Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic Chemistry 34 (2006) 90–98
BIOORGANIC CHEMISTRY
www.elsevier.com/locate/bioorg

3-Amidoquinuclidine derivatives: Synthesis of compounds and inhibition of butyrylcholinesterase

Renata Odžak *, Srđanka Tomić

Laboratory of Organic Chemistry, Department of Chemistry, Faculty of Science, University of Zagreb, Horvatovac 102A, HR-10 000 Zagreb, Croatia

Received 27 October 2005

Abstract

The synthesis of racemic and enantiomerically pure 3-butanamidoquinuclidines ((±)-Bu, (*R*)-Bu and (*S*)-Bu), (**1**–**3**) and 3-benzamidoquinuclidines ((±)-Bz, (*R*)-Bz, and (*S*)-Bz), (**4**–**6**) is described. The *N*-quaternary derivatives, *N*-benzyl-3-butanamidoquinuclidinium bromides ((±)-BnlBu, (*R*)-BnlBu and (*S*)-BnlBu), (**7**–**9**) and *N*-benzyl-3-benzamidoquinuclidinium bromides ((±)-BnlBz, (*R*)-BnlBz and (*S*)-BnlBz), (**10**–**12**) were subsequently synthesized. The interaction of the four enantiomerically pure quaternary derivatives with horse serum butyrylcholinesterase (BChE) was tested. All tested compounds inhibited the enzyme. The best inhibitior of the enzyme was (*S*)-BnlBz with a $K_i = 3.7$ μM. The inhibitor potency decreases in order (*S*)-BnlBz > (*R*)-BnlBz ≫ (*R*)-BnlBu > (*S*)-BnlBu.

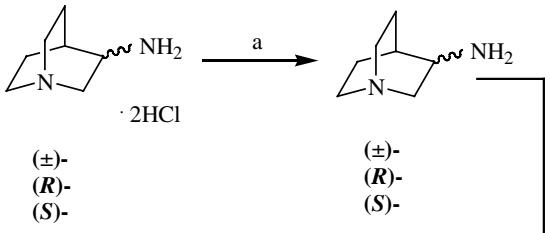
Keywords: 3-Amidoquinuclidines; Quaternary 3-amidoquinuclidines; Synthesis; Butyrylcholinesterase inhibition

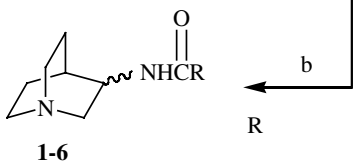
1. Introduction

Quinuclidine (1-azabicyclo[2.2.2]octane) is a constituent of a large number of natural compounds such as the Cinchona, Iboga, Voacanga, Ajmaline and Sarpagine alkaloids

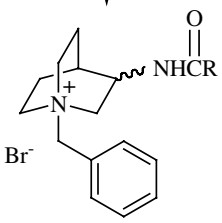
* Corresponding author. Fax: +385 1 4606401.
E-mail address: rodzak@chem.pmf.hr (R. Odžak).

0045-2068/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bioorg.2006.01.004





R	(±)-		(*R*)-		(*S*)-	
-$CH_2CH_2CH_3$	**1**	92%	**2**	92%	**3**	86%
-C_6H_5	**4**	90%	**5**	84%	**6**	93%



R	(±)-		(*R*)-		(*S*)-	
-$CH_2CH_2CH_3$	**7**	94%	**8**	92%	**9**	87%
-C_6H_5	**10**	80%	**11**	80%	**12**	91%

Scheme 1. (a) KOH, K_2CO_3, (b) $(CH_3CH_2CH_2CO)_2O$ or $(C_6H_5CO)_2O$, (c) $C_6H_5CH_2Br$, dry diethyl ether.

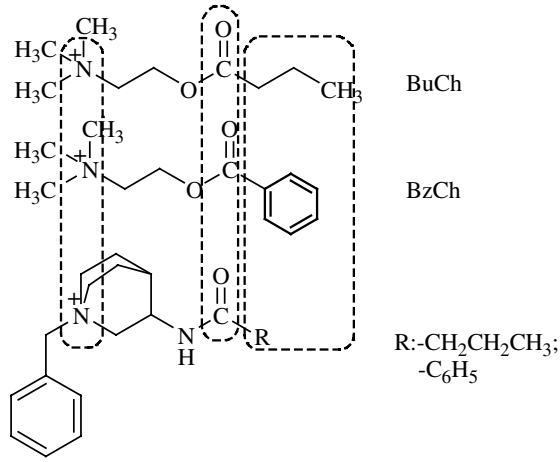


Fig. 1. Comparison of structures of BuCh and BzCh with quaternary derivatives of 3-amidoquinuclidines.

[1–3]. In addition, many synthetic physiologically active substances with antiarythmic and antihistaminic activities contain the quinuclidine moiety [4]. Synthetic 3-substituted quinuclidine derivatives are of special interest due to their various pharmacological properties. Many esters of quinuclidine-3-ol were also extensively studied and recognized as bioactive substances, and as such some of them are commercially available as therapeutics such as aceclidine [4]. 3-Amidoquinuclidine derivatives are classical 5-hydroxytryptamine$_3$ (5-HT$_3$) receptor antagonists since they contain the generally recognized pharmacophore, a basic nitrogen, carbonyl group and an aromatic ring. Some known examples which contain the 3-amidoquinuclidine moiety are zacopride and RG 12915 [5]. Compounds containing the 3-substituted quinuclidine moiety have also been found to be potential antidotes for organophosphate poisons, among which warfare agents are of special interest [6–10].

3-Substituted quinuclidines contain an asymmetric carbon atom and any classical synthesis starting from quinuclidin-3-ol or 3-aminoquinuclidine leads to racemates. Racemates of pharmaceuticals are rarely acceptable in therapies since enantiomers of a given bioactive compound can cause different biological effects ranging from lower activity of the undesired enantiomer to increased toxicity. Therefore, many investigations concentrate on the resolution of racemic quinuclidin-3-ol derivatives using chemical [11,12] and biocatalytic [13,14] methods. One of the enzymes tested as a biocatalyst in the resolution of esters of quinuclidin-3-ol was butyrylcholinesterase (BChE, EC 3.1.1.8) from horse serum [13,15–18]. Only a few examples were reported concerning the resolution of 3-amidoquinuclidines using chemical [19,20] and biocatalytic methods [21].

In this work we report the synthesis of some 3-amidoquinuclidines in racemic and enantiomerically pure forms as well as the synthesis of their quaternary salts (Scheme 1). Benzyl bromide was used as quaternization agent because the kinetics of a reaction of quaternary amides with BChE can be monitored by following the production of benzoic acid by UV detector in HPLC.

The racemic non-quaternary and quaternary 3-amidoquinuclidines (**1**, **4**, **7**, and **10**) were tested as substrates of some hydrolytic enzymes including BChE but no hydrolysis was observed. Instead, inhibition of BChE from horse serum by (*R*)- and (*S*)-enantiomers of quaternary 3-amidoquinuclidines was observed. This finding is not surprising because *N*-quaternary derivatives of 3-amidoquinuclidine may be looked upon as bicyclic analogues of butyrylcholine (BuCh) and benzoylcholine (BzCh) which are good substrates for BChE, and therefore their interaction with the enzyme could have been expected (Fig. 1).

2. Experimental

2.1. General methods and chemicals

Melting points were determined in open capillaries using a Büchi B-540 apparatus and are uncorrected. Optical rotations (in degrees) were measured on an Optical Activity AA-10 automatic polarimeter at ambient temperature in methanol. Elemental analyses were performed with a Perkin-Elmer PE 2400 Series II CHNS/O Analyser. IR spectra were recorded with a Perkin-Elmer FTIR 1725 X spectrometer. ^{1}H and ^{13}C 1D and 2D NMR spectra were recorded on a Bruker AV300 or Bruker AV600 spectrometers at room temperature. Chemical shifts are given in ppm downfield from TMS as internal standard.

The (±)-, (*R*)-, and (*S*)-3-aminoquinuclidine dihydrochlorides (Aldrich) were used for the synthesis of the appropriate 3-amidoquinuclidines with butyric (Aldrich) and benzoic (Merck) acid anhydrides.

2.2. Synthesis of compounds

The (±)-, (*R*)-, and (*S*)-3-aminoquinuclidines were prepared in high yields (>90%) by treating commercial (±)-, (*R*)-, and (*S*)-3-aminoquinuclidine dihydrochlorides (200 mg, 1.00 mmol) with saturated aqueous solutions of KOH (7.13 mmol in 0.4 mL of H_2O). The aqueous reaction solution was then extracted with chloroform (10×2 mL). The extracts were dried over K_2CO_3, filtered and evaporated under reduced pressure. The samples were stored over $CaCl_2$ under reduced pressure.

2.2.1. Synthesis of 3-amidoquinuclidines

2.2.1.1. 3-Butanamidoquinuclidines. A mixture of (±)-3-aminoquinuclidine (3.20 mmol) and butyric acid anhydride (15.8 mmol) was heated with stirring for 2 h at 110–120 °C. Water (2 mL) was added to the reaction mixture after cooling and pH values were adjusted to ~10 with saturated aqueous K_2CO_3 followed by extraction with chloroform (5×20 mL). The extracts were dried over Na_2SO_4, filtered and evaporated under reduced pressure. The residue was distilled under reduced pressure (oil pump) to give:

(±)-3-Butanamidoquinuclidine as white crystals (**1**, 92%), mp 120–122 °C. IR (KBr): $\tilde{\nu} = 3369$, 2949, 2868, 1635, 1524, 1127, 768 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6): $\delta = 0.85$ (t, $J = 7.38$, 3H, $CH_3CH_2CH_2$), 1.25–1.29 (m, 1H, H-8), 1.45–1.56 (m, 4H, $CH_3CH_2CH_2$ and H-5), 1.68–1.72 (m, 2H, H-4, and H-8), 2.06 (t, $J = 7.29$, 2H, $CH_3CH_2CH_2$), 2.37–2.41 (m, 1H, H-2), 2.57–2.68 (m, 3H, H-6, and H-7), 2.73–2.78 (m, 1H, H-7), 3.00–3.04 (m, 1H, H-2), 3.68–3.71 (m, 1H, H-3), 7.81 (d, $J = 6.78$, 1H, CONH) ppm. ^{13}C NMR (150 MHz, DMSO-d_6): $\delta = 13.52$ ($CH_3CH_2CH_2$), 18.79 (C-5), 19.81 (C-8), 25.60 ($CH_3CH_2CH_2$), 25.65 (C-4), 37.24 ($CH_3CH_2CH_2$), 45.97 (C-3), 46.25 (C-6), 46.85 (C-7), 54.46 (C-2), 171.82 (C=O) ppm. Anal. Calcd for $C_{11}H_{20}N_2O$ (196.29): C, 67.31; H, 10.27; N, 14.27. Found: C, 67.05; H, 9.92; N, 13.92%.

(*R*)-3-butanamidoquinuclidine (**2**, 92%); $[\alpha]_D^{25}$ +28° ($c = 0.99$, MeOH); mp 89.3–90.8 °C.

(*S*)-3-butanamidoquinuclidine (**3**, 86%); $[\alpha]_D^{25}$ −27° ($c = 1.0$, MeOH); mp 84.2–84.9 °C.

2.2.1.2. 3-Benzamidoquinuclidines. The same reaction procedure as described for **1** was followed using benzoic acid anhydride. Diethyl ether (2 mL) and water (2 mL) were added to a cold reaction mixture. After extraction with diethyl ether, the pH value of the water phase was adjusted to ~10 with saturated aqueous K_2CO_3 and extracted with chloroform (5×10 mL). The chloroform extracts were dried over Na_2SO_4 and evaporated under reduced pressure to give:

(±)-3-Benzamidoquinuclidine as white crystals (**4**, 90%), mp 158.2–161.9 °C. IR (KBr): $\tilde{\nu} = 3287$, 2934, 2866, 1630, 1539, 1153, 692 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): $\delta = 1.49$–1.53 (m, 1H, H-5), 1.67–1.73 (m, 3H, H-5, and H-8), 2.03–2.06 (m, 1H, H-4), 2.63 (dd, $J = 4.73$, $J = 14.13$, 1H, H-2), 2.75–2.98 (m, 4H, H-6, and H-7), 3.38–3.46 (m, 1H, H-2), 4.13–4.15 (m, 1H, H-3), 6.45 (d, $J = 5.78$, 1H, CONH), 7.47–7.49 (m, 3H, H-3 *Bz*, H-4 *Bz*, and H-5 *Bz*), 7.77 (d, $J = 7.06$, 2H, H-2 *Bz*, and H-6 *Bz*) ppm. ^{13}C **NMR** (75 MHz, $CDCl_3$): $\delta = 20.07$ (C-5), 25.56 (C-4), 25.60 (C-8),

46.49 (C-6), 46.97 (C-3), 47.23 (C-7), 55.81 (C-2), 126.72 (C-2 *Bz* and C-6 *Bz*),128.38 (C-3 *Bz* and C-5 *Bz*), 131.28 (C-4 *Bz*), 134.48 (C-1 *Bz*), 167.37 (C=O) ppm. Anal. Calcd for $C_{14}H_{18}N_2O$ (230.31): C, 73.01; H, 7.88; N, 12.16. Found: C, 72.76; H, 7.71; N, 11.86%.

(*R*)-3-Benzamidoquinuclidine (**5**, 84%); $[\alpha]_D^{25}$ +40° ($c = 0.99$, MeOH); mp 152.4–153.8 °C.

(*S*)-3-Benzamidoquinuclidine (**6**, 93%); $[\alpha]_D^{25}$ −39° ($c = 1.0$, MeOH); mp 148.9–150.4 °C.

2.2.2. Synthesis of N-quaternary derivatives

To the solution of the appropriate 3-butanamidoquinuclidine (**1**–**3**, 7.6 mmol) in dry diethyl ether equimolar amounts of benzyl bromide were added at room temperature. The reaction mixture was kept in the dark overnight to obtain a solid. Recrystallization from acetonitrile gave:

(±)-*N*-Benzyl-3-butanamidoquinuclidinium bromide as white crystals (**7**, 94%), mp 153.2–154.8 °C. IR (KBr): $\tilde{\nu} = 3426$, 2961, 2868, 1656, 1538, 1143, 769 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): $\delta = 0.84$ (t, $J = 7.31$, 3H, $CH_3CH_2CH_2$), 1.44–1.56 (m, 4H, $CH_3CH_2CH_2$ and H-5), 1.87–1.98 (m, 2H, H-8), 2.05–2.09 (m, 3H, $CH_3CH_2CH_2$ and H-4), 3.05–3.15 (m, 1H, H-2), 3.35–3.39 (m, 4H, H-6 and H-7), 3.78–3.86 (m, 1H, H-2), 4.07–4.16 (m, 1H, H-3), 4.46 (s, 2H, CH_2 *Bnl*), 7.52 (s, 5H, *Bnl*), 8.22 (d, $J = 5.57$, 1H, CONH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 13.57$ ($CH_3CH_2CH_2$), 18.25 (C-5), 18.58 (C-8), 22.26 ($CH_3CH_2CH_2$), 24.68 (C-4), 36.94 ($CH_3CH_2CH_2$), 44.29 (C-3), 53.36 (C-6 and C-7), 59.48 (C-2), 66.30 (CH_2 *Bnl*), 127.46 (C-1 *Bnl*), 129.00 C-3 *Bnl* and C-5 (*Bnl*), 130.22 (C-4 *Bnl*), 133.09 (C-2 *Bnl* and C-6 *Bnl*), 172.42 (C=O) ppm. Anal. Calcd for $C_{18}H_{27}BrN_2O$ (367.32): C, 58.86; H, 7.41; N 7.63. Found: C, 58.54; H, 7.35; N, 7.63%.

(*R*)-*N*-Benzyl-3-butanamidoquinuclidinium bromide (**8**, 92%); $[\alpha]_D^{25}$ +30° ($c = 1$, MeOH); mp 179.4–180 °C.

(*S*)-*N*-Benzyl-3-butanamidoquinuclidinium bromide (**9**, 87%); $[\alpha]_D^{25}$ −29° ($c = 1.02$, MeOH); mp 168.2–169.5 °C.

The same reaction procedure as described for **7** was followed using the appropriate 3-benzamidoquinuclidine (**4**–**6**, 0.43 mmol).

(±)-*N*-Benzyl-3-benzamidoquinuclidinium bromide as white crystals, (**10**, 80%) mp 243.3–244.7 °C. IR (KBr): $\tilde{\nu} = 3468$, 3409, 3258, 2927, 2865, 1630, 1543, 1317, 1054, 696 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): $\delta = 1.75$–1.77 (m, 1H, H-5), 1.91–2.14 (m, 2H, H-8), 2.43–2.44 (m, 2H, H-4, and H-5), 3.28–3.30 (m, 1H, H-2), 3.34–3.47 (m, 4H, H-6, and H-7), 3.85–3.89 (m, 1H, H-2), 4.30–4.31 (m, 1H, H-3), 4.45 (s, 2H, CH_2, *Bnl*), 7.39–7.51 (m, 8H, *Bnl*, and H-3, H-4, and H-5 *Bz*), 7.80 (d, $J = 7.16$, 2H, H-2 and H-6 *Bz*), 8.58 (d, $J = 5.93$, 1H, CONH) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 18.39$ (C-5), 22.33 (C-8), 24.88 (C-4), 45.38 (C-3), 53.37 (C-6), 53.56 (C-7), 58.60 (C-2), 66.38 (CH_2*Bnl*), 124.82 (C-1 *Bnl*), 127.50 (C-3 *Bnl* and C-5 *Bnl*), 128.22 (C-3 *Bz* and C-5 *Bz*), 128.97 (C-2 *Bnl* and C-6 *Bnl*), 130.18 (C-4 *Bnl*), 131.51 (C-4 *Bz*), 133.10 (C-2 *Bz* and C-6 *Bz*), 133.82 (C-1 *Bz*), 166.91 (C=O) ppm. Anal. Calcd for $C_{21}H_{25}BrN_2O$ (401.34): C, 62.85; H, 6.28; N, 6.98. Found: C, 63.26; H, 6.40; N, 7.05%.

(*R*)-*N*-Benzyl-3-benzamidoquinuclidinium bromide (**11**, 80%); $[\alpha]_D^{25}$ +7° ($c = 1.04$, MeOH); mp 221.0–221.5 °C.

(*S*)-*N*-Benzyl-3-benzamidoquinuclidinium bromide (**12**, 91%); $[\alpha]_D^{25}$ −5° ($c = 1$, MeOH); mp 236.0–236.5 °C.

2.3. Inhibition of butyrylcholinesterase

BChE (EC, 3.1.1.8), type IV-S, lyophilized from horse serum (Sigma Chemical Co.) was used without further purification. Benzoylcholine chloride (BDH Chemicals Ltd.) was used as a substrate for BChE. HPLC analyses (Thermo Separation Products, Spectra SYSTEM 2000) were performed on an RP-18 column (Waters, SymmetryShield, 5 μm, 150 × 3.9 mm i.d.) at 40 °C. The mobile phase used was water–methanol–acetonitrile–acetic acid–triethylamine (60:25:15:0.33:0.2) at a flow-rate of 1.0 mL min^{-1}. The reactions were carried out in a Heidolph UNIMAX 1100 shaker.

The kinetics of the reaction between enantiomers of *N*-quaternary quinuclidinamides **8**, **9**, **11**, and **12** and BChE were monitored by following the production of benzoic acid at 230 nm using HPLC. All experiments were performed in a total volume of 1.0 mL and an enzyme concentration of 1.5×10^{-9} M (19.8 U/mg solid, 0.016 mg mL^{-1}). The enzymatic reaction was stopped by addition of aliquots (20 μL) of the reaction mixture to the HPLC mobile phase (200 μL). Two to three measurements were made with each substrate concentration (0.1–0.5 mM). K_M values were obtained by non-linear regression of the experimental data to the Michaelis–Menten equation. The dissociation constant of enzyme–inhibitor complex was determined from Hunter-Downs plot, using 0.1–0.5 mM concentrations of the inhibitor.

3. Results and discussion

Racemic and chiral (*R*)- and (*S*)-3-amidoquinuclidine derivatives (**1**–**6**) were synthesized by the classical reaction of the appropriate amine and anhydride. Their quaternary salts were prepared as well (**7**–**12**) with benzyl bromide as quaternization agent. The structure and purity of all compounds were determined by elemental analyses, IR, ^{1}H and ^{13}C NMR and 2D NMR spectroscopy. Optical purities were checked by optical rotation measurements.

Racemic non-quaternary and quaternary (**1**, **4**, **7**, and **10**) 3-amidoderivatives were used to evaluate selective hydrolysis catalyzed by some hydrolases such as PLE (an esterase), subtilisin and chymotrypsin (proteases) and an amidase from human serum [22] but no hydrolysis was observed. BChE was also used as a catalyst since it was previously shown that BChE accepts as substrates and catalyzes the hydrolysis of compounds with diverse structures such as heroin, aspirin and succinylcholine [23]. In the case of quinuclidines, BChE has been previously used for the hydrolysis of the racemic quinuclidine-3-yl butyrate [14], quinuclidin-3-yl benzoates and their *N*-methyl and *N*-benzyl derivatives [16,17]. Quaternary quinuclidinium benzoates were better substrates for BChE than their non-quaternized analogues [17]. That is not surprising because *N*-quaternary derivatives of quinuclidine esters and amides have structural similarities with BzCh (Fig. 1). However, hydrolysis of the compounds **1**, **4**, **7** and **10** was not observed in the experimental conditions used in this work.

It was shown that the enantiomers of *N*-benzyl derivatives (**8**, **9**, **11**, and **12**) act as reversible inhibitors of BChE. Inhibition was determined with benzoylcholine (BzCh) as the enzyme's substrate. The measured activity of BChE at different concentrations of BzCh showed that in a given concentration range the Michaelis–Menten kinetics was followed. The value of K_M of 0.17 ± 0.01 mM was obtained from Lineweaver–Burk plot

(Fig. 2) for BzCh as substrates of BChE. This is in accord with previously published data [17].

We started with the assumption that our compounds were competitive inhibitors, i.e., that they compete for the same active site on the enzyme as does the substrate (BzCh). In this case in the Lineweaver–Burk plot the same ordinal intercept ($1/v$) is expected in the presence or absence of the inhibitor. The effect of the competitive inhibitor, therefore, is to produce an apparent increase in K_M values. The kinetics of inhibition by the (*R*)-enantiomer (**11**) indicated that this compound is a competitive inhibitor and therefore binds to the catalytic site of BChE. On the other hand the (*S*)-enantiomer (**12**) does not have the same ordinal intercept ($1/v$) as the substrate which indicates that this enantiomer was not exclusively bound to the catalytic site of the enzyme (Fig. 2). The same results were obtained in the case of *N*-benzyl-3-butanamidoquinuclidines (**8** and **9**).

The concentrations of the inhibitor which were chosen inhibited the enzyme between 20 and 80%. The constants were evaluated from the kinetics of competition between BzCh and inhibitors of BChE. Activities of the enzymes were measured at different substrate concentrations (S) in the absence (v_0) and presence (v_i) of the given inhibitor concentration (I). The concentration constant (K_{app}) was calculated for each substrate. The enzyme-inhibitor dissociation constants K_i were evaluated from the Hunter-Downs equation using linear regression analysis [24] where K_{app} is the apparent enzyme–inhibitor dissociation constant at a given substrate concentration (S) and K_M is the Michaelis constant for the substrate.

$$K_{app} = \frac{v_i I}{v_0 - v_i} = K_i + \frac{K_i}{K_M} S \tag{1}$$

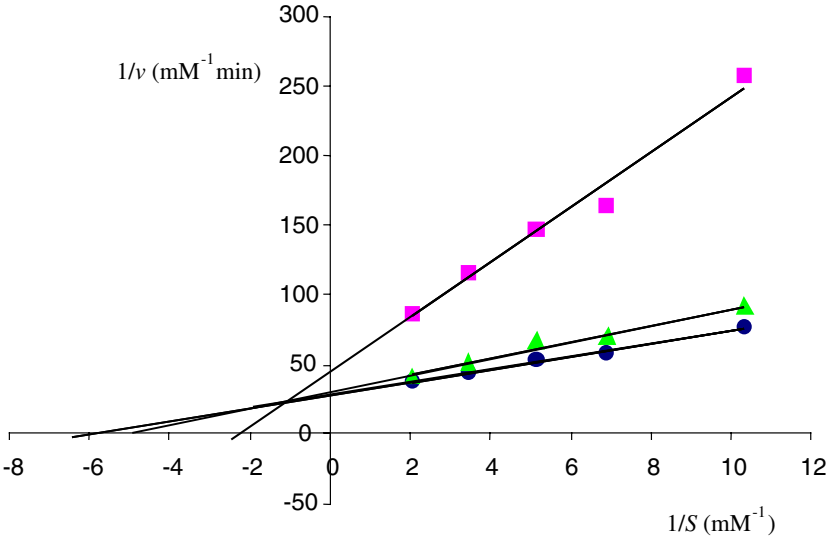


Fig. 2. Lineweaver–Burk plot in the absence (●) and presence of inhibitor **11** (▲) and **12** (■).

Table 1
Reversible inhibition of BChE measurement with benzoylcholine as a substrate in the presence of compounds as inhibitors

Compound	K_i[a] (μM)	K_M[b] (mM)
8	159.88 ± 0.47	0.30
9	241.96 ± 0.08	0.36
11	25.92 ± 0.01	0.19
12	3.70 ± 0.00	0.45

[a] K_i is the enzyme–inhibitor dissociation constant calculated according to Eq. (1). Each measurement was repeated two to three times.
[b] K_M were obtained from Lineweaver–Burk plot. Relative standard deviations of the K_M values were on average 15%.

The Hunter-Downs plot of the reversible inhibition of BChE by the quaternary enantiomers of 3-amidoquinuclidine derivatives displayed a linear relationship between the apparent dissociation constant and the substrate concentration between 0.1 and 0.5 mM. The enzyme–inhibitor dissociation constant was obtained for all tested compounds. The most potent inhibitor (with the lowest dissociation constant) was the (*S*)-enantiomer of *N*-benzyl-3-benzamidoquinuclidine (**12**) with $K_i = 3.7 \pm 0.0$ μM. This could have been expected since previously reported data showed that the (*S*)-enantiomer of *N*-benzylquinuclidine-3-yl benzoate was the poorest substrate but an efficient inhibitor of all tested enantiomers of *N*-methyl and *N*-benzylquinuclidine-3-yl benzoates, with $K_i = 3.3 \pm 0.2$ μM [16]. The (*R*)-enantiomer of *N*-benzyl-3-benzamidoquinuclidine (**11**) was a 7-fold weaker inhibitor than its (*S*)-enantiomer. The least potent inhibitor was the (*S*)-enantiomer of the quaternary derivative of butyric acid which was 65-fold weaker than the most potent inhibitor **12**. Both enantiomers of quaternary 3-benzamidoquinuclidines (**11** and **12**) were more potent inhibitors of BChE than enantiomers of quaternary 3-buatanamidoquinuclidines (**8** and **9**). The obtained kinetic constants for inhibitors are displayed in Table 1.

4. Conclusion

We have synthesized racemic and enantiomerically pure amides of 3-aminoquinuclidine with butyric and benzoic acid anhydrides. Their quaternary *N*-benzyl derivatives were prepared as well and tested as possible inhibitors of BChE. For all tested quaternary compounds inhibition of BChE was observed. The most potent inhibitor was the (*S*)- enantiomer of *N*-benzyl derivatives of 3-benzamidoquinuclidine (**12**) which was 7-fold more potent than the (*R*)-enantiomer (**11**). Both enantiomers of quaternary derivatives of 3-butanamidoquinuclidine were weaker inhibitors than enantiomers of quaternary derivatives of 3-benzamidoquinuclidine. Thus, the (*S*)-*N*-benzyl derivatives of butyric acid (**8**) was 65-fold less potent than the compound **12**.

Acknowledgments

Grateful thanks are due to Dr Ines Primožič, Faculty of Science, University of Zagreb for the useful advice regarding the kinetic study. We are very grateful to Dr Vera Simeon-Rudolf, Institute for Medical Research and Occupational Health, Zagreb, for her kind

help with chemicals and helpful comments. This work was supported by the Ministry of Science, Education and Sports of the Republic of Croatia, Project No. 0119610.

References

[1] R.B. Woodward, R.B. Turner, in: R.H.F. Manske, H.L. Holmes (Eds.), The Alkaloids, vol. III, Academic, New York, 1953, p. 1 (Chapter 16).
[2] W.I. Taylor, in: R.H.F. Manske, H.L. Holmes (Eds.), The Alkaloids, vol. VIII, Academic, New York, 1965, p. 203 (Chapter 9).
[3] W.I. Taylor, in: R.H.F. Manske, H.L. Holmes (Eds.), The Alkaloids, vol. VIII, Academic, New York, 1965, p. 237 (Chapter 22).
[4] M.D. Mashkovsky, L.N. Yakhontov, M.E. Kaminka, E.E. Mikhlina, Prog. Drug. Res. 27 (1983) 9–61.
[5] M. Langlois, J.L. Soulier, M. Allainmat, S. Shen, C. Gallais, Bioorg. Med. Chem. Lett. 3 (1993) 1555–1558.
[6] G.H. Sterling, P.H. Doukas, R.J. Sheldon, J.J. O'Neill, Biochem. Pharmacol. 37 (1988) 379–384.
[7] G.H. Sterling, P.H. Doukas, C. Jackson, R. Caccese, K.J. O'Neill, J.J. O'Neill, Biochem. Pharmacol. 45 (1993) 465–472.
[8] G. Amitai, D. Balderman, R. Bruckstein-Davidovici, M. Spiegelstein, US Patent 4,675,326, 1987.
[9] G. Amitai, I. Rabinovitz, G. Zomber, R. Chen, G. Cohen, R. Adani, L. Raveh, Proceedings of the Fifth International Symposium on Protection Against Chemical and Biological Warfare Agents, Stockholm, Defence Research Establishment, Umea, 1995, pp. 247–254.
[10] V. Simeon-Rudolf, E. Reiner, M. Škrinjarić-Špoljar, B. Radić, A. Lucić, I. Primožič, S. Tomić, Arch. Toxicol. 72 (1998) 289–295.
[11] B. Ringdahl, R.S. Jope, D.J. Jeden, Biochem. Pharmacol. 33 (1984) 2819–2822.
[12] L.H. Sternbach, S. Kaiser, J. Am. Chem. Soc. 74 (1952) 2219–2221.
[13] A. Kalir, E. Sali, E. Shirin, Isr. J. Chem. 9 (1971) 267–268.
[14] M. Rehavi, S. Maayani, M. Sokolovsky, Life Sci. 21 (1977) 1293–1302.
[15] D.C. Muchmore, US Patent 5,215,918, 1993.
[16] I. Primožič, T. Hrenar, S. Tomić, Z. Meić, Eur. J. Org. Chem. (2003) 295–301.
[17] I. Primožič, T. Hrenar, S. Tomić, Z. Meić, J. Phys. Org. Chem. 15 (2002) 608–614.
[18] I. Primožič, T. Hrenar, S. Tomić, Z. Meić, Croat. Chem. Acta 76 (2003) 93–99.
[19] M. Langlois, C. Meyer, J.L. Soulier, Synth. Commun. 22 (1992) 1895–1911.
[20] B.A. Kowalczyk, J.C. Rohloff, C.A. Dvorak, J.O. Gardner, Synth. Commun. 26 (1996) 2009–2015.
[21] A.N. Collins, G.N. Sheldrake, J. Crosby, Chirality in Industry, Wiley, London, 1992, pp. 478–503.
[22] Z. Valinger, B. Ladešić, J. Tomašić, Biochim. Biophys. Acta 701 (1982) 63–71.
[23] O. Lockridge, Pharmacol. Ther. 47 (1990) 35–60.
[24] W.N. Aldrige, E. Reiner, in: E.L. Tatum, I.A. Neuberger (Eds.), Frontiers in Biology, vol. 26, North-Holland, Amsterdam, 1972.