Constrained analogues of tocainide as potent skeletal muscle sodium channel blockers towards the development of antimyotonic agents

Alessia Catalano a, Alessia Carocci a, Filomena Corbo a, Carlo Franchini a, *, Marilena Muraglia a, Antonio Scilimati a, Michela De Bellis b, Annamaria De Luca b, Diana Conte Camerinob, Maria Stefania Sinicropic, Vincenzo Tortorellaa

a Department of Medicinal Chemistry, University of Bari, via E. Orabona n. 4, 70125 Bari, Italy
b Department of Pharmacology, University of Bari, via E. Orabona n. 4, 70125 Bari, Italy
c Department of Pharmaceutic Sciences, University of Calabria, Arcavacata di Rende (CS) 87036, Italy

Received 11 December 2007; received in revised form 15 January 2008; accepted 16 January 2008
Available online 31 January 2008

Abstract

1-Benzyl-N-(2,6-dimethylphenyl)piperidine-3-carboxamide and 4-benzyl-N-(2,6-dimethylphenyl)piperazine-2-carboxamide, two conformationally restricted analogues of tocainide, were designed and synthesized as voltage-gated skeletal muscle sodium channel blockers. They showed, with respect to tocainide, a marked increase in both potency and use-dependent block.

© 2008 Published by Elsevier Masson SAS.

Keywords: Sodium channel blockers; Myotonia; Benzylation; Tocainide

1. Introduction

Sodium channels are involved in many cellular functions and are altered in many pathological conditions, as in the channelopathies. Therefore, drugs targeting sodium channels constitute important therapeutic interventions for a number of diseases. Among these, tocainide (1a, Fig. 1), a well-known sodium channel blocker, is a class Ib antiarrhythmic drug once used in the treatment of symptomatic life-threatening ventricular arrhythmias [1,2]. It has also a marked analgesic effect in trigeminal neuralgia in humans [3,4] and antinociceptive effect in rats [5]. Furthermore, tocainide has been proposed as a clinically useful antimyotonic drug being able to block sodium channels in a use-dependent manner, i.e., with an increased potency in condition of high-frequency discharges of action potentials [6]. Myotonic syndromes are hereditary disorders of the skeletal muscle caused by missense mutations in the human skeletal muscle sodium channel Na,1.4 that prevent the normal fast inactivation of sodium channel [7–9]. Currently, tocainide is among the few drugs clinically used for the symptomatic treatment of muscle hyperexcitability in myotonic syndromes. However, its use as antimyotonic is hindered by unwanted adverse side-effects [10]. Thus, there is a need to develop new safer use-dependent sodium channel blockers with an improved pharmacological profile. A few years ago, a comprehensive model of the sodium channel was reported, showing that the increase in lipophilicity and molecular size of antiarrhythmic drugs can reinforce hydrophobic interactions with the binding site during use-dependent block [11,12]. As a part of our program aimed at developing new antimyotonic drugs using tocainide as a “lead compound”, a series of tocainide analogues were designed with the purpose of identifying novel potent voltage- and use-dependent skeletal muscle sodium channel blockers with very high affinity constants for the inactivated channels [13,14]. In particular, potency and use-dependent behaviour were found to be strongly increased by constraining the amino terminal group of 1a in both a rigid alpha- and beta-proline cycle.
A further improvement was still achieved by introducing a benzyl on the amino group of proline-derived compounds (2c and 3b) [15,16]. To confirm the importance of both molecular rigidity and presence of the benzyl group, we designed and synthesized two compounds, 4 and 5, having a chemical structure that combines alpha- and beta-proline features (2 and 3) in a six-membered ring.

The introduction of an N-benzyl versus the more classic N-alkyl was designed as an attempt to investigate the effect of the presence of a second aromatic moiety in the molecule, which could establish specific additional hydrophobic interactions with the binding site in the protein. As we have previously reported [16], lipophilicity seems to affect the tonic block; in fact, all the N-alkyl derivatives showed higher tonic block values than their corresponding unsubstituted precursors. This, in turn, is related to the increase of the block potency of the channel. Recently, docking studies [17] on tocainide (1a) and N-benzyltocainide (1b) showed that the absence of an alkyl linked to the amino group of the tocainide led to the reduction of the van der Waals interactions with the side chain of Phe1579. In fact, the benzyl group in 1b adopts an off-centered parallel orientation relative to the aromatic ring of the side chain of Phe1579. The additional energy of interaction explains the higher binding affinity of 1b than tocainide (1a).

Thus, the two tocainide derived compounds were designed with the aim to merge the characteristics of 2c and 3b, and with the consideration that several local anaesthetics currently used in therapy, such as mepivacaine, bupivacaine and ropivacaine, are substituted 2-piperidine carboxanilides, structurally related to our compounds [18] and that 2-piperazine carboxanilides were already investigated as anaesthetic and antiarrhythmic drugs [19,20].

2. Chemistry

Compounds 4a,b and 5a,b were synthesized as depicted in Scheme 1. N-t-Boc derivatives 8 and 9 were prepared by reacting 6 (or 7) and 2-tert-butoxycarbonylimino-2-phenylacetanitride (Boc-ON) in a mixture of dioxane/water in the presence of Et3N. Then, 8 (or 9) was reacted with 2,6-dimethylaniline in the presence of IIDQ (2-isobutoxy-1-isobutoxycarbonyl-1,2-dihydroquinoline) to afford the corresponding carboxanilides 4c and 5c, which were, in turn, deprotected by treatment with 3 N HCl or 48% HBr to give 4a and 5a, respectively. The free amines were converted into their N-benzyl derivatives 4b and 5b by reacting 4a and 5a with benzyl bromide. We attributed the preferential benzylation of the nitrogen atom in the beta position of 5a to several factors: the sterical hindrance of the xylididic moiety; the electron-withdrawing effect of the same moiety that determines a reduction of basicity of the alpha nitrogen atom and, finally, the possibility of the same nitrogen atom to be involved in a five-membered pseudo-cycle with the carbonyl oxygen.

3. Pharmacology

The effects of the newly synthesized N-benzyl analogues of tocainide in which the asymmetric carbon atom is constrained...
4. Results and discussion

The research herein described is based upon our previous works dealing with tocainide derivatives with enhanced potency and selectivity for use-dependent inhibition of the skeletal muscle Na\textsubscript{V}1.4 channel. In particular, additional tonic potency was attained by introduction of the hydrophobic benzyl group, whereas ring constraint (proline ring) affected both potency and use-dependence of channel block. The present work shows that similar potency and use-dependence inhibition values are still possible by expanding the proline, from five- to a six-membered ring, also incorporating an additional nitrogen atom. In fact, by constraining the stereogenic center of tocainide in a rigid piperidine and piperazine cycle, as in 4b and 5b, respectively, a marked increase of the potency for producing both tonic and use-dependent blocks of I\textsubscript{Na} was obtained. Tonic block IC\textsubscript{50} values of the two anilide derivatives 4b and 5b were similar being about 15- and 18-fold more potent than tocainide at -100 mV, respectively. During the 10 Hz stimulation, the potency of both compounds increased. Indeed, the IC\textsubscript{50} values for use-dependent block of 4b and 5b were about 61- and 114-fold lower, respectively (Table 1), than tocainide. In particular, a very high increase in potency for use-dependent block was observed with the 5b analogue, with a ratio (IC\textsubscript{50} tonic block/IC\textsubscript{50} use-dependent block at 10 Hz = 31.6:2.4) of 13. In summary, the synthesis of constrained analogues of tocainide has been proposed. The synthetic routes herein described, though not innovative and with not very high overall yields, gave access to 4b and 5b which present themselves as valuable pharmacological tools, both being more potent and use-dependent than tocainide. In particular, 5b showed a marked increase in both potency and use-dependence of action; compared to tocainide, it was 18- and 114-fold more potent in tonic and phasic block experiments, respectively. In conclusion, the candidates 4b and 5b will be subjected to a complete pharmacological characterization as antimiotonic agents possibly overcoming tocainide’s adverse side-effects. Further in vivo studies on the compounds 4b and 5b will be performed. Moreover, given that a stereoselective site for sodium channel blockers on adult skeletal muscle fibers has been evidenced [14], we have recently started the synthesis of the optical isomers of 4b and 5b; further studies are in progress to find a correlation between potency, stereoselectivity and conformational characteristics of the compounds presented in this paper.

Table 1

Concentrations for half-maximal tonic and phasic (use-dependent) blocks (IC\textsubscript{50}, \textmu M) of sodium currents, potency ratio (IC\textsubscript{50} tocainide/IC\textsubscript{50} derivatives), clog \textit{P} and p\textit{K}e of tocainide (1a) and its analogues (4b, 5b)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Tonic block \textsuperscript{a} (IC\textsubscript{50})</th>
<th>Phasic block \textsuperscript{b} (IC\textsubscript{50})</th>
<th>clog \textit{P}</th>
<th>p\textit{K}e</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a (Tocainide)</td>
<td>580.7 ± 37.9</td>
<td>273.3 ± 23.3</td>
<td>0.76 ± 0.48</td>
<td></td>
</tr>
<tr>
<td>4b</td>
<td>38.5 ± 1.4</td>
<td>4.5 ± 0.3</td>
<td>3.53 ± 0.34</td>
<td></td>
</tr>
<tr>
<td>5b</td>
<td>31.6 ± 0.8</td>
<td>2.4 ± 0.4</td>
<td>2.81 ± 0.46</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a} Tonic block: block of sodium channel at resting conditions, evaluated during infrequent depolarizing pulses.

\textsuperscript{b} Phasic block: cumulative sodium current reduction by the drug at 10 Hz stimulation frequency, obtained by concentration–response curves.

\textsuperscript{c} Calculated using Advanced Chemistry Development (ACD) Software Solaris V4.76.
5. Experimental protocols

5.1. Chemistry

Yields refer to purified products and were not optimized. All chemicals were purchased from Sigma—Aldrich or Lancaster in the highest quality commercially available. The structures of the compounds were confirmed by routine spectrometric analyses. For compounds not previously described, complete spectroscopic characterization is given; for known compounds only spectra not described in the literature are given. Melting points were determined on a Gallenkamp melting point apparatus.1H 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 as solvent. Chemical shifts are given in parts per million (ppm) relative to solvent resonance: δ 7.26 (1H NMR) and δ 77.3 (13C NMR). Absolute values of J are given in hertz. EIMS spectra were recorded on a Hewlett-Packard 6890-5973 MSD gas chromatograph/mass spectrometer at low resolution. Elemental analyses were performed with a Eurovector Euro EA 3000 analyzer. Flash chromatography was performed on silica gel (Kieselgel 60 F254, Merck). TLC analyses were performed on precoated silica gel on glass plates (Kieselgel 60 F254, Merck). PLC analyses were performed on precoated silica gel on aluminum sheets (Kieselgel 60 F254, Merck). Other spectroscopic data were in agreement with the literature [22].

5.1.1. 1-(tert-Butyloxycarbonyl)piperidine-3-carboxylic acid (8)

Yield 75%; white solid, mp: 160–162 °C, lit [22] 149–151 °C (abs EtOH/H2O); GC–MS (70 eV); m/z (rel. int.): 229 [M+1, 10], 57 (100); 13C NMR: δ = 24.3 (1C), 27.4 (1C), 28.6 (3C), 41.3 (1C), 44.1 (1C), 45.7 (1C), 80.2 (1C), 155.0 (1C), 179.1 ppm (1C). Other spectroscopic data were in agreement with the literature.[22]

5.1.2. 1,4-Bis(tert-butyloxycarbonyl)piperazine-2-carboxylic acid (9)

Yield 75%; white solid, mp: 140–150 °C, lit [23] 143.0–144.5 °C for the (S)-enantiomer; 13C NMR: δ = 28.4 (6C), 40.3 (1C), 41.6 (1C), 53.6 (1C), 54.9 (1C), 80.9 (1C), 81.3 (1C), 154.8 (1C), 155.4 (1C), 174.3 ppm (1C). Other spectroscopic data were in agreement with those reported for the (S)-enantiomer [23].

5.1.3. tert-Butyl 3-[(2,6-dimethylphenyl)amino]carbonyl-piperidine-1-carboxylate (4e)

IIDQ (2.46 mL, 8.3 mmol), 2,6-dimethylaniline (0.93 mL, 7.6 mmol) and Et3N (1.4 mL, 10.4 mmol) were successively added to a stirring solution of 8 (1.60 g, 6.9 mmol) in CHCl3 (220 mL). The reaction mixture was heated under reflux for 6 h. The solvent was removed under reduced pressure and the residue, taken up with EtOAc, was washed three times with 2 N HCl, twice with 2 N NaOH, and then dried over anhydrous Na2SO4. Flash chromatography (eluent EtOAc/petroleum ether 2:8) of the residue gave 0.39 g (17% yield) of 4e as a white solid, mp: 59–61 °C; FT-IR (KBr): 3421, 2988, 2944, 2860, 1675, 1488, 1467, 1368, 1300, 1270, 1149, 957, 989, 858 cm−1; 1H NMR: δ = 1.45 (s, 9H, partially overlapped to triplet at 1.42–1.60 ppm, t-Bu), 1.42–1.60 (m, 1H, partially overlapped to singlet at 1.45 ppm), 1.64–1.75 (m, 1H), 1.84–1.90 (m, 1H), 2.18 (s, 6H, CH2–Ar), 2.40–2.60 (m, 1H), 2.95–3.15 (m, 1H), 3.25–3.40 (m, 1H), 3.65–3.85 (m, 1H), 3.95–4.10 (m, 2H), 6.95–7.10 (m, 3H, Ar), 7.35 ppm (br s, 1H, exchange with D2O, NH+); 13C NMR: δ = 18.6 (2C), 24.4 (1C), 28.0 (2C), 28.6 (3C), 43.2 (1C), 44.7 (1C), 46.3 (1C), 80.3 (1C), 127.5 (1C), 128.4 (3C), 135.6 (1C), 155.2 (1C), 171.8 ppm (1C); GC–MS (70 eV) m/z (rel. int.): 332 [M]+, 1, 121 (100).

5.1.4. Di-tert-butyl 2-[(2,6-dimethylphenyl)amino]carbonyl)piperazine-1,4-dicarboxylate (5e)

Prepared as reported above for the synthesis of 4e, but starting from 9. Yield 51%; white crystals, mp: 189–190 °C (EtOAc/petroleum ether); FT-IR (KB): 3273, 2979, 2885, 1675, 1478, 1403, 1366, 1250, 1169, 1041, 974, 868 cm−1; 1H NMR: δ = 1.44 (s, 9H, t-Bu), 1.50 (s, 9H, t-Bu), 2.19 (s, 6H, CH2–Ar), 2.95–3.40 (m, 3H), 3.80–4.10 (m, 2H), 4.50–4.85 (m, 2H), 6.95–7.15 (m, 3H, Ar), 7.39 ppm (br s, 1H, NH+); 13C NMR: δ = 18.7 (2C), 28.5 (6C), 43.3 (2C), 60.6 (2C), 80.6 (1C), 81.9 (1C), 127.7 (2C), 128.5 (3C), 135.5 (1C), 154.8 (2C), 168.0 ppm (1C); GC–MS (70 eV) m/z (rel. int.): 333 [M]+ – 100, 1, 85 (100).

5.1.5. N-(2,6-Dimethylphenyl)piperidine-3-carboxamide (4a)

To a solution of 4c (0.40 g, 1.2 mmol) in EtOAc (9.5 mL), 3 N HCl (3.1 mL) was added. The reaction mixture was stirred at room temperature for 3 h. The solvent was removed under reduced pressure to give a white solid (0.41 g) which was recrystallized from EtOH/Et2O to afford 0.21 g (65% yield) of the desired amine hydrochloride (4a HCl): mp: 199–201 °C (abs EtOH/Et2O), lit [18] mp: 196–210 °C (abs EtOH/Et2O). Calcd for C14H13NO2·HCl: C 61.87, H 5.91, N 10.31; found C 62.14, H 7.96, N 10.34. Compound 4a as free amine was recovered by extraction of the corresponding hydrochloride: FT-IR (CHCl3): 3421, 2988, 2944, 2860, 1675, 1488, 1467, 1368, 1300, 1270, 1149, 957, 989, 858 cm−1; 1H NMR: δ = 1.51–1.62 (m, 1H, NH+), 1.72–2.04 (m, 3H), 2.19 (s, 6H, CH2–Ar), 2.46–3.04 (m, 5H), 3.10–3.24 (m, 1H), 7.03 (s, 3H, Ar), 9.53 ppm (br s, 1H, NH–CO); 13C NMR: δ = 18.8 (2C), 23.4 (1C), 27.5 (1C), 42.5 (1C), 46.6 (1C), 48.4 (1C), 127.4 (1C), 127.8 (2C), 128.6 (2C), 135.2 (1C), 173.7 ppm (1C); GC–MS (70 eV) m/z (rel. int.): 232 [M]+, 20, 84 (100).

5.1.6. N-(2,6-Dimethylphenyl)piperazine-2-carboxamide (5a)

To a solution of 5c (0.90 g, 2.1 mmol) in EtOAc (12 mL), 48% HBr (48 mL) was added. The reaction mixture was stirred at room temperature for 3 h. The solvent was...
evaporated under reduced pressure and the aqueous phase was washed with EtOAc, made alkaline with 2 N NaOH and extracted with EtOAc. The organic phase was dried over anhydrous Na2SO4. The solvent was removed under pressure to give 5a (0.22 g, 45% yield) as a white solid: mp: 172–174 °C, lit [19] 171–172 °C (CHCl3/C6H6); FT-IR (KBr): 3251, 3201, 2932, 2850, 2805, 1657, 1594, 1531, 1477, 1224, 1118, 897, 769, 759 cm⁻¹. ¹H NMR: δ = 1.95 (s, 2H, exchange with D2O, CH3-7), 2.74–3.14 (m, 5H), 3.28 (dd, J = 3.1 and 12.1 Hz, 1H), 3.55 (dd, J = 3.1 and 8.0 Hz, 1H), 7.07 (s, 3H, Ar), 7.16 ppm (s, 3H, Ar), 7.58 ppm (s, 1H: exchange with D2O, N pyridine protons), 7.02 (s, 3H, Ar), 7.16 ppm (s, 3H, Ar), 7.58 ppm (s, 1H: exchange with D2O, N pyridine protons), 2.88–3.02 (m, 2H), 3.04–3.16 (m, 1H), 3.54 (2d, J = 13.0 Hz, 2H, benzylic protons), 3.65 (dd, J = 7.1 and 3.6 Hz, 1H), 7.02–7.10 (m, 3H, Ar), 7.22–7.34 (m, 5H, Ar), 8.55–8.64 ppm (br s, 1H: exchange with D2O, NH–CO); ¹³C NMR: δ = 18.9 (2C), 44.2 (1C), 53.6 (1C), 56.2 (1C), 58.9 (1C), 63.5 (1C), 127.3 (2C), 127.5 (2C), 128.4 (2C), 128.6 (2C), 129.4 (2C), 135.3 (1C), 137.7 (1C), 170.8 ppm (1C); GC–MS (70 eV) m/z (rel. int.): 233 [M⁺, 1], 85 (100). Compound 5a was purified by recrystallization of the corresponding hydrochloride salt, obtained by adding to the free amine a few drops ofaq, HCl and then, removing the water azeotropically (toluene/abs EtOH); mp >250 °C (abs EtOH/ Et2O). Calcd for C20H25N3O+HCl 250.1; 1HN M R : rel. int.: 322 [M⁺, 1], 85 (100). Compound 5b was obtained as reported above for the preparation of 4b, but starting from 5a. Compound 5b was obtained as a yellow oil containing also the dibenzylderivative of 5a, which were separated by preparative chromatography (PLC) using silica gel on glass plates (elucent EtOAc/petroleum ether 1:1) to give 5b in 21% yield as a slightly yellowish oil: FT-IR (neat): 3356, 3201, 3066, 3031, 2983, 2942, 2822, 1676, 1601, 1497, 1445, 1373, 1304, 1248, 1145, 1047, 1028, 846, 792 cm⁻¹. ¹H NMR: δ = 1.93 (br s, 1H: exchange with D2O, NH–CO); 13C NMR: δ = 18.9 (2C), 44.2 (1C), 53.6 (1C), 56.2 (1C), 58.9 (1C), 63.5 (1C), 127.3 (2C), 127.5 (2C), 128.4 (2C), 128.6 (2C), 129.4 (2C), 135.3 (1C), 137.7 (1C), 170.8 ppm (1C); GC–MS (70 eV) m/z (rel. int.): 223 [M⁺ – 100], 1, 91 (100). Compound 5b-HCl was obtained as above for 4b-HCl in 17% yield: mp: 241–243 °C (abs EtOH/ Et2O). Calcd for C20H25N3O-HCl·1.67H2O % C 61.61, H 7.58, N 10.78; found C 61.60, H 7.55, N 10.79.

5.2. Pharmacology

5.2.1. Recording of Na⁺ current and pulse protocols

The actions of the two anilide derivatives of tocainide 4b and 5b were tested in vitro on sodium currents (INa) of single fibers of frog *serritenninosus* muscle by vaseline-gap voltage clamp method, as described in detail elsewhere [24]. The tonic block (TB) exerted by each compound was calculated as percentage reduction of the maximal peak sodium transient (INa,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 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.

5.2.2. 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 INa,max (IC50) were determined by using a non-linear least-squares fit of the concentration–response curves to the following logistic equation:

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

de where effect = percentage change of INa, −100 = maximal percentage block of INa, K = IC50 of tested compound, n = logistic slope factor, and [drug] = molar concentration of the tested compound [24].
Acknowledgment

This work was carried out under the framework of the National Projects “Progettazione, Sintesi e Valutazione Biologica di Nuovi Farmaci Cardiovascolari” supported by the Ministero dell’Università e della Ricerca (MiUR, Rome) shared also with the University of Bari.

References