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3-Amidoquinuclidine Derivatives: Synthesis and Interaction with Butyrylcholinesterase

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Racemates as well as (*R*)- and (*S*)-enantiomers of 3-pivalamidoquinuclidine (PivQ) and 3-acetamidoquinuclidine (AcQ) were prepared. Their quaternary racemic and enantiomerically pure *N*-benzyl derivatives (BnlPivQ and BnlAcQ) were synthesized as well. The amides were tested as substrates and inhibitors of butyrylcholinesterase (BChE) from horse serum (EC 3.1.1.8). No hydrolysis was observed under the experimental conditions applied. On the contrary, inhibition of BChE by (*R*)- and (*S*)-enantiomers of *N*-benzylquinuclidinium amides of pivalic acid was observed. The (*S*)-BnlPivQ with K_i = 41.57 μmol dm^{-3} was 3-fold more potent inhibitor than the (*R*)-enantiomer. On the other hand, preliminary results indicated that both enantiomers of *N*-benzylquinuclidinum amides of acetic acid may possibly be inhibitors as well as activators depending on the concentrations of benzoylcholine (BzCh) used as a substrate of BChE.

Keywords
• 3-amidoquinuclidine
• quaternary derivatives of 3-amidoquinuclidine
• butyrylcholinesterase
• chiral inhibitors

INTRODUCTION

Quinuclidine compounds display a wide variety of biological activities. They have also been shown to be potential antidotes against poisoning by organophosphorus compounds such as pesticides and chemical warfare agents.[1] 3-Substituted quinuclidines have been found especially interesting with respect to their pharmacological properties. Several esters of quinuclidin-3-ol are even commercially available as therapeutic agents.[2] 3-Amidoquinuclidine derivatives are also classical 5-hydroxytryptamine$_3$ (5-HT$_3$) receptor antagonists (since they contain the generally recognized pharmacofore, *i.e.,* basic nitrogen, carbonyl group and aromatic ring) such as *e.g.* zacopride and RG 12915.[3] Since 3-aminoquinuclidine contains an asymmetric carbon atom, preparation of its amides leads to racemates. Racemates of pharmaceuticals are regarded with suspicion, since enantiomers of a given bioactive compound can cause different biological effects, ranging from lower activity of a less active enantiomer to no response at all or even to increased toxicity.[4] However, the resolution of 3-amidoquinuclidine derivatives to pure enantiomers was shown to be difficult. Only few examples using chemical[5–7] and biocatalytic[8] methods have been described so far.

In this paper, we report on the synthesis of (±)-, (*R*)-, and (*S*)-3-pivalamidoquinuclidine derivatives and (±)-, (*R*)-, and (*S*)-3-acetamidoquinuclidine. We also report on the synthesis of their *N*-benzyl derivatives ((±)-, (*R*)-, (*S*)-BnlPivQ and (±)-, (*R*)-, (*S*)-BnlAcQ) (Figure 1).

N-quaternary derivatives of 3-amidoquinuclidines and esters of quinuclidin-3-ol may be looked upon as bicyclic analogues of acethylcholine and benzoylcholine, which are good substrates of BChE. Therefore, their in-

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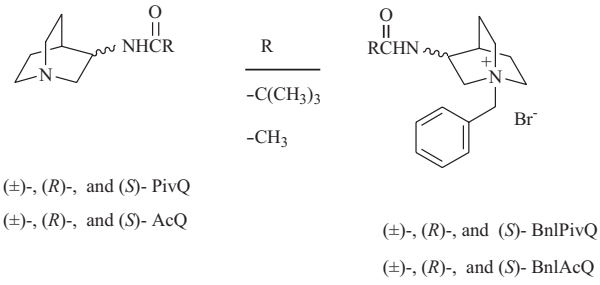


Figure 1. Structures of quinuclidine derivatives.

teraction with the enzyme could have been expected. BChE was also found to be suitable as a biocatalyst for the resolution of racemic quaternary quinuclidinium esters.[9–12] For these reasons, racemic nonquaternary and quaternary quinuclidine amides were tested as possible substrates primarily of BChE and then of some other hydrolytic enzymes. Under the experimental conditions described in this work, no hydrolysis catalyzed by these enzymes occurred. But, *N*-benzyl derivatives of 3-amidoquinuclidine were shown to inhibit BChE from horse serum. To get more information on the inhibition mechanism of that enzyme, the dissociation constants of the enzyme-inhibitor complexes were determined.

EXPERIMENTAL

Synthesis of Compounds

General Remarks. – Melting points were determined in open capillaries using a Büchi B-540 apparatus and are uncorrected. Specific optical rotation values were determined on an Optical Activity AA-10 automatic polarimeter at 589 nm in methanol at ambient temperature (~ 24 °C). Elemental analyses were performed with a Perkin-Elmer PE 2400 Series II CHNS/O Analyzer. 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 and Bruker AV600 spectrometer at ambient temperature. Chemical shifts are given in ppm downfield from TMS as internal standard. The (±)-, (*R*)- and (*S*)-3-aminoquinuclidine dihydrochlorides (Aldrich) were used for synthesis of the appropriate 3-amidoquinuclidines with pivalic and acetic acid anhydrides (Aldrich).

The (±)-, (*R*)- and (*S*)-3-aminoquinuclidine were prepared in high yields (>90 %) by treating commercial (±)-, (*R*)- and (*S*)-3-aminoquinuclidine dihydrochlorides (200 mg, 1.00 mmol) with saturated aqueous solution of KOH (0.4 g, 7.13 mmol in 0.4 mL 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.

Synthesis of Nonquaternary Derivatives

(±)-3-Aminoquinuclidine (0.126 g, 0.1 mmol) was added to pivalic acid anhydride (246 mL, 1.20 mmol) in dry THF (1 mL). The reaction mixture was heated under stirring overnight at 70 °C. Water was added to the reaction mixture and the pH value was fixed at ≈1 with conc. HCl, then extracted with diethyl ether (4 × 10 mL). After extraction with diethyl ether, the pH value of the water phase was fixed at ≈10 with 50 % aqueous solution of NaOH and extracted with chloroform (5 × 10 mL). Chloroform extracts were dried over Na_2SO_4, filtered and evaporated under reduced pressure to give:

(±)-3-(2,2-Dimetilpropanamido)quinuclidine ((±)-PivQ). – Obtained as white crystals; 188 mg (90 %); m.p. 132.2–133.4 °C; IR (KBr) ν_{max}/cm^{-1}: 3369 (N–H), 2949 (C–H), 1635 (C=O), 1127 (C–N); ^{1}H NMR (DMSO-d$_6$) δ/ppm: 1.11 (s, 9H, (C$\underline{H}_3$)$_3$C), 1.21–1.26 (m, 1H, H-5), 1.44–1.56 (m, 2H, H-8), 1.65–1.72 (m, 2H, H-4 and H-5), 2.55–2.68 (m, 4H, H-6, H-7 and H-2), 2.81–2.85 (m, 1H, H-7), 2.94–2.99 (m, 1H, H-2), 3.65–3.68 (m, 1H, H-3), 7.14 (d, J = 6.47 Hz, 1H, CONH); ^{13}C NMR (DMSO-d$_6$) δ/ppm: 19.96 (C-5), 25.62 (C-4), 25.74 (C-8), 27.50 ((C$\underline{H}_3$)$_3$C), 38.05 ((CH$_3$)$_3$C$\underline{}$), 46.49 (C-6), 46.57 (C-3), 46.80 (C-7), 53.59 (C-2), 177.45 (C=O).

Anal. Calcd. for $C_{12}H_{22}N_2O$ (M_r = 210.32): C 68.53, H 10.54, N 13.32 %; found: C 68.22, H 10.19, N 12.96 %.

*(*R*)-3-(2,2-Dimetilpropanamido)quinuclidine ((*R*)-PivQ).* – Obtained from (*R*)-3-aminoquinuclidine (0.126 g, 0.99 mmol) and pivalic acid anhydride as white crystals; 188 mg (90 %); m.p. 131.2–132.4 °C; $[\alpha]_D^{25}$ +29° (*c* = 1.01, MeOH).

*(*S*)-3-(2,2-Dimetilpropanamido)quinuclidine ((*S*)-PivQ).* – Obtained from (*S*)-3-aminoquinuclidine (0.125 g, 0.99 mmol) and pivalic acid anhydride as white crystals; 192 mg (92 %); m.p. 132.5–133.5 °C; $[\alpha]_D^{25}$ –30° (*c* = 1.02, MeOH).

The mixture of (±)-3-aminoquinuclidine (0.300 g, 2.40 mmol) and acetic acid anhydride (1.12 mL, 11.9 mmol) was refluxed for 3 hours. Distillation under reduced pressure was used to remove the excess of acetic acid anhydride. The residue upon distillation was neutralized with saturated aqueous solution of K_2CO_3 (5 mL) and extracted with chloroform (5 × 10 mL). The extracts were dried over Na_2SO_4, filtered and evaporated under reduced pressure. The obtained black oil was distilled under reduced pressure of 0.01 mmHg to give:

(±)-3-Acetamidoquinuclidine ((±)-AcQ). – Obtained as white crystals; 374 mg (93 %); m.p. 120–122 °C; IR (KBr) ν_{max}/cm^{-1}: 3290 (N–H), 2949 (C–H), 1654 (C=O), 1132 (C–N); ^{1}H NMR (DMSO-d$_6$) δ/ppm: 1.22–1.30 (m, 1H, H-5), 1.41–1.60 (m, 2H, H-8), 1.67–1.75 (m, 2H, H-4 and H-5), 1.81 (s, 3H, CH$_3$), 2.34–2.40 (dd, J = 4.67 Hz; J = 13.44 Hz, 1H, H-2), 2.46–2.79 (m, 4H, H-6 and H-7), 2.97–3.06 (m, 1H, H-2), 3.66–3.71 (m, 1H, H-3), 7.88 (d, J = 6.46 Hz, 1H, CONH); ^{13}C NMR (DMSO-d$_6$) δ/ppm: 19.87 (C-5), 22.67 (C-4), 25.66 (C-8), 25.71 (CH$_3$), 46.11 (C-3), 46.28 (C-6), 46.92 (C-7), 54.58 (C-2), 168.98 (C=O).

Anal. Calcd. for $C_9H_{16}N_2O$ (M_r = 168.24): C 64.25, H 9.59, N 16.65 %; found: C 64.05, H 9.24, N 16.32 %.

*(*R*)-3-Acetamidoquinuclidine ((*R*)-AcQ).* – Obtained from (*R*)-3-aminoquinuclidine (0.100 g, 0.79 mmol) and acetic acid anhydride as white crystals; 123 mg (84 %); m.p. 134.5–135.6 °C; $[\alpha]_D^{25}$ +37° (*c* = 1.01, MeOH).

*(*S*)-3-Acetamidoquinuclidine ((*S*)-AcQ).* – Obtained from (*S*)-3-aminoquinuclidine (0.120 g, 0.95 mmol) and acetic acid anhydride as white crystals; 156 mg (89 %); m.p. 125.9–127 °C; $[\alpha]_D^{25}$ –35° (*c* = 0.98, MeOH).

Synthesis of N*-quaternary Derivatives*

To the solution of the appropriate 3-amidoquinuclidine ((±)-PivQ, 0.050 g, 0.43 mmol or (±)-AcQ, 0.470 g, 2.6 mmol) in dry acetone (1 mL), equimolar amounts of benzyl bromide were added under stirring at room temperature. The reaction mixture was kept in the dark overnight to obtain a solid. Recrystallization from acetonitrile gave:

*(±)-*N*-benzyl-3-(2,2)-dimetilpropanamidoqinuclidinium bromide ((±)-BnlPivQ).* – Obtained as white crystals; 78 mg (86 %); m.p. 166–167 °C; IR (KBr) ν_{max}/cm^{-1}: 3231 (N–H), 2962 (C–H), 1652 (C=O), 1527 (C=C), 1063 (C–N); ^{1}H NMR (DMSO-d$_6$) δ/ppm: 1.11 (s, 9H, ((CH$_3$)$_3$C), 1.70–1.80 (m, 2H, H-5), 1.87–1.97 (m, 2H, H-8), 2.02–2.08 (m, 1H, H-4), 3.25–3.50 (m, 5H, H-2, H-6 and H-7), 3.76–3.83 (m, 1H, H-2), 4.07–4.15 (m, 1H, H-3), 4.46 (s, 2H, CH$_2$ *Bnl*), 7.52 (s, 5H, *Bnl*), 7.62 (d, *J* = 5.52 Hz, 1H, CONH); ^{13}C NMR (DMSO-d$_6$) δ/ppm: 18.24 (C-5), 22.14 (C-8), 24.74 (C-4), 27.24 ((CH$_3$)$_3$C), 38.01 ((CH$_3$)$_3$C), 44.88 (C-3), 53.27 (C-6), 53.43 (C-7), 58.36 (C-2), 66.31 (CH$_2$ *Bnl*), 127.45 (C-1 *Bnl*), 128.95 (C-3 *Bnl* and C-5 *Bnl*), 130.15 (C-4 *Bnl*), 133.05 (C-2 *Bnl* and C-6 *Bnl*), 177.96 (C=O).

Anal. Calcd. for $C_{19}H_{29}BrN_2O$ (M_r = 381.35): C 59.84, H 7.66, N 7.35 %; found: C 59.93, H 7.58, N 7.15 %.

*(*R*)-*N*-benzyl-3-(2,2)-dimetilpropanamidoqinuclidinium bromide ((*R*)-BnlPivQ).* – Obtained from (*R*)-3-(2,2-dimetilpropanamido)quinuclidine (0.088 g, 0.42 mmol) and benzyl bromide as white crystals, 157 mg (98 %); m.p. 123.5–125 °C; $[\alpha]_D^{25}$ +21° (*c* = 1.0, MeOH).

*(*S*)-*N*-Benzyl-3-(2,2)-dimetilpropanamidoqinuclidinium bromide ((*S*)-BnlPivQ).* – Obtained from (*S*)-3-(2,2-dimetilpropanamido)quinuclidine (0.092 g, 0.44 mmol) and benzyl bromide as white crystals, 156 mg (93 %); m.p. 135.5–137 °C; $[\alpha]_D^{25}$ –21° (*c* = 1.02, MeOH).

*(±)-*N*-benzyl-3-acetamidoqinuclidinium bromide ((±)-BnlAcQ).* – Obtained as white crystals; 813 mg (90 %); m.p. 218–218.5 °C; IR (KBr) ν_{max}/cm^{-1}: 3218 (N–H), 2953 (C–H), 1664 (C=O), 1523 (C=C), 1151 (C–N); ^{1}H NMR (DMSO-d$_6$) δ/ppm: 1.77–1.87 (m, 3H, H-4 and H-8), 1.94–2.01 (m, 3H, CH$_3$), 2.95–2.97 (m, 1H, H-2), 3.27–3.38 (m, 6H, H-5, H-6 and H-7), 3.72–3.76 (m, 1H, H-2), 4.02–4.04 (m, 1H, H-3), 4.39 (s, 2H, CH$_2$ *Bnl*), 7.43–7.47 (m, 5H, *Bnl*), 8.24 (d, *J* = 6.13 Hz, 1H, CONH); ^{13}C NMR (DMSO-d$_6$) δ/ppm: 18.21 (C-5), 22.25 (C-8), 22.34 (C-4), 24.59 (CH$_3$), 44.41 (C-3), 53.32 (C-6), 53.35 (C-7), 59.53 (C-2), 66.30 (CH$_2$ *Bnl*), 127.37 (C-1 *Bnl*), 128.94 (C-3 *Bnl* and C-5 *Bnl*), 130.16 (C-4 *Bnl*), 133.01 (C-2 *Bnl* and C-6 *Bnl*), 169.58 (C=O).

Anal. Calcd. for $C_{16}H_{23}BrN_2O$ (M_r = 339.27): C 56.46, H 6.83, N 8.26 %; found: C 56.65, H 6.72, N 8.37 %.

*(*R*)-*N*-benzyl-3-acetamidoqinuclidinium bromide ((*R*)-BnlAcQ).* – Obtained from (*R*)-3-acetamidoquinuclidine (0.050 g, 0.272 mmol) and benzyl bromide as white crystals; 83 mg (86 %); m.p. 203–206 °C; $[\alpha]_D^{25}$ +37° (*c* = 1.0, MeOH).

*(*S*)-*N*-benzyl-3-acetamidoqinuclidinium bromide ((*S*)-BnlAcQ).* – Obtained from (*S*)-3-acetamidoquinuclidine (0.080 g, 0.434 mmol) and benzyl bromide as white crystals; 111 mg (72 %); m.p. 199.5–202 °C; $[\alpha]_D^{25}$ –35° (*c* = 1, MeOH).

Substrate Hydrolysis

For the hydrolysis of prepared racemic amides, butyrylcholinesterase from horse serum, BChE, (E.C. 3.1.1.8, lyophilized, Sigma Chem. Co.) and a series of hydrolytic enzymes: proteinase from *bacillus subtilis,* (EC 3.4.24.4, Fluka), α-chymotrypsin from bovine pancreas (EC 3.4.21.1, Fluka), esterase from porcine liver, PLE, (EC 3.1.1.1, Sigma), lipase from hog pancreas, PPL, (EC 3.1.1.3, Fluka), lipase from wheat germ, (EC 3.1.1.3, Sigma) and amidase from human serum, (EC 3.5.1.28, *N*-acethylmuramyl-L-alanine amidase) were tested as possible biocatalysts. Substrates (±)-PivQ, (±)-AcQ, (±)-BnlPivQ and (±)-BnlAcQ (0.044 mmol) were dissolved in phosphate buffer (300 μL, 0.2 mol, dm^{-3}; Na_2HPO_4/NaH_2PO_4; pH = 7.4) in all reactions except in that with the amidase. In the hydrolysis with amidase, the substrates (0.044 mmol) were dissolved in Tris/HCl buffer (300 μL, pH = 8) with $MgCl_2$. The enzymes (3 mg of each; 100 μL of the amidase) were then added. The reactions were carried out in a Heidolph UNIMAX 1010 shaker at 30 °C. Reactions were followed by TLC on aluminium oxide with $CHCl_3$: MeOH = 9 : 1 as eluent.

Enzyme Inhibition

Inhibition of the enzyme by enantiomers of *N*-benzyl-3-amidoquinuclidine derivatives was measured with benzoylcholine chloride (BDH Chemicals Ltd.) as the substrate of BChE. BChE (EC, 3.1.1.8), type IV-S, lyophilized from horse serum (Sigma Chemical Co.) was used without further purification. 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 at room temperature.

Interactions of *N*-quaternary quinuclidinamides, (*R*)-BnlPivQ, (*S*)-BnlPivQ, (*R*)-BnlAcQ and (*S*)-BnlAcQ, with BChE were monitored by following the production of benzoic acid from benzoylcholine hydrolysis at 230 nm by a UV detector (HPLC). All experiments were performed in a total volume of 1.0 mL and an enzyme concentration of 1.5 nmol dm^{-3} (0.016 mg mL^{-1}). The enzymatic reaction was stopped by the 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 mmol dm^{-3}). The K_M values were obtained by non-linear regression of the experimental data to the Michaelis-Menten equation. The dissociation constant of the enzyme-inhibitor complex was determined from the Hunter-Downs plot, using 0.1–0.5 mmol dm^{-3} concentrations of substrates.[13]

Enzyme-inhibitor Dissociation Constants

Reversible inhibition of BChE was measured for all enantiomers of *N*-benzyl derivatives. Inhibition was determined with benzoylcholine (BzCh) as a substrate of the enzyme. To study the competition between *N*-benzyl-3-amidoquinuclidinium derivatives and the substrate, activities of the enzymes were measured at different substrate concentrations [S] in the absence (v_0) and in the presence (v_i) of a given inhibitor concentration [I]. Concentrations of the inhibitor were chosen to inhibit the enzyme between 20–80 %. For each substrate concentration, the apparent dissociation constants (K_{app}) were calculated. The enzyme-inhibitor dissociation constants (K_i) were evaluated from the Hunter-Downs equation using linear regression analysis[13] 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.

$$K_{app} = \frac{v_i [I]}{v_0 - v_i} = K_i + \frac{K_i}{K_M}[S] \qquad (1)$$

RESULTS AND DISCUSSION

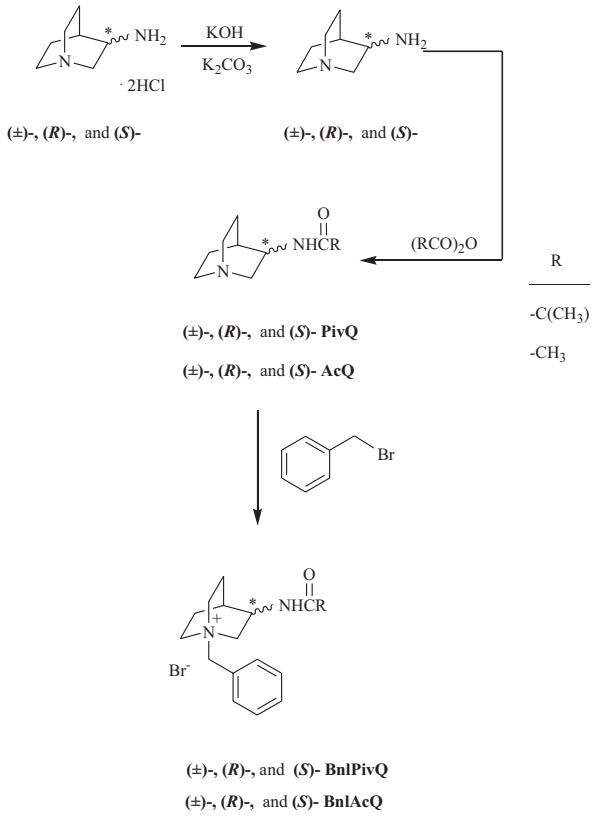
Synthesis of Quinuclidine Derivatives

3-Amidoquinuclidines, racemates as well as both enantiomers were synthesized from the appropriate 3-aminoquinuclidine with pivalic or acetic acid anhydrides. Racemic and enantiomerically pure 3-aminoquinuclidines were obtained from the appropriate 3-aminoquinuclidine dihydrochlorides (Scheme 1). *N*-Quaternary derivatives of 3-amidoquinuclidine were synthesized by quaternization of appropriate chiral amides with benzyl bromide (Scheme 1).

Synthesized compounds were identified and characterized by IR, one- and two-dimensional ^1H and ^{13}C NMR spectroscopies, elemental analyses and by determining the melting points and optical rotation values.

Hydrolysis of Derivatives of 3-amidoquinuclidines

One of the enzymes tested as a biocatalyst for the hydrolysis of synthesized racemic amides was butyrylcholinesterase (BChE, EC 3.1.1.8). BChE is a serine hydrolase that has a structure similar to that of acetylcholinesterase.[14] The active site of this enzyme consists of several major domains: (i) esteratic site containing active serine as a part of the catalytic triade (Ser-His-Glu); (ii) acyl pocket, a



Scheme 1.

hydrophobic region that accommodates the acyl group of an ester or amide; (iii) choline subsite consisting of tryptophan for molecular recognition of the substrate's quaternary ammonium group; and (iv) oxyanion hole, formed by the main chain N–H dipoles interacting with the carbonyl oxygen of the substrate.

Although this enzyme has not been much investigated as a catalyst in organic synthesis, it was shown that it could accept as substrates compounds with diverse structures such as *e.g.* some drugs like heroin, aspirin and succinylcholine.[15] In the case of quinuclidines, BChE has been previously used for the hydrolysis of the racemic quinuclidine-3-yl butyrate,[9] quinuclidin-3-yl benzoates and their *N*-methyl and *N*-benzyl derivatives.[10,11] Quaternary quinuclidinium benzoates were better substrates for BChE than their nonquaternized analogues.[10] This is not surprising because *N*-quaternary derivatives of quinuclidine esters and amides have structural similarities with BzCh and acetylcholine, which are good substrates of BChE (Figure 2).

We tested racemic 3-amidoquinuclidines as substrates for the possible hydrolysis by BChE. The catalytic steps in amides hydrolysis would involve initial formation of an enzyme-substrate complex, followed by an acylation step, and finally water would cause deacylation of the enzyme.[16] However, no hydrolysis was observed under experimental conditions described in this work. The attempts to achieve selective hydrolysis of racemic tertiary

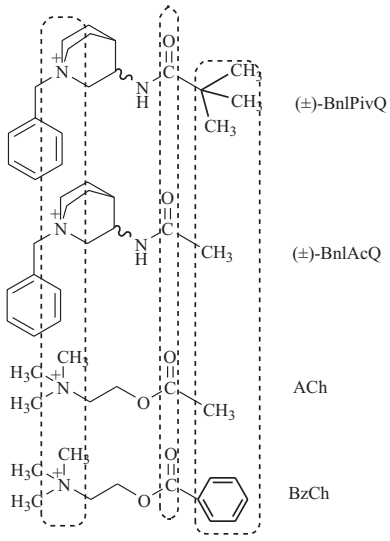


Figure 2. Comparison of amide, benzoylcholine and acethylcholine structures.

and quaternary derivatives of 3-amidoquinuclidine catalyzed by some other hydrolytic enzyme such as PPL and lipase from wheat germ, PLE (an esterase), subtilisin and chymotrypsin (proteases) and an amidase from human serum,[17] were unsuccessful.

Inhibition of BChE by Quaternary-3-amidoquinuclidines Derivatives

Since it was shown earlier that the enantiomers of *N*-quaternary quinuclidinium esters were good reversible inhibitors of BChE,[10–12] it can be assumed that the enantiomers of *N*-quaternary derivatives of 3-amidoquinuclidines act as reversible inhibitors of BChE as well. In our previous work,[18] *N*-quaternary 3-benzamido- and 3-butanamidoquinuclidinium compounds were tested as inhibitors of BChE. The degree of inhibition was shown to be dependant on the structure of the acyl group. We confirmed that the (*S*)-enantiomer of *N*-benzyl-3-benzamidoquinuclidinium bromide was the most potent inhibitor with K_i = 3.7 μmol dm^{-3}. Therefore, we tested (*R*)-BnlPivQ, (*S*)-BnlPivQ, (*R*)-BnlAcQ and (*S*)-BnlAcQ as inhibitors of BChE with BzCh as the substrate.

The measured activity of BChE at different concentrations of BzCh showed that the Michaelis-Menten kinetics was followed in a given concentration area. The value of K_M of (0.16 ± 0.02) mmol dm^{-3} was obtained from the Lineweaver-Burk plot (Figure 3) for BzCh as the substrate of BChE. This value is in accord with previously published data.[10]

The measured activity of BChE at different concentrations of both enantiomers of quaternary 3-pivalamidoquinuclidines (*R*)-BnlPivQ and (*S*)-BnlPivQ showed that the Michaelis-Menten kinetics was followed as well. The apparent increase of K_M values which were obtained from the Lineweaver-Burk plot was observed for both enantiomers of quaternary 3-pivalamidoquinuclidines (Figure 3). This led to the assumption that these 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 kinetics of inhibition by (*R*)-BnlPivQ indicated that this compound is a competitive inhibitor and therefore binds to the catalytic site of BChE. On the other hand, the (*S*)-BnlPivQ does not have the same ordinal intercept as the substrate, which indicates that this enantiomer may be a mixed noncompetitive inhibitor that binds to both the enzyme and enzyme-substrate forms of the enzyme (Figure 3). Further studies would be necessary to determine the mechanism of action of the (*S*)-BnlPivQ.

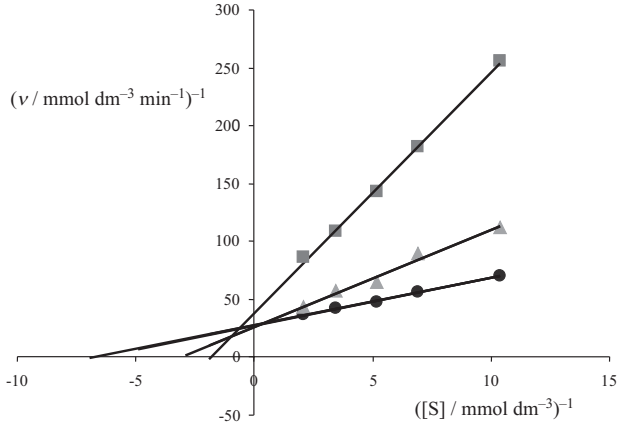


Figure 3. Lineweaver-Burk plot in the absence (●) and presence of inhibitor (*R*)-BnlPivQ (▲) and (*S*)-BnlPivQ (■). Concentration of (*R*)-enantiomer is 0.138 mmol dm^{-3} while that of (*S*)-enantiomer is 0.666 mmol dm^{-3}.

The Hunter-Downs plots of the reversible inhibition of BChE by the quaternary enantiomers of 3-pivalamidoquinuclidines (*R*)-BnlPivQ and (*S*)-BnlPivQ displayed curves that indicated competition between the inhibitors and BzCh. A linear relationship between the apparent dissociation constants and the substrate concentration between 0.1 and 0.5 mmol dm^{-3} was obtained for both enantiomers (*R*)-BnlPivQ and (*S*)-BnlPivQ (Figure 4). For these enantiomers, the enzyme-inhibitor dissociation constant (K_i) was obtained, which indicated that both enantiomers were good inhibitors of BChE. The more potent inhibitor (with the lowest dissociation constant) was the (*S*)-enantiomer with K_i = (41.57 ± 0.09) μmol dm^{-3} and it had 3-fold higher affinity for BChE than the (*R*)-enantiomer.

The obtained inhibition constants for compounds (*R*)-BnlPivQ and (*S*)-BnlPivQ are displayed in Table I.

The measured activity of BChE at different concentrations of both enantiomers of quaternary 3-acetamido-

TABLE I. Reversible inhibition of BChE measured with BzCh as a substrate in the presence of *N*-quaternary derivatives of 3-pival-amidoquinuclidine as inhibitors (constants ± SD)

Compound	[I]	[BzCh]	K_i [(a)]	K_M[(b)]
	mmol dm^{-3}	mmol dm^{-3}	μmol dm^{-3}	mmol dm^{-3}
(*R*)-BnlPivQ	0.138	0.1–0.5	128.61 ± 0.14	0.32
(*S*)-BnlPivQ	0.666	0.1–0.5	41.57 ± 0.09	0.53

[(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 the Lineweaver-Burk plot. Relative standard deviations of the K_M values were on average 15 %.

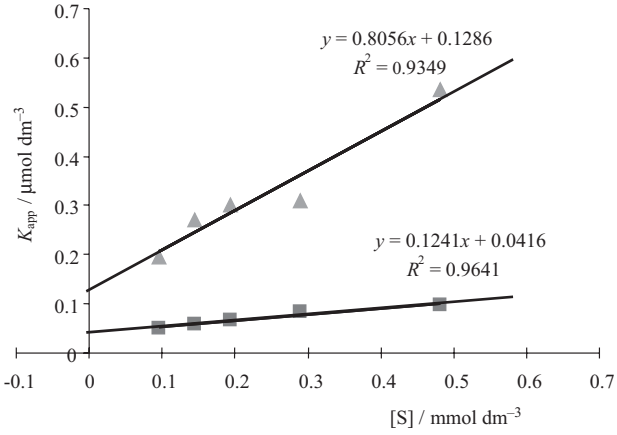


Figure 4. Reversible inhibition of butyrylcholinesterase by (*R*)-BnlPivQ (▲) and (*S*)-BnlPivQ (■). Points indicate the calculated average K_{app} from the measured activities with benzoylcholine in the presence of compounds using Eq. (1). Concentration of (*R*)-enantiomer is 0.138 mmol dm^{-3} and that of (*S*)-enantiomer is 0.666 mmol dm^{-3}.

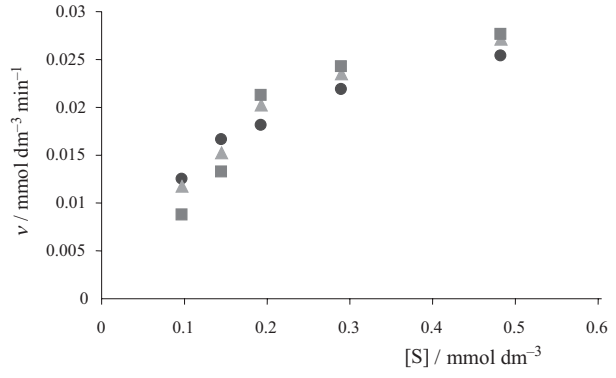


Figure 5. Measured activities of BChE in the absence (●) and presence of inhibitor (*R*)-BnlAcQ (▲) and (*S*)-BnlAcQ (■).

quinuclidines (*R*)-BnlAcQ and (*S*)-BnlAcQ showed that the Michaelis-Menten kinetics was followed as well. Preliminary results indicated that in the concentration area of BzCh between 0.1 and 0.15 mmol dm^{-3} these enantiomers may possibly act as inhibitors of BChE while in the higher concentration area they may act as possible activators of the enzyme (Figure 5). Therefore, K_i and K_M values for (*R*)-BnlAcQ and (*S*)-BnlAcQ could not be determined.

CONCLUSIONS

We have synthesized racemic and enantiomerically pure amides of 3-aminoquinuclidine with pivalic and acetic acid anhydride. Their quaternary racemic and enantiomerically pure *N*-benzyl derivatives were also prepared. Nonquaternary and quaternary synthesized racemic amides were tested as possible substrates of BChE and some other hydrolytic enzymes. However, hydrolysis was not observed. Enantiomerically pure quaternary derivatives were then tested as inhibitors of BChE. Both enantiomers of *N*-benzyl-3-pivalamidoquinuclidines were good inhibitors of BChE. However, preliminary experiments indicate that both enantiomers of *N*-benzyl-3-acetamido-quinuclidines may be inhibitors and activators of BChE depending on the concentration of BzCh.

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SAŽETAK

Derivati 3-amidokinuklidina: Priprava i interakcija s butirilkolinesterazom

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Pripravljeni su racemati te (*R*)- i (*S*)-enantiomeri 3-pivalamidokinuklidina (PivQ) i 3-acetamidokinuklidina (AcQ). Također su sintetizirani i njihovi *N*-benzilni derivati (BnlPivQ and BnlAcQ). Amidi su testirani kao supstrati i inhibitori butirilkolinesteraze iz konjskog seruma (EC 3.1.1.8). Hidroliza nije uočena u opisanim eksperimentalnim uvjetima. Uočena je, međutim, inhibicija BChE sa (*R*)- i (*S*)-enantiomerima *N*-benzilkinuklidinijevih amida pivalinske kiseline. (*S*)-BnlPivQ s K_i = 41,57 μmol dm^{-3} je 3 puta jači inhibitor u odnosu na (*R*)-enantiomer. S druge strane, preliminarni rezultati ukazuju da su oba enantiomera *N*-benzilkinuklidinijevih amida octene kiseline mogući inhibitori i aktivatori ovisno o koncentracijama benzoilkolina (BzCh) kao supstrata BChE.