a well known class IB anti-arrhythmic drug, which presents almost twice the activity of the parent compound on cardiac voltage-gated sodium channels. Given the different activity of mexiletine enantiomers on sodium currents (being the \textit{R}-isomer the eutomer), it is conceivable that (\textit{R})- and (\textit{S})-MHM could differ in pharmacodynamic and pharmacokinetic properties, too. Herein we report the efficient synthesis of MHM enantiomers that could represent useful tools for further investigations on stereospecific requirements of the voltage-gated sodium channel binding site. MHM enantiomers and all the homochiral intermediates were fully characterized. The ee values for (\textit{R})- and (\textit{S})-MHM were >99%, as assessed by capillary electrophoresis using \textbeta-cyclodextrin sulfated sodium salt as a chiral selector.

Keywords: Sodium channel blockers, asymmetric synthesis, \textit{m}-Hydroxymexiletine, anti-arrhythmics, chirality.

INTRODUCTION

Differentiation between stereoisomers is a fundamental phenomenon as chiral compounds interact in a stereoselective way with each other. Therefore, chiral molecules play an important part in many aspects of life sciences, medical sciences, synthetic chemistry, food chemistry, as well as many other fields. Several drugs are now marketed or being developed as single enantiomers in place of a previous racemic mixture, a process known as “chiral switching” [1]. The potential advantages of chiral switching include an improved therapeutic index through increased potency and selectivity and decreased side-effects. The well-known history of thalidomide is a striking example of the toxicity of one enantiomer [2]. Thalidomide was developed and sold by the German pharmaceutical company Grunenthal in 1957. The drug was prescribed for use as a sedative and for the treatment of morning sickness. No remarkable toxicity was observed when tested on rodents, and thalidomide was distributed in over 40 countries. However, studies by McBride [3] and Lenz [4] independently reported its teratogenicity in 1961 and 1962, respectively. The use of thalidomide during early pregnancy led to serious embryotoxic effects, such as limb defects (amelia and phocomelia); this was due to thalidomide chirality: the eutomer is a powerful tranquilizer, while the distomer can disrupt fetal development causing severe handicap [5]. Over 10,000 children were affected worldwide. Our interest in chirality was focused on mexiletine, 1-(2,6-dimethylphenoxy)-2-propanamine (Mex, Fig. 1), that is a class IB orally effective antiarrhythmic agent which shows a narrow therapeutic index. Its clinical usefulness is due to its ability to block gated sodium channels more potently in situations of excessive burst of action potentials (use-dependent or phasic block). This occurs in diseased tissues, rather than under conditions of physiological excitability (tonic block). Its primary use has been the treatment of ventricular arrhythmias, but it can also be used in chronic pain [6-9] and myotonia [10-12]. Mexiletine was clinically used as a racemic mixture; nowadays, the use and commerce of mexiletine hydrochloride (Mexitil, Boehringer Ingelheim) in Italy has been discontinued, because high doses caused drowsiness, confusion, nausea, hypotension, sinus bradycardia, paresthesia, seizures, bundle branch block, atrioventricular heart block, ventricular arrhythmias, asystole, cardiovascular collapse, and coma [13,14]. No therapeutic drug substitution for mexiletine has been proposed till now. Thus, with the aim of finding a substitute for mexiletine, which could show the same activity but lower side effects, we proposed a metabolite switch from mexiletine to \textit{m}-hydroxymexiletine (MHM, 3-(2-aminopropoxy)-2,4-dimethylphenol, Fig. 1) [15-17]. MHM is a minor metabolite of mexiletine, which in humans accounts for approximately 2% of an administered oral dose of mexiletine, but its great importance is due to the fact that it is equipotent to mexiletine on voltage-gated skeletal muscle sodium channels and even 2-fold more potent than the parent compound on the cardiac ones [15-17]. Herein, we propose a synthetic route to (\textit{R})- and (\textit{S})-MHM, which might feed studies in view of a chiral switch from MHM to one of its enantiomers provided that they show differences in pharmacodynamics and/or pharmacokinetics. This is conceivable given that the two enantiomers of mexiletine show differences in pharmacodynamic effects and pharmacokinetic profiles. The prevention of ventricular tachycardia and tonic block of the skeletal muscle channels effects of \textit{R}-mexiletine are both higher than that of its antipode [18-22].

MATERIALS AND METHODS

Drug Synthesis

The synthesis of MHM enantiomers was obtained according to Scheme 1. To a stirring solution of (\textit{R})-3 (0.97...
g, 2.80 mmol) in trifluoroacetic acid (10 mL) at room temperature, NaNO₃ (0.28 g, 3.36 mmol) was added. After 1 h, excess TFA was removed under vacuum and the residue was taken up with EtOAc, washed with water and brine and dried over anhydrous Na₂SO₄. Purification of the residue by column chromatography on silica gel (EtOAc/petroleum ether 1:9) gave 0.91 g of a white solid (84%), which was crystallized from EtOAc/hexane to give 0.48 g of (R)-5 as a slightly yellowish oil: [α]₂⁰D = −54 (c 2.2, CHCl₃); IR (CHCl₃); 1775, 1710 (C=O); ¹H NMR (CDCl₃): δ 1.54 (d, J = 7.2 Hz, 3H, C-5,6); 13C NMR (CDCl₃): δ 11.8 (1C), 15.3 (1C), 16.6 (1C), 47.0 (1C), 72.5 (1C), 119.7 (1C), 123.5 (2C), 125.4 (1C), 129.9 (2C), 132.0 (1C), 134.4 (2C), 135.1 (1C), 149.0 (1C), 154.3 (1C), 168.6 (2C); GC-MS m/z (%) 324 (M⁺, 22), 188 (100).

(S)-5 was prepared via the above reaction starting from (S)-4. Yield: 69%; [α]₂⁰D = + 49 (c 1.9, CHCl₃). Spectrometry data were in agreement with those reported for (R)-5.

To a stirring suspension of (R)-5 (0.49 g, 1.51 mmol) in 0.25 N HCl (20 mL), a solution of NaNO₂ (104 mg, 1.51 mmol) in H₂O (10 mL) was added. After 1 h, H₂O (20 mL) was added and the temperature brought to 70 °C. After 30 min the aqueous suspension was extracted with EtOAc. The organic layers were dried over Na₂SO₄ and solvent evaporated under vacuum, giving 0.33 g of (R)-6 as a brown oil (68%); [α]₂⁰D = −49 (c 2.1, CHCl₃); IR (CHCl₃); 3684 (OH), 1774, 1709 (C=O); ¹H NMR (CDCl₃): δ 1.54 (d, J = 7.1 Hz, 3H, C₅H₅), 2.08 (s, 3H, ArO CH₂(C-6), 2.10 (s, 3H, ArO CH₂(C-2), 3.87 (dd, J = 9.3, 5.8 Hz, 1H, CHH), 4.33 (apparent t, 1H, CHH), 4.78–4.94 (m, 1H, CH), 6.37 (d, J = 8.0 Hz, 1H, ArO HC-4), 6.76 (d, J = 8.2 Hz, 1H, ArO HC-5), 7.66–7.74 (m, 2H, Ar HC-6, 7.80–7.90 (m, 2H, Ar HC-7); ¹³C NMR (CDCl₃): δ 11.2 (1C), 15.4 (1C), 16.0 (1C), 47.3 (1C), 72.2 (1C), 111.3 (1C), 116.0 (1C), 120.7 (1C), 123.4 (2C), 128.6 (1C), 132.2 (2C), 134.2 (2C), 144.0 (1C), 155.7 (1C), 168.7 (2C); GC-MS ml/z (%) 324 (M⁺, 22), 188 (100).

Scheme I. Synthesis of MHM enantiomers.
exch D₂O, 1H, OH), 6.46 (d, J = 8.0 Hz, 1H, ArO HC-4), 6.79 (d, J = 8.2 Hz, 1H, ArO HC-5), 7.65–7.76 (m, 2H, Ar HC-5,6), 7.80–7.90 (m, 2H, Ar HC-4,7); 13C NMR (CDCl₃): δ 9.2 (1C), 15.4 (1C), 16.0 (1C), 47.3 (1C), 72.1 (1C), 111.0 (1C), 117.5 (1C), 122.8 (1C), 123.5 (2C), 128.2 (1C), 132.2 (2C), 134.2 (2C), 153.1 (1C), 156.0 (1C), 168.8 (2C); GC-MS m/z (%) 325 (M⁺, 7), 188 (100).

(S)-6 was prepared via the above reaction starting from (S)-5. Yield: 71%; [α]²⁰ D = + 44 (c 2.3, CHCl₃). Spectrometry data were in agreement with those reported for (R)-6.

To a stirred solution of (R)-6 (0.32 g, 0.98 mmol) in absolute EtOH (11 mL), glacial AcOH (2.94 mmol) and aqueous hydrazine (2.94 mmol) were added and 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 x 20 mL); then the aqueous phase was treated with 2 N NaOH (40 mL) and brought to 9 < pH < 11 with 2 M Na₂CO₃ and extracted twice with EtOAc. The combined organic layers were dried (Na₂SO₄) and concentrated under vacuum. The final product, (R)-MHM, was a reddish solid (0.18 g, 95%) which was recrystallized from Et₂O: mp 101–102 °C; >99% ee; IR (KBr): 3353, 3276 (NH₂, OH); 1H NMR (CD₃OD): δ 1.19 (d, J = 6.6 Hz, 3H, CH₃CH), 2.10 (s, 3H, ArO CH₃C-2), 2.16 (s, 3H, ArO CH₃C-6), 3.25–3.35 (m overlapped to CD₃OD signal, 1H, CH), 6.46 (d, J = 8.2 Hz, 1H, Ar HC-4), 6.78 (d, J = 8.2 Hz, 1H, Ar HC-5); 13C NMR (CD₂OD): δ 9.5 (1C), 16.0 (1C), 19.2 (1C), 48.1 (1C), 78.5 (1C), 111.6 (1C), 118.7 (1C), 122.3 (1C), 128.9 (1C), 155.7 (1C), 157.3 (1C). Anal. calcd for C₈H₁₇NO₂: C, 67.66; H, 8.78; N, 7.17. Found C, 67.42; H, 8.74; N, 7.04.

(S)-MHM was prepared via the above reaction starting from (S)-6. Yield: 80%; mp 95–96 °C (Et₂O); >99% ee; Spectrometry data were in agreement with those reported for (R)-MHM. Anal. calcd for C₈H₁₇NO₂·0.33 H₂O: C, 65.64; H, 8.85; N, 6.96. Found C, 65.12; H, 8.49; N, 7.07.

Analytical Instruments

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 ¹³C NMR spectra were recorded on a Varian VX Mercury spectrometer operating at 300 and 75 MHz for ¹H and ¹³C, respectively, using CDCl₃ and CD₂OD as solvents. Chemical shifts are reported in parts per million (ppm) relative to the residual non-deuterated solvent resonance: CDCl₃, δ 7.26 (¹H NMR) and δ 77.3 (¹³C NMR); CD₂OD, δ 3.30 (¹H NMR) and δ 49.0 (¹³C NMR). J values are given in Hz. GC-MS 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 [α]D20 values are given in units of 10-1 deg cm2 g-1. Electrophoretic runs were performed by CZE on a P/ACE TM MDQ Capillary Electrophoresis System (Beckman Coulter). A fused silica capillary of 60 cm (effective length 50 cm) and 0.05 mm i.d. (Quadrex Corp.) thermostated at 15 °C was used as a separation tube. The samples (0.1 mg/mL) were pressure injected (0.5 psi/s) and detected at 214 nm; applied voltage was 20 kV. Phosphate buffer (0.033 M, pH = 7.0), in the presence of sulfated β-cyclodextrin (20 mg/mL) as a chiral selector, was used as background electrolyte (BGE). Chromatographic separations were performed on silica gel columns by flash chromatography (Kieselgel 60, 0.040–0.063 mm, Merck, Darmstadt, Germany) as previously described [23]. TLC analyses were performed on precoated silica gel on aluminum sheets (Kieselgel 60 F254, Merck). Melting points were determined on a Gallenkamp apparatus in open glass capillary tubes and are uncorrected.

Chemicals

Chemicals were purchased from Sigma-Aldrich or Lancaster. Compounds (R)- and (S)-2 and 3 were prepared as previously described [24].

RESULTS AND DISCUSSION

The synthetic sequence to (R)- and (S)-MHM (Scheme 1) exploits the one previously reported for the racemate [17]. Homochiral aminopropanols 1 were protected with phthalic anhydride in quantitative yield to give the phthalimido alcohols 2 which were reacted with 4-chloro-2,6-dimethylphenol under Mitsunobu conditions [15,24,25]. Nitration of compounds 3 [26] gave nitroderivatives 4. The successive dechlorination reaction [27] brought to anilines 5. Diazonium salt of these compounds gave phenols 6 which were deprotected by hydrazinolysis to give (R)- and (S)-MHM. All compounds were characterized by routine spectrometric and spectroscopic analyses. Ee values of MHM enantiomers were evaluated by capillary electrophoresis using β-cyclodextrin sulfated sodium salt as a chiral selector and were >99% for both enantiomers (Fig. 2).

CONCLUSION

In this paper we report the efficient synthesis of MHM enantiomers. This work is an extension of our previous work, in which we reported the synthesis of racemic MHM [17]. MHM is a minor metabolite of mexiletine, which is active on voltage-dependent sodium channels: in particular, it is equipotent to mexiletine on skeletal muscle channels and 2-fold more potent of the parent compound on the cardiac ones [15-17]. All the intermediates for the synthesis of (R)- and (S)-MHM were fully characterized. MHM has been already proposed for a metabolite switch [15,16] from mexiletine, which has turned out to be tainted with common toxicity. Moreover, the use of prodrugs of mexiletine and its active metabolites has been recently suggested for the treatment of neuropathic pain and arrhythmias [15,28]. Herein we would propose a chiral switch [1] from MHM to one of its enantiomers provided that the two enantiomers differ in pharmacodynamics and/or pharmacokinetics. This is true for mexiletine enantiomers: (–)-(R)-M is the eutomer both on cardiac and skeletal muscle voltage-gated sodium channels. Thus, given the higher activity of MHM, it might be assumed that similar differences might be found for MHM enantiomers. In this view, it could be interesting to analyze if metabolic hydroxylation to MHM is enantioselective. In vitro studies, performed with human liver microsomes, demonstrated that hydroxylation to the main metabolic products of mexiletine is enantioselective: in particular, aliphatic hydroxylation to hydroxymethylmexiletine is predominant for (–)-(R)-M, whereas aromatic hydroxylation to para-hydroxymexiletine is favored for the (+)-(S)-enantiomer [29-31]. MHM enantiomers were used to explore the efficiency of new chiral solvating agents (CSAs). In particular, the (R)-enantiomer presented a downfield sense of the 1H NMR signal non-equivalence, in agreement with what observed for the other studied mexiletine analogues [32]. Actually, recent studies showed that the binding of mexiletine enantiomers to human serum proteins was enantioselective [33,34]. Herein, we synthesized non-racemic MHM enantiomers as tools that can be used to study the enantioselectivity of this class of voltage-gated sodium channel blockers.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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ABBREVIATIONS

BGE = Background electrolyte
CSAs = Chiral solvating agents
CZE = Capillary zone electrophoresis
GC-MS = Gas chromatography-tandem mass spectrometry
IR = Infrared
MHM = m-hydroxymexiletine
NMR = Nuclear Magnetic Resonance
TFA = Trifluoroacetic acid
TLC = Thin layer chromatography

REFERENCES


