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PILSICAINIDE AND ITS OXYMETHYLENE ANALOG: FACILE ALTERNATIVE SYNTHESES AND *IN VITRO* TESTING ON HUMAN SKELETAL MUSCLE SODIUM CHANNELS

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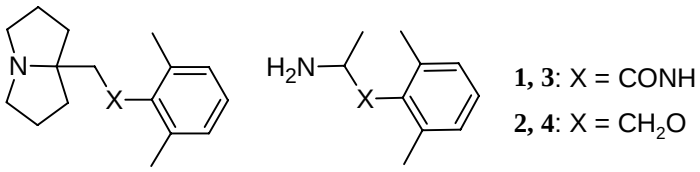
Abstract – Facile, alternative synthetic routes gave access to both pilsicainide [*N*-(2,6-dimethylphenyl)-2-tetrahydro-1*H*-pyrrolizin-7a(5*H*)-ylacetamide, **1**], a well-known I_C antiarrhythmic drug, and its oxymethylene analog **2**. Both compounds were tested on human skeletal muscle voltage-gated sodium channels, hNav1.4, transfected in tsA201 cells. 7a-[2-(2,6-Dimethylphenoxy)ethyl]hexahydro-1*H*-pyrrolizine (**2**) behaved as a bioisostere of **1**, exerting a 4-fold more potent use-dependent block.

INTRODUCTION

Pilsicainide [*N*-(2,6-dimethylphenyl)-2-tetrahydro-1*H*-pyrrolizin-7a(5*H*)-ylacetamide] (**1**, Figure 1) is a synthetic drug available in Japan endowed with class I_C antiarrhythmic properties[1] related to the inhibition of cardiac muscle sodium channels. Like other I_C antiarrhythmic drugs, pilsicainide may cause serious side effects such as ventricular fibrillation and tachycardia.[2,3] Ozeki at al.[4] reported the first case of pilsicainide intoxication associated with dehydration. The authors speculated the reason was pilsicainide rapid absorption from the gastrointestinal tract and its resistance to metabolic transformations which cause accumulation in patients with impaired renal function induced by

dehydration. Severe side effects occurred also when pilsicainide was administered to elderly patients affected by renal disfunctions.[5,6]

Figure 1



1, 2 **3, 4**

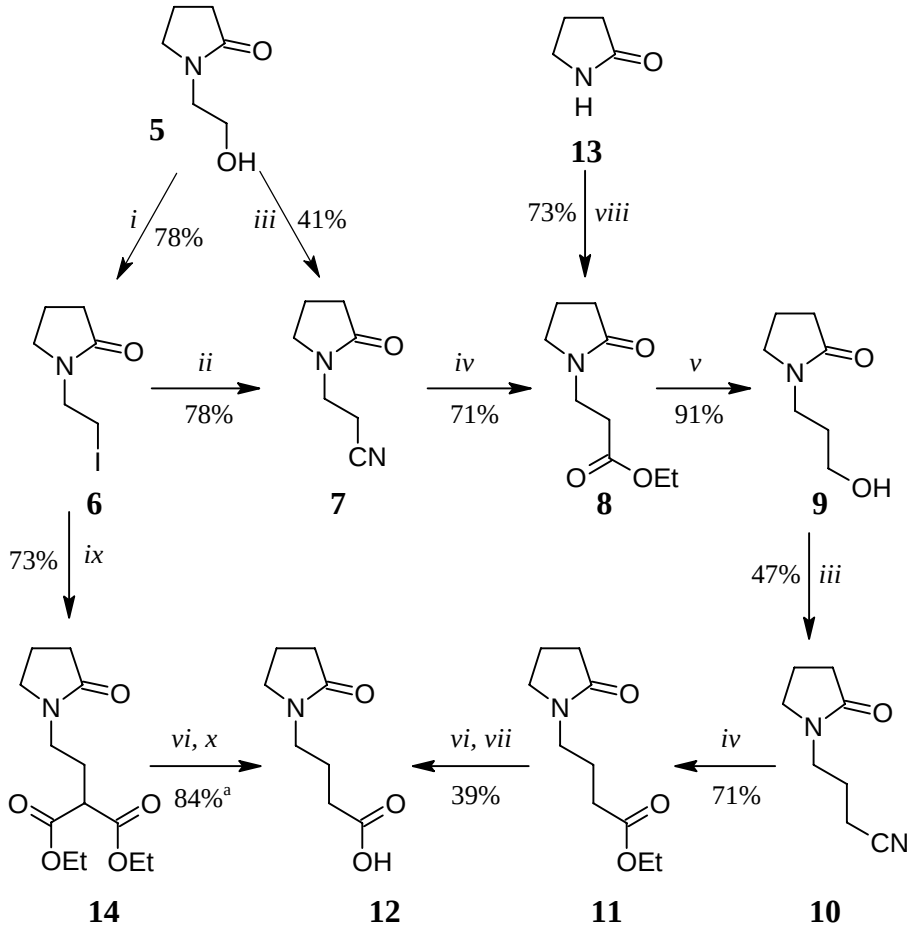
Thus, close blood level monitoring and dosage adjustment are required whenever pilsicainide is administered to patients with impaired renal function due to ageing or dehydration.[7] These findings prompted us to look for a pilsicainide-like drug whose liver metabolization might be favoured resulting in a safer use in the above reported clinical cases. In the domain of I_B antiarrhythmic drugs, a well-known bioisosteric relationship holds between tocainide (**3**) and its oxymethylene analog mexiletine (**4**), the latter being endowed with a more favorable toxicological profile.[8] Furthermore, mexiletine is subjected to extensive metabolization.[9] Thus, we decided to investigate the pilsicainide oxymethylene analog (**2**) as a possible bioisostere of **1**. To verify our design, we needed (a) a facile access to **2** and (b) clues in favor of its sodium channel blocking activity. Herein we present how both goals were pursued: facile, alternative synthetic routes to **1** and **2** were developed and **2** was proved to be at least four times more potent than its prototype **1** when the blocking activity on human skeletal muscle voltage-gated sodium channels, hNav1.4, transfected in tsA201 cells was tested.

RESULTS AND DISCUSSION

The key intermediate for the synthesis of both compounds **1** and **2** is 4-(2-oxopyrrolidin-1-yl)butanoic acid (**12**, Scheme 1). It was obtained following different synthetic routes, all of which use freely purchasable compounds as starting materials, in this resulting more convenient than those previously reported, both starting from γ-butyrolactone,[10,11] which is a regulated chemical. Both literature

procedures has the advantage to give **12** in a single step. However the first one, consisting in the reaction of γ-butyrolactone with potassium cyanate,[10] in our hands gave **12** only in low yield (20%), while the second one, using the sodium salt of 2-pyrrolidone as a reactant, is tainted by the hazard of explosions.[12]

Scheme 1



Reagents and conditions: (i) I_2, 1,2-bis(diphenylphosphino)ethane, anhyd toluene, 130 °C; (ii) NaCN, anhyd DMF, 50 °C; (iii) $Me_2C(OH)CN$, DEAD, Ph_3P, anhyd Et_2O, –20 °C; (iv) Me_3SiCl, abs EtOH, 50 °C; (v) $LiAlH_4$, anhyd THF, rt; (vi) KOH, EtOH, 60 °C; (vii) 2 N HCl; (viii) CH_2CHCO_2Et, CsF, $Si(OEt)_4$, rt; (ix) $CH_2(CO_2Et)_2$, EtONa, anhyd THF, 60 °C; (x) 6 N HCl, 60 °C, then 220 °C.
[a]Yields refer to crystallized product.

The first entry starts with the replacement of the hydroxy group of 1-(2-hydroxyethyl)pyrrolidin-2-one (**5**) by iodine which was easily performed modifying a literature procedure:[13] the use of 1,2-bis(diphenylphosphino)ethane in lieu of triphenylphosphine allowed the easy isolation of **6**. In fact, the bis(phosphine oxide) by-product formed in the reaction, being more polar than triphenylphosphine oxide, may be precipitated treating the crude product with EtOAc and then removed by filtration.[14] After concentration, flash chromatography on the filtrate gave pure **6**. The cyanoderivative **7**, which is now

commercially available, was obtained via two alternative routes, starting from **5**[15] and **6**[16] respectively, and was fully characterized. The transformation of nitrile **7** into the corresponding ethyl ester **8** was obtained following a literature procedure[17] and reduction of **8** with $LiAlH_4$ gave alcohol **9**. Nitrile **10** and ethyl ester **11** were obtained starting from **9** via the same reactions that had led to the inferior homologs **7** and **8**. Alkaline hydrolysis of the ethyl ester **11**, followed by treatment with acid, gave the key intermediate **12**. This route may be more conveniently entered submitting γ-butyrolactame **13** to conjugate addition to ethyl acrylate, extending a modified Michael addition procedure previously reported for **13** superior cyclohomologs and acetamides.[18]

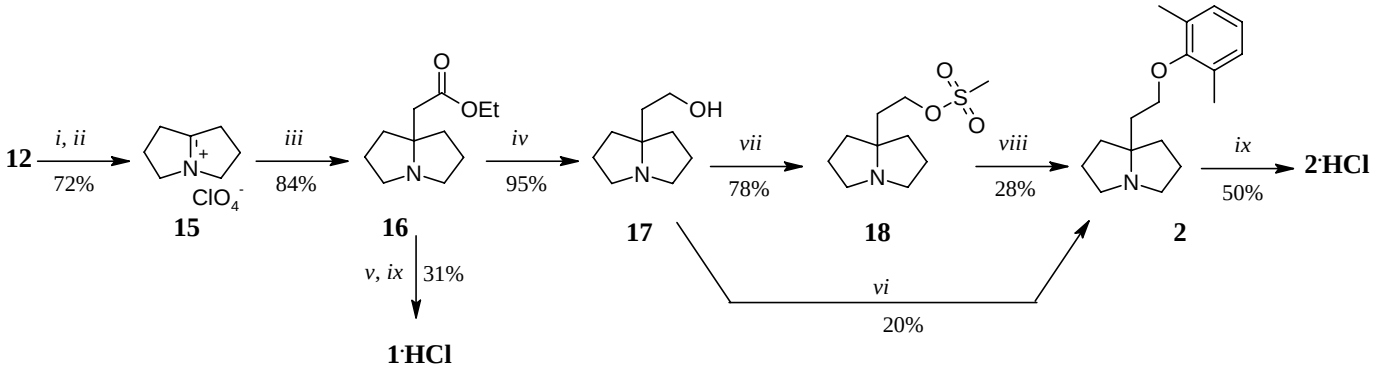
The intermediate **12** can be more directly accessed by submitting to hydrolysis and decarboxylation the diester **14**, which was, in turn, obtained by submitting the iodide **6** to malonic ester synthesis.

The acid **12** represents the key intermediate for the synthesis of **2** (Scheme 2). Pyrrolizine derivatives **15**, **16**, and **1** (pilsicainide) were prepared as previously described.[19,20] The free amine **1** was converted into its hydrochloride salt (**1**·**HCl**) by treatment with 2 N HCl. Perhydropyrrolizineethanol **17** was obtained by reduction of **16** following the procedure reported in the literature for the corresponding methyl ester.[21] 7a-[2-(2,6-Dimethylphenoxy)ethyl]hexahydro-1*H*-pyrrolizine (**2**), the oxymethylene analog of **1**, was obtained by condensating the alcohol **17** with 2,6-dimethylphenol under Mitsunobu conditions.[22] As an alternative, **2** was obtained by a Williamson-type etherification of **18**, the mesyl derivative of **17**, with 2,6-dimethylphenol.[23] The free amine **2** was converted into its hydrochloride salt (**2**·**HCl**) by treatment with 2 N HCl.

The activity of the hydrochloride salts of **1** and **2** (**1**·**HCl** and **2**·**HCl**, respectively) was tested in vitro on voltage-gated sodium currents recorded in tsA201 cells transfected with the human skeletal muscle sodium channel, hNav1.4, using the whole-cell patch-clamp method. Sodium currents were elicited by

depolarizing the cell from the holding potential of −120 mV to −30 mV at two stimulation frequency, 0.1 Hz for determination of tonic block and 10 Hz for use-dependent block determination.

Scheme 2



Reagents and conditions: (i) *soda lime*, 170 °C; (ii) $HClO_4$, EtOH; (iii) $EtOCOCH_2CO_2K$, abs EtOH, reflux; (iv) $LiAlH_4$, anhyd THF, rt; (v) 2,6-dimethylaniline, NaH, anhyd dioxane, 100 °C; (vi) 2,6-dimethylphenol, PPh_3, DIAD, anhyd THF, rt; (vii) $MeSO_2Cl$, Et_3N, anhyd CH_2Cl_2, 0 °C; (viii) 2,6-dimethylphenol, EtONa, anhyd THF/anhyd CH_2Cl_2, 60 °C; (ix) HCl.

The IC_{50} values (Table 1) were calculated by fitting the concentration/effect relationships with a first-order binding function, and are reported with the S.E. of the fit.[24]

Table 1. In vitro blocking activity of the hydrochloride salts of pilsicainide (**1·HCl**) and its oxymethylene analog **2·HCl** on voltage-gated sodium currents recorded in tsA201 cells transfected with the human skeletal muscle sodium channel, hNav1.4 (see experimental section for details).

Compound	IC_{50} at 0.1 Hz (μM)	IC_{50} at 10 Hz (μM)
Pilsicainide hydrochloride (**1·HCl**)	189 ± 10	76 ± 7
2·HCl	76 ± 6	18 ± 2

CONCLUSIONS

Alternative entries to **12**, a key intermediate in the synthesis of pilsicainide (**1**), have been proposed. They all start from commercially available compounds which are not included in the regulated substance lists and avoid erratic[10] or hazardous[11,12] reactions. The same routes gave access to the oxymethylene analog of **1** — 7a-[2-(2,6-dimethylphenoxy)ethyl]hexahydro-1*H*-pyrrolizine (**2**). The route that passes

through the malonic synthesis of **14** was the most efficient in our hands and is to be preferred when considering also the hazard accompanying the reaction between γ-butyrolactone and the sodium salt of **13**.[11,12] Finally, the route starting from either alcohol **5** or lactame **13**, passes through several pyrrolidinone intermediates which are possibly useful for the preparation of cognition enhancers, smart drugs generally referred to as "racetams".[25,26]

When tested in vitro on voltage-gated sodium currents recorded in tsA201 cells transfected with the human skeletal muscle sodium channel, hNav1.4, using the whole-cell patch-clamp method, both **1**·**HCl** and **2**·**HCl** displayed both tonic and phasic blocking activities. However, **2**·**HCl** was more potent than its prototype **1**·**HCl** in both protocols used. In particular, it was four times more potent than **1**·**HCl** under use-dependent block conditions. On the basis of these preliminar results, **2** may be considered as a bioisostere of pilsicainide. Further pharmacological investigations will explore its therapeutical potential usefulness.

EXPERIMENTAL

General. 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 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 NMR and ^{13}C NMR spectra were recorded on a Bruker 300-MHz spectrometer (ASPECT 3000), 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 solvent resonance: $CDCl_3$, δ 7.26 (^{1}H NMR) and δ 77.3 (^{13}C NMR); DMSO-d_6, δ 2.50 (^{1}H NMR); CD_3OD, δ 3.30 (^{1}H NMR) and δ 47.8 (^{13}C NMR). *J* values are given in Hz. 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. GC was performed on a Varian 3800 gas chromatograph equipped with a flame ionization detector and a Jew Scientific DB-5 capillary column (30 m, 0.25 mm i. d., 0.25 μm film thickness). 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.[27] TLC analyses were performed on precoated silica gel on aluminium sheets (Kieselgel 60 F_{254}, Merck).

In vitro testing. In vitro testing was performed as previously described.[24] Briefly, the tsA201 cells, a subclone of HEK293 cells, were transiently transfected with the full-length hNav1.4 cDNA, encoding a human skeletal muscle sodium channel, using the calcium-phosphate precipitation method. The cells were co-transfected with a lower amount of cDNA encoding the plasma membrane receptor CD8. For patch-clamp recordings, 36–72 h after transfection, successfully transfected cells were recognized using Dynal microbeads coated with anti-CD8 antibody (Dynal A.S., Oslo, Norway).

Whole-cell sodium currents were recorded at rt (20–22 °C) using an Axopatch 1D amplifier (Axon Instruments, Union City, CA, USA). Voltage-clamp protocols and data acquisition were performed with pClamp 6.0 software (Axon Instruments) through a 12-bit A-D/D-A Digidata 1200 interface (axon Instruments). The external solution contained (mM): 150 NaCl, 4 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 5 Hepes and 5 glucose; the pH was set to 7.4 with NaOH. The pipette solution contained (mM): 120 CsF, 10 CsCl, 10 NaCl, 5 EGTA, and 5 Hepes; The pH was set to 7.2 with CsOH. Currents were low-pass filtered at 2 kHz (–3dB) by the four pole Bessel filter of the amplifier and digitized at 10–20 kHz.

After rupturing the patch membrane, sodium currents were elicited by a 25 ms-long test pulse to –30 mV from a holding potential of –120 mV applied at 0.1 Hz frequency, until stabilization of current amplitude was achieved (typically 5 min). Then the drug was applied at the desired concentration around the cell through a plastic capillary, and effects of drug on sodium currents was measured first at 0.1 Hz

stimulation frequency then at 10 Hz. Blocking effects were fully reversed upon drug wash-out. Little or no run-down was observed during the experiments.

Analysis was performed off-line. The mean ratio I_{DRUG}/I_{CTRL} calculated from at least 3 cells was reported as a function of drug concentration and the relationship was fitted by a first-order binding function, $I_{DRUG}/I_{CTRL} = 1/(1 + ([DRUG]/IC_{50})$, allowing the calculation of half-maximum inhibitory concentration values.

1-(2-Iodoethyl)pyrrolidin-2-one (6). This compound had been previously obtained by means of a Finkelstein reaction on the corresponding chloride but attempts to isolate it by distillation had given samples analyzed only closely to the theoretical composition at the best (bp 125–127.5 °C, 0.5 mmHg).[28] To a stirred solution of compound **5** (2.00 g, 15.5 mmol) in dry toluene (40 mL) under N_2 atmosphere, 4.00 g (10.0 mmol) of 1,2-bis(diphenylphosphino) ethane were added. The reaction mixture was brought to 130 °C and then 5.12 g (20.2 mmol) of iodine were added portionwise. The mixture was refluxed for 1 h, then absolute EtOH (1 mL) was added in two portions at ca. 30 min intervals. After evaporation of the solvent, the residue was taken up with $CHCl_3$ and washed twice with saturated $Na_2S_2O_3$ and then twice with H_2O. The organic layer was dried (Na_2SO_4) and concentrated under vacuum, then EtOAc was added, and the precipitate formed was filtered off. The filtrate was evaporated and the residue was purified by flash chromatography (EtOAc) to give 2.90 g of **6** as a colorless oil (78%): bp 160 °C, 1 mmHg; IR (neat): 1685 (C=O) cm^{-1}; ^{1}H NMR (300 MHz): δ 2.08 (apparent quintet, *J* = 7.6 Hz; t upon irradiation at 2.45, *J* = 6.9 Hz; t upon irradiation at 3.49, *J* = 8.1 Hz, 2H, $CH_2CH_2CH_2$), 2.45 (t, *J* = 8.1 Hz; s upon irradiation at 2.08, 2H, CH_2CO), 3.25 (t, *J* = 7.1 Hz; s upon irradiation at 3.67, 2H, CH_2I), 3.50 (t, *J* = 7.0 Hz; s upon irradiation at 2.08, 2H, $CH_2CH_2CH_2N$), 3.67 (t, *J* = 7.0 Hz; s upon irradiation at 3.25, 2H, CH_2CH_2I); ^{13}C NMR (75 MHz): δ 1.02 (1C), 18.3 (1C), 31.0 (1C), 45.3 (1C), 47.7 (1C), 175.5 (1C); MS (70 eV) *m/z* (%) 239 (M^+, 7), 112 (100).

3-(2-Oxopyrrolidin-1-yl)propanenitrile (7). Method A. To a stirred solution of triphenylphosphine (3.04 g, 11.6 mmol) in anhydrous Et_2O (15 mL) at –20 °C, under nitrogen atmosphere, a solution of diethyl azodicarboxylate (1.83 mL, 11.6 mmol) in anhydrous Et_2O (15 mL) was added dropwise. The reaction was stirred for 20 min under cooling, then a solution of **5** (3 g, 23.2 mmol) in 10 mL of anhydrous Et_2O and anhydrous THF (1:1) was added dropwise. After stirring for 30 min at the same temperature, a solution of acetone cyanohydrin (1.06 mL, 11.6 mmol) in anhydrous Et_2O (20 mL) was added and the mixture was stirred overnight at rt, then the precipitate formed was filtered off. The filtrate was evaporated and the residue was purified by flash chromatography (EtOAc) to give 0.44 g of **7** as a pale yellow oil (41%). **Method B.** To a stirred solution of **6** (5.70 g, 23.8 mmol) in anhydrous DMF (50 mL), NaCN (2.34 g, 47.7 mmol) was added. The reaction was heated to 50 °C and stirred for 5 h. The solvent was then evaporated under vacuum, taken up with CH_2Cl_2 and then washed with water. The organic layer was dried (Na_2SO_4) and concentrated under vacuum. Purification of the crude oil residue by flash chromatography (EtOAc) gave 2.60 g of **7** as a pale yellow oil (78%): IR (neat): 2249 (C≡N), 1681 (C=O) cm^{-1}; ^{1}H NMR (300 MHz): δ 2.06 (apparent quintet, J = 7.6 Hz, 2H, $CH_2CH_2CH_2$), 2.38 (t, J = 8.0 Hz, 2H, CH_2CO), 2.60 (t, J = 6.5 Hz, 2H, CH_2CN), 3.54 (apparent t, J = 7.0 Hz, 4H, CH_2NCH_2); ^{13}C NMR (75 MHz): δ 16.7 (1C), 18.3 (1C), 30.8 (1C), 39.1 (1C), 48.1 (1C), 118.3 (1C), 175.7 (1C); MS (70 eV) *m/z* (%) 138 (M^+, 41), 98 (100).

Ethyl 3-(2-oxopyrrolidin-1-yl)propanoate (8). Method A: To a stirred solution of **7** (1.46 g, 10.6 mmol) in absolute EtOH (4.10 mL, 70.4 mmol), under nitrogen atmosphere, chlorotrimethylsilane (4.10 mL, 32.3 mmol) was added. The reaction mixture was heated at 50 °C for 16 h. After being cooled to rt, water (0.40 mL, 23.3 mmol) was added to the mixture and followed by the addition of Na_2CO_3 (1.12 g, 10.6 mmol) and CH_2Cl_2 (15 mL). The mixture was dried (Na_2SO_4) and evaporated. Purification of the crude oil residue by flash chromatography (EtOAc) gave 1.40 g of **8** as a slightly yellowish oil (71%). **Method B:** To a suspension of CsF (0.18 g, 1.17 mmol) in **13** (0.89 mL, 11.7 mmol), tetraethyl

orthosilicate (2.61 mL, 11.7 mmol) was added, then ethyl acrylate (1.40 mL, 12.9 mmol) was added dropwise. The reaction mixture was stirred for 4h at rt. Purification of the crude oil by flash chromatography (EtOAc/petroleum ether 1:1) gave 1.57 g of **8** as a colorless oil (73%): IR (neat): 1732 (C=O, ester), 1683 (C=O, amide) cm^{-1}; ^1H NMR (300 MHz COSY): δ 1.25 (t, *J* = 7.1 Hz, 3H, C*H*$_3$), 2.00 (apparent quintet, *J* = 7.6 Hz, 2H, $CH_2CH_2CH_2$), 2.35 (t, *J* = 8.1 Hz, 2H, CH_2CON), 2.55 (t, *J* = 6.9 Hz, 2H, CH_2COO), 3.41 (t, *J* = 7.0 Hz, 2H, $CH_2CH_2CH_2N$), 3.57 (t, *J* = 6.9 Hz, 2H, CH_2CH_2COO), 4.13 (q, *J* = 7.1 Hz, 2H, CH_3CH_2); ^{13}C NMR (75 MHz): δ 14.4 (1C), 18.2 (1C), 31.0 (1C), 32.9 (1C), 38.8 (1C), 47.8 (1C), 60.9 (1C), 171.9 (1C), 175.3 (1C); MS (70 eV) *m/z* (%) 185 (M^+, 36), 98 (100).

1-(3-Hydroxypropyl)pyrrolidin-2-one (9). To a stirred solution of **8** (0.20 g, 1.08 mmol) in dry THF (10 mL) $LiAlH_4$ (0.04 g, 1.08 mmol) under N_2 atmosphere was added. The mixture was stirred at rt for 1 h. The reaction was quenched by the careful addition of few drops of water until the end of gas evolution. The residue was removed by filtration and the filtrate was dried over anhydrous Na_2SO_4 and concentrated under vacuum to give 0.14 g (91%) of a colorless oil: full characterization of this compound can be found in the literature[29] and our data are in agreement all but ^1H NMR signals relative to H_2C-4 and H_2C-2'. On the basis of a COSY experiment on 3',3'-D_2-**9** (obtained by reduction of **8** with $LiAlD_4$), m at 1.68–1.74 and t at 2.05 (*J* = 7.7 Hz) are to be assigned to H_2C-2' and H_2C-4, respectively.

4-(2-Oxopyrrolidin-1-yl)butanenitrile (10). Prepared as above described for **7** (Method A) starting from **9**. IR (neat): 2246 (C≡N), 1666 (C=O) cm^{-1}; ^1H NMR (300 MHz COSY; signals unambiguously assigned on the basis of a COSY experiment on 2,2-D_2-**10** obtained from 3',3'-D_2-**9**): δ 1.88 (apparent quintet, *J* = 7.0 Hz, 2H, CH_2CH_2CN), 2.0–2.1 (m, 2H, CH_2CH_2CO), 2.35 (t overlapping t at 2.37, *J* = 7.2 Hz, 2H, CH_2CN), 2.37 (t overlapping t at 2.35, *J* = 8.1 Hz, 2H, CH_2CO), 3.36 (t overlapping t at 3.39, *J* = 6.8 Hz, 2H, $CH_2CH_2CH_2CN$), 3.39 (t overlapping t at 3.36, *J* = 7.0 Hz, 2H, CH_2NCO); ^{13}C NMR (75

MHz): δ 15.1 (1C), 18.1 (1C), 23.7 (1C), 31.0 (1C), 41.8 (1C), 47.6 (1C), 119.4 (1C), 175.9 (1C); MS (70 eV) *m/z* (%) 152 (M^+, 27), 98 (100).

Ethyl 4-(2-oxopyrrolidin-1-yl)butanoate (11). Prepared as above described for **8** starting from **10**. IR (neat): 1734 (C=O, ester), 1685 (C=O, amide) cm^{-1}; ^1H NMR (300 MHz; signals unambiguously assigned on the basis of a COSY experiment on 2,2-D_2-**11** obtained from 2,2-D_2-**10**): δ 1.22 (t, *J* = 7.2 Hz, 3H, C*H*$_3$), 1.82 (apparent quintet, *J* = 7.4 Hz, 2H, C*H*$_2$CH$_2$COO), 1.99 (apparent quintet, *J* = 7.6 Hz, 2H, C*H*$_2$CH$_2$CON), 2.28 (t overlapping t at 2.34, *J* = 7.5 Hz, 2H, C*H*$_2$COO), 2.34 (t overlapping t at 2.28, *J* = 8.1 Hz, 2H, C*H*$_2$CON), 3.28 (t, *J* = 7.2 Hz, 2H, C*H*$_2$CH$_2$CH$_2$COO), 3.36 (t, *J* = 7.0 Hz, 2H, C*H*$_2$NCO), 4.10 (q, *J* = 7.2 Hz, 2H, C*H*$_2$CH$_3$); ^{13}C NMR (75 MHz): δ 14.5 (1C), 18.2 (1C), 22.9 (1C), 31.2 (1C), 31.9 (1C), 42.2 (1C), 47.3 (1C), 60.7 (1C), 173.3 (1C), 175.4 (1C); MS (70 eV) *m/z* (%) 199 (M^+, 35), 112 (100).

4-(2-Oxopyrrolidin-1-yl)butanoic acid (12). Method A: To an alcoholic stirred solution of 15 % KOH (10 mL), compound **11** (0.15 g, 0.75 mmol) and H_2O (0.2 mL) were added. The reaction mixture was heated at 60 °C for 1 h. The solvent was removed under vacuum and the residue taken with 6 N HCl and extracted five times with CH_2Cl_2. The combined organic phases were dried and evaporated to give 0.05 g (39%) of a slightly yellowish solid. **Method B:** To an alcoholic stirred solution of 15 % KOH (30 mL), compound **14** (2.70 g, 9.96 mmol) and H_2O (0.5 mL) were added. The reaction mixture was heated at 60 °C for 1 h and then acidified with 37% HCl. The solvent was removed under vacuum and the residue was heated at 220 °C for 20 min. The crude residue was taken up three times with hot acetone. The combined organic phases were evaporated and the residue was taken up with $CHCl_3$, dried (Na_2SO_4) and evaporated to give a yellow solid which was recrystallized from EtOAc to give 1.43 g (84%) of a slightly yellowish solid: mp 86–87 °C (EtOAc); IR (KBr): 3200–2400 (OH), 1718 (C=O, carboxylic acid), 1644 (C=O, amide) cm^{-1}; ^1H NMR (300 MHz COSY, DMSO-d_6): δ 1.64 (apparent quintet, *J* =

7.2 Hz, 2H, C*H*$_2$CH$_2$COO; 0.03 ppm upfield shifted upon treatment with NaOD), 1.88 (apparent quintet, *J* = 7.4 Hz, 2H, C*H*$_2$CH$_2$CON), 2.15 (t overlapping t at 2.18, *J* = 7.5 Hz, 2H, C*H*$_2$COO; 0.07 ppm upfield shifted upon treatment with NaOD), 2.18 (t overlapping t at 2.15, *J* = 7.5 Hz, 2H, C*H*$_2$CON), 3.14 (t, *J* = 7.1 Hz, 2H, C*H*$_2$CH$_2$CH$_2$COO), 3.28 (t, *J* = 7.1 Hz; 2H, C*H*$_2$NCO), 12.04 (s, 1H, COO*H*); ^{1}H NMR (300 MHz): δ 1.84 (apparent quintet, *J* = 7.2 Hz, 2H, C*H*$_2$CH$_2$COO), 2.01 (apparent quintet, *J* = 7.6 Hz, 2H, C*H*$_2$CH$_2$CON), 2.33 (t, *J* = 7.2 Hz, 2H, C*H*$_2$COO), 2.41 (t, *J* = 8.1 Hz; s upon irradiation at 2.01, 2H, C*H*$_2$CON), 3.32 (t, *J* = 7.1 Hz, 2H, C*H*$_2$CH$_2$CH$_2$COO), 3.40 (t, *J* = 7.1 Hz; s upon irradiation at 2.01, 2H, C*H*$_2$NCO), 10.2 (br s, 1H, COO*H*); ^{13}C NMR (75 MHz): δ 18.0 (1C), 22.7 (1C), 31.1 (1C), 31.6 (1C), 42.2 (1C), 47.6 (1C), 176.3 (1C), 176.8 (1C); MS (70 eV) *m/z* (%) 171 (M^+, 24), 98 (100). Anal. Calcd for $C_8H_{13}NO_3$ (171.19): C, 56.13; H, 7.65; N, 8.18. Found C, 56.01; H, 7.68; N, 8.09.

Diethyl [2-(2-oxopyrrolidin-1-yl)ethyl]malonate (14). To a suspension of NaOEt (0.63 g, 9.26 mmol) in anhydrous THF (5 mL), diethyl malonate (1.4 mL, 9.22 mmol) was added and the mixture was heated at 60 °C. Then, a solution of **6** (2.00 g, 8.37 mmol) in anhydrous THF (10 mL) was added dropwise. After 1.5 h the solvent was removed under vacuum and the residue was taken up with EtOAc, washed with 0.3 N HCl, H_2O and 5% $NaHCO_3$. The organic layer was dried (Na_2SO_4) and concentrated under vacuum. Purification of the residue by flash chromatography (EtOAc) gave 1.64 g (73%) of a colorless oil: IR (neat): 1731 (C=O, ester), 1685 (C=O, amide) cm^{-1}; ^{1}H NMR (300 MHz COSY): δ 1.26 (t, *J* = 7.1 Hz, 6H, CH$_2$C*H*$_3$), 2.00 (apparent quintet, *J* = 7.6 Hz, 2H, CH$_2$C*H*$_2$CH$_2$), 2.11 (apparent q, *J* = 7.2 Hz, 2H, C*H*$_2$CH), 2.35 (t, *J* = 8.1 Hz, 2H, C*H*$_2$CO), 3.25–3.35 (m, 3H, NC*H*$_2$CH$_2$C*H*), 3.38 (t, *J* = 7.2 Hz, 2H, C*H*$_2$NCO), 4.10–4.25 (m, 4H, C*H*$_2$CH$_3$); ^{13}C NMR (75 MHz): δ 14.2 (2C), 18.1 (1C), 26.6 (1C), 31.1 (1C), 40.8 (1C), 47.3 (1C), 49.8 (1C), 61.8 (2C), 169.2 (2C), 175.4 (1C); MS (70 eV) *m/z* (%) 271 (M^+, 4), 112 (100).

2-Tetrahydro-1*H*-pyrrolizin-7a(5*H*)-ylethanol (17). It was prepared by reduction of compound **16** and obtained as a pure, colorless oil after distillation (bp 70 °C, 2 mmHg); yield: 95%; IR (neat): 3361 (OH) cm^{-1}; ^{1}H NMR (300 MHz COSY): δ 1.56–1.69 (m, 4H, CC*H*$_2$CH$_2$O + CC*H*HCH$_2$CH$_2$), 1.72–1.86 (m, 6H, CH$_2$C*H*$_2$CH$_2$ + CCH*H*CH$_2$CH$_2$), 2.50–2.62 (m, 2H, C*H*HN), 2.98–3.10 (m, 2H, CH*H*N), 3.76–3.85 (m, 2H, C*H*$_2$O), 7.41 (br s, 1H, O*H*); ^{13}C NMR (75 MHz): δ 25.0 (2C), 38.5 (2C), 40.1 (1C), 55.3 (2C), 60.6 (1C), 74.2 (1C); MS (70 eV) *m/z* (%) 154 (M^+ – 1, 5), 110 (100).

2-[Tetrahydro-1*H*-pyrrolizin-7a(5*H*)-yl]ethyl methanesulfonate (18). To a stirred solution of **17** (0.30 g, 1.93 mmol) in dry CH_2Cl_2 (10 mL) under N_2 atmosphere at 0 °C, 0.33 mL (2.89 mmol) of Et_3N were added. Then a solution of 0.21 mL (2.13 mmol) of $MeSO_2Cl$ in dry CH_2Cl_2 (8 mL) was added dropwise. The reaction mixture was kept at 0 °C for 3 h, then a saturated aqueous $NaHCO_3$ (10 mL) was added. The aqueous solution was azeotropically evaporated and the white solid obtained was washed four times with hot acetone (15 mL x 4) and filtrated. The combined organic phases were evaporated and the residue was taken up with $CHCl_3$, dried (Na_2SO_4), and concentrated *in vacuo* to give 0.35 g (78%) of the title compound as a yellow oil: ^{1}H NMR (300 MHz COSY): δ 2.08 (ddd, *J* = 13.2, 11.2, 6.3 Hz, 2H, CC*H*HCH$_2$CH$_2$), 2.23 (br ddd, *J* = 13.2, 6.3, 2.2 Hz, 2H, CCH*H*CH$_2$CH$_2$), 2.34 (t, *J* = 9.2 Hz, 2H, CC*H*$_2$CH$_2$O), 2.40–2.60 (m, 2H, CH$_2$C*H*HCH$_2$), 2.68 (s overlapping m at 2.65–2.90, 3H, C*H*$_3$), 2.65–2.90 (m overlapping s at 2.68, 2H, CH$_2$CH*H*CH$_2$), 3.36 (apparent dt, *J* = 11.8, 5.8 Hz, 2H, C*H*HN), 396 (br ddd, *J* = 12.0, 6.4, 2.0 Hz, 2H, CH*H*N), 4.22 (t, *J* = 9.2 Hz, 2H, C*H*$_2$O); ^{13}C NMR (75 MHz): δ 26.0 (1C), 29.7 (2C), 36.7 (2C), 39.8 (1C), 57.4 (1C), 62.0 (2C), 92.9 (1C); MS (70 eV) *m/z* (%) 138 (M^+ – 95, 6), 110 (100).

7a-[2-(2,6-Dimethylphenoxy)ethyl]hexahydro-1*H*-pyrrolizine (2). Method A: To a stirred solution of triphenylphosphine (0.76 g, 2.90 mmol) in dry THF (20 mL) under N_2 atmosphere, a solution of compound **17** (0.30 g, 1.93 mmol) in dry THF (10 mL) was added, and then 2,6-dimethylphenol (0.35 g,

2.90 mmol). A solution of DIAD (0.57 mL, 2.90 mmol) in dry THF (20 mL) was then added dropwise. The mixture was stirred at rt for 24 h. The solvent was then evaporated under reduced pressure, then the residue was taken up with EtOAc and extracted three times with 2 N HCl. The aqueous phases were made alkaline with 6 N NaOH and extracted with $CHCl_3$. The organic layer was dried (Na_2SO_4) and concentrated under vacuum and the residue was purified by flash chromatography using silica gel ($CHCl_3$/MeOH 9:1) to give 0.10 g of a colorless oil (20%). **Method B:** To a solution of NaOEt (0.13 g, 1.91 mmol) in anhydrous THF (5 mL), 2,6-dimethylphenol (0.20 g, 1.64 mmol) was added and the mixture was heated at 60 °C. Then, a solution of **18** (0.35 g, 1.50 mmol) in 10 mL of anhydrous THF and anhydrous CH_2Cl_2 (1:1) was added dropwise. After 1.5 h the solvent was removed under vacuum and the residue was taken up with 2 N HCl and washed with EtOAc. The aqueous phase was made alkaline with 2 N NaOH and extracted with EtOAc to give a mixture of the free amine **2** and the parent alchol **17** (0.155 g, 70:30, 28% yield of the desired product **2** as stated by GC analysis). ^{1}H NMR (300 MHz COSY): δ 1.50–1.65 (m, 2H, CC*H*HCH$_2$CH$_2$), 1.65–1.92 (m, 6H, CCH*H*CH$_2$CH$_2$ + CH$_2$C*H*$_2$CH$_2$), 1.99 (t, *J* = 7.3 Hz, 2H, CC*H*$_2$CH$_2$O), 2.28 (s, 6H, C*H*$_3$), 2.54–2.65 (m, 2H, C*H*HN), 2.95–3.05 (m, 2H, CH*H*N), 3.84 (t, *J* = 7.3 Hz, 2H, C*H*$_2$O), 6.86–6.94 (m, 1H, Ar *H*C-4) 6.95–7.04 (m, 2H, Ar *H*C-3,5); ^{13}C NMR (75 MHz): δ 16.7 (2C), 25.2 (2C), 37.9 (2C), 42.3 (1C), 55.5 (2C), 70.5 (1C), 72.0 (1C), 123.7 (1C), 128.9 (2C), 131.1 (2C), 156.7 (1C); MS (70 eV) *m/z* (%) 259 (M$^+$, 5), 110 (100).

7a-[2-(2,6-Dimethylphenoxy)ethyl]hexahydro-1*H*-pyrrolizine Hydrochloride (2·HCl). It was obtained by treating the free amine with a few drops of 2 N HCl and azeotropically removing water. Yield: 50%; mp 147–149 °C (THF); ^{1}H NMR (300 MHz COSY, CD_3OD): δ 2.05–2.40 (m, 8H, CC*H*$_2$C*H*$_2$CH$_2$), 2.27 (s overlapping m at 2.05–2.40, 6H, C*H*$_3$), 2.34 (t overlapping m at 2.05–2.40, *J* = 6.2 Hz, 2H, CC*H*$_2$CH$_2$O), 3.16–3.28 (m, 2H, C*H*HN), 3.58–3.72 (m, 2H, CH*H*N), 3.96 (t, *J* = 6.1 Hz, 2H, C*H*$_2$O), 6.86–6.96 (m, 1H, Ar *H*C-4), 6.96–7.05 (m, 2H, Ar *H*C-3,5); ^{13}C NMR (75 MHz, CD_3OD): δ 15.4 (2C), 24.0 (2C), 35.9 (2C), 37.4 (1C), 55.0 (2C), 68.0 (1C), 81.5 (1C), 124.2 (1C), 128.8 (2C),

130.5 (2C), 155.7 (1C). Anal. Calcd for $C_{17}H_{25}NO{\cdot}HCl{\cdot}0.33H_2O$ (301.86): C, 67.64; H, 8.90; N, 4.64. Found C, 67.63; H, 8.90; N, 4.91.

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REFERENCES AND NOTES

1. K. Okishige, M. Nishizaki, K. Azegami, M. Igawa, N. Yamawaki, and K. Aonuma, *Am. Heart J.*, 2000, **140**, 437.
2. J. Morganroth, J. L. Anderson, and G. D. Gentzkow, *J. Am. Coll. Cardiol.*, 1986, **8**, 607.
3. M. Kawabata, K. Hirao, T. Horikawa, K. Suzuki, K. Motokawa, F. Suzuki, K. Azegami, and K. Hiejima, *J. Electrocardiol.*, 2001, **34**, 65.
4. S. Ozeki, T. Utsunomiya, S. Matsuo, and K. Yano, *Jpn. Circ. J.*, 1999, **63**, 219.
5. T. Toeda, R. Susa, T. Saigawa, T. Abe, Y. Yamaguchi, K. Fuse, and H. Murooka, *Jpn. Heart J.*, 2000, **41**, 405.
6. G. Hiasa, H. Okayama, K. Nishimura, and T. Sumimoto, *Internal Medicine*, 2005, **44**, 55.
7. T. Kitahashi and I. Furuta, *J. Pharm. Biomed. Anal.*, 2002, **29**, 625.
8. B. Herweg and J. S. Steinberg, *Card. Electrophysiol. Rev.*, 2000, **4**, 255.
9. L. Labbé and J. Turgeon, *Clin. Pharmacokinet.*, 1999, **37**, 361.
10. W. Reppe and co-workers *Justus Liebigs Ann. Chem.*, 1955, **596**, 199.
11. S. Miyano, S. Fujii, O. Yamashita, N. Toraishi, and K. Sumoto, *J. Heterocycl. Chem.*, 1982, **19**, 1465.
12. M. Oka, T. Suzuki, and Y. Baba, E. P. Patent 0703233, 1996 (*Chem. Abstr.*, 1996, **125**, 10610).
13. M. D. Rozwadowska, *Tetrahedron: Asymmetry*, 1993, **4**, 1619.
14. I. A. O'Neil, S. Thompson, C. L. Murray, and S. B. Kalindjian, *Tetrahedron Lett.*, 1998, **39**, 7787.
15. M. C. Aesa, G. Baán, L. Novák, and C. Szántay, *Synth. Commun.*, 1996, **26**, 909.
16. L. Friedman and H. Shechter, *J. Org. Chem.*, 1960, **25**, 877.
17. F.-T. Luo and A. Jeevanandam, *Tetrahedron Lett.*, 1998, **39**, 9455.
18. K. H. Ahn and S. J. Lee, *Tetrahedron Lett.*, 1994, **35**, 1875.
19. S. Miyano, T. Somehara, M. Nakao, and K. Sumoto, *Synthesis*, 1978, 701. CAUTION: warming of solid hexahydropyrrolizinium perchlorate must be avoided because traces of chlorates, always to be

feared when working with perchlorates, may cause explosions.

20. S. Miyano, K. Sumoto, F. Satoh, K. Shima, M. Hayashimatsu, M. Morita, K. Aisaka, and T. Noguchi, *J. Med. Chem.*, 1985, **28**, 714.
21. S. Miyano, O. Yamashita, S. Fujii, T. Somehara, and K. Sumoto, *Heterocycles*, 1981, **16**, 755.
22. O. Mitsunobu, *Synthesis*, 1981, 1.
23. S. Miyano, K. Sumotu, F. Satoh, and M. Hayashimatsu, J. P. Patent 59184178, 1984 (*Chem. Abstr.*, 1985, **102**, 45772).
24. J.-F. Desaphy, A. De Luca, M. P. Didonna, A. L. George Jr., and D. Camerino Conte, *J. Physiol.*, 2004, **554**, 321.
25. D. E. Butler, I. C. Nordin, Y. J. L'Italien, L. Zweisler, P. H. Poschel, and J. G. Marriott, *J. Med. Chem.*, 1984, **27**, 684.
26. A. H. Gouliaev, J. B. Mønster, M. Vedsø, and A. Senning, *Org. Prep. Proced. Int.*, 1995, **27**, 273.
27. W. C. Still, M. Kahn, and A. Mitra, *J. Org. Chem.*, 1978, **43**, 2923.
28. B. Puetzer, L. Katz, and L. Horwitz, *J. Am. Chem. Soc.*, 1952, **74**, 4959.
29. D. J. Bergmann, E. M. Campi, W. R. Jackson, and A. F. Patti, *Aust. J. Chem.*, 1999, **52**, 1131.