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Enantiomers of Quinuclidin-3-ol Derivatives: Resolution and Interactions with Human Cholinesterases

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The (*R*)- and (*S*)-enantiomers of quinuclidin-3-ol and quinuclidin-3-yl acetate as well as their quaternary *N*-methyl and *N*-benzyl derivatives were synthesized in order to study the stereoselectivity of human erythrocyte acetylcholinesterase (EC 3.1.1.7) and plasma butyrylcholinesterase (EC 3.1.1.8). The compounds were tested as substrates and inhibitors of cholinesterases. Both cholinesterases hydrolyze the derivatives of quinuclidin-3-yl acetate with a preference for the (*R*)- over (*S*)-enantiomers. In contrast to the hydrolysis of the enantiomers of acetates, the inhibition of acetylcholinesterase and butyrylcholinesterase by the (*R*)- and (*S*)-enantiomers of quinuclidin-3-ol derivatives does not reveal enantiomeric preference of the enzymes. The (*R*)- and (*S*)-acetates also act as nonstereoselective inhibitors of the enzyme-induced hydrolysis of acetylthiocholine. The best substrate is (*R*)-*N*-methyl-3-acetoxyquinuclidinium iodide with $k_{cat} = 1.5 \times 10^6$ min^{-1} and $k_{cat} = 5.5 \times 10^4$ min^{-1} for acetylcholinesterase and butyrylcholinesterase, respectively. The (*R*)- and (*S*)-*N*-benzylquinuclidinium derivatives are the most potent inhibitors of both enzymes.

INTRODUCTION

Quinuclidine, 1-azabicyclo[2.2.2]octane, is a structural feature of several natural and synthetic, physiologically active compounds, many of which have been recognized as pharmacological agents.[1] Quinuclidine-3-ol and its esters have been found to be of special interest. Quinuclidine-3-ol and its esters may be looked upon as bicyclic analogues of acetylcholine, and therefore their interaction with acetylcholinesterase (AChE; EC 3.1.1.7) and butyrylcholinesterase (BChE; EC 3.1.1.8) can be expected. Since inhibition of AChE by phosphorylation is a key step in poisoning by organophosphorus (OP) compounds such as pesticides and warfare agents, it is reasonable to expect that these quinuclidine derivatives may interfere in the poisoning process and act as potential antidotes. It was previously found that quaternized quinuclidine-3-ol and its conjugates with imidazolium and pyridinium oximes were reversible inhibitors of AChE, while their carbamoylated derivatives progressively inhibited AChE.[2,3] It was also shown that these compounds protected mice against soman and tabun poisoning.[4,5]

Since quinuclidine-3-ol contains an asymmetric carbon atom, preparation of its esters leads to racemates. The use of racemates of bioactive compounds requires cautiousness since enantiomers can cause different biological effects, from lower activity of one of the enantiomers to increased toxicity of the unwanted enantiomer. There-

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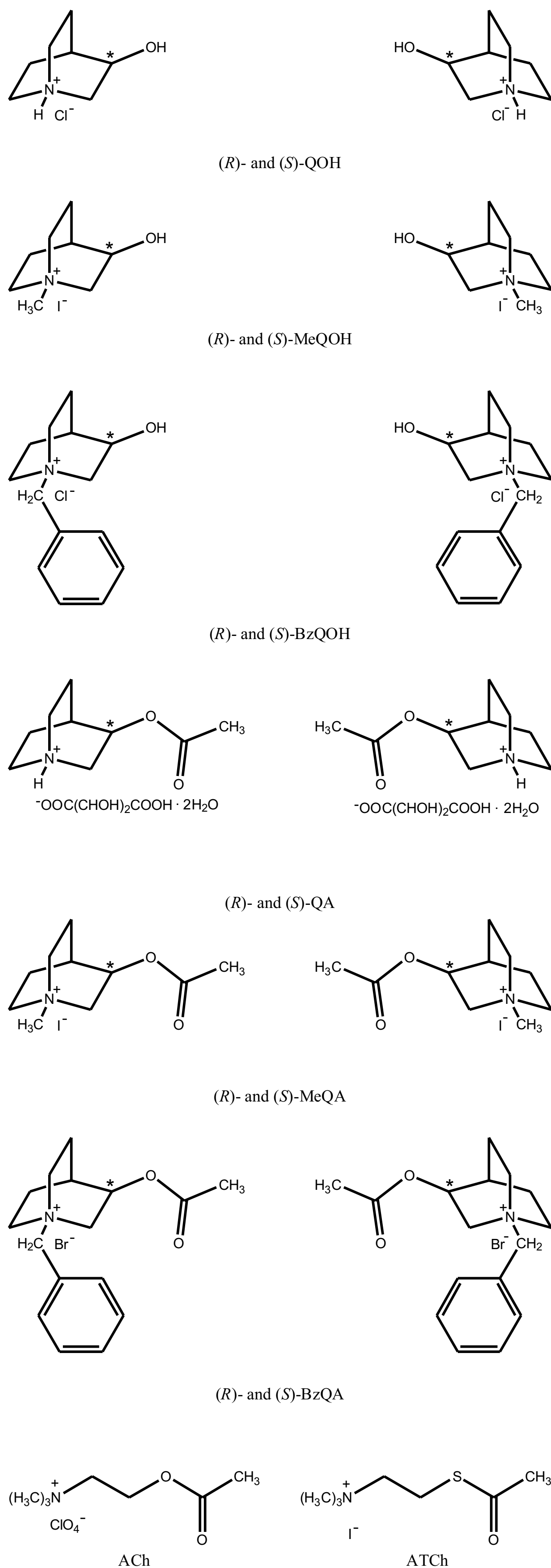

Figure 1. Structures and abbreviations of the quinuclidinium derivatives, acetylcholine and acetyltiocholine.

fore, many chemical and some biocatalytic methods were used for the separation of quinuclidine-3-ol derivatives.[6,7]

AChE and BChE revealed high stereoselectivity in inhibition by chiral progressive inhibitors as well as in their reactivation after being inhibited by chiral compounds.[8,9] The aim of this work was to study the stereoselectivity of AChE and BChE in reactions with enantiomers of quinuclidin-3-yl acetates as substrates as well as in the reversible inhibition by enantiomers of quinuclidin-3-ol derivatives (Figure 1). For that purpose (*R*)- and (*S*)- alcohols and their esters were prepared and characterized.

EXPERIMENTAL

Substrates and Inhibitors

Substrates included the synthesized (*R*)-3-acetoxyquinuclidinium L-tartarate dihydrate ((*R*)-QA), (*S*)-3-acetoxyquinuclidinium D-tartarate dihydrate ((*S*)-QA), (*R*)- and (*S*)-*N*-methyl-3-acetoxyquinuclidinium iodides (MeQA), (*R*)- and (*S*)-*N*-benzyl-3-acetoxyquinuclidinium chlorides (BzQA) as well as commercially available acetylcholine perchlorate (ACh) (BDH Chemicals Ltd., England) and acetylthiocholine iodide (ATCh) (Fluka-BioChemika, Switzerland) (Figure 1). Alcohols tested as inhibitors included the prepared (*R*)- and (*S*)-quinuclidin-3-ols (QOH), (*R*)- and (*S*)-*N*-methyl-3-hydroxyquinuclidinium iodides (MeQOH) and (*R*)- and (*S*)-*N*-benzyl-3-hydroxyquinuclidinium chlorides (BzQOH) (Figure 1). All quinuclidinium acetates were also tested as inhibitors. Stock solutions of ACh and of acetates, as further solutions, were prepared in 0.9 % sodium chloride. Stock solutions of ATCh (0.1 M) were prepared in water and those of quinuclidinium alcohols in 0.1 M phosphate buffer, pH = 7.4; further solutions were also prepared in phosphate buffer.

Synthesis of Compounds

General Remarks: Melting points were determined in open capillaries using a Büchi B-540 melting point apparatus and are uncorrected. Specific optical rotation values were determined with an Optical Activity LTD automatic polarimeter AA-10 at 589 nm in methanol and ambient temperature (≈ 24 °C). Elemental analyses were performed with a Perkin-Elmer PE 2400 Series II CHNS/O Analyser. ^{1}H and ^{13}C 1D and 2D NMR spectra were recorded with a Varian XL-GEM 300 spectrometer at ambient temperature. Chemical shifts are given in ppm downfield from TMS as internal standard.

(*R*)- and (*S*)-QOH were prepared from the commercially available racemic QOH. Esterification of racemic QOH with acetic anhydride produced the racemic acetate (QA). QA was treated with L- or D-tartaric acid to obtain diastereomeric mixtures.[10] Diastereoisomers were separated by several recrystallizations and then hydrolyzed with aqueous sodium hydroxide to obtain pure QOH enantiomers. (*R*)- and (*S*)-QOH were then the starting compounds in the prep-

aration of quaternary (*R*)- and (*S*)-MeQOH using methyl iodide as a quaternization agent.[2,10] Compounds (*R*)-BzQOH [m.p. 230.2–231.3 °C, $[\alpha]_D^{25}$ = –23.3° (*c* = 3.0; MeOH)] and (*S*)-BzQOH [m.p. 229.8–230.3 °C, $[\alpha]_D^{25}$ = +24.1° (*c* = 3.0; MeOH)] were prepared using the method previously described in the synthesis of the racemic salt.[11] ESMS: *m*/*z* ($C_{14}H_{20}NO^+$, calcd. 218.31) found 218.4. ^{1}H NMR (DMSO-d$_6$) δ/ppm: 1.60–2.05 (m, 5H, H_5, H_8 and H_4); 3.01–3.11 (m, 1H, H_2); 3.24–3.45 (m, 4H, H_6 and H_7); 3.61–3.71 (m, 1H, H_2); 3.90–4.10 (m, 1H, H_3); 4.45–4.56 (m, 2H, –CH_2–Ph); 5.77 (d, ^3J = 3.5 Hz, 1H, –O<u>H</u>), 7.48–7.52 (m, 5H, Ph). ^{13}C NMR (DMSO-d$_6$) δ/ppm: 17.73 (C_5); 20.21 (C_8); 28.55 (C_4); 51.99 (C_6); 53.98 (C_7); 59.25 (C_2); 65.12 (–<u>C</u>H_2–Ph); 67.67 (C_3); 127.34 (C_1 Ph); 129.28 (C_3, C_5 Ph); 130.44 (C_4 Ph); 133.54 (C_2, C_6 Ph).

Anal. Calcd. for $C_{14}H_{20}ClNO$ (M_r = 253.77): C 66.26, H 7.94, N 5.52; found: C 66.50, H 8.08, N 5.75 %.

Compounds (*R*)-BzQA [m.p. 204.2–205.3 °C, $[\alpha]_D^{25}$ = –11.9° (*c* = 3.0; MeOH)] and (*S*)-BzQA [m.p. 204.5–205.1 °C, +12.3° (*c* = 3.0; MeOH)] were prepared by the addition of two equivalents of benzyl bromide to the solution of the appropriate chiral quinuclidin-3-yl acetate in dry acetone. Precipitated white crystals were repeatedly washed with dry ether (yield 96 %), ESMS: *m*/*z* ($C_{16}H_{22}NO_2^+$, calcd. 260.16) found 260.1. ^{1}H NMR (DMSO-d$_6$) δ/ppm: 1.78–2.13 (m, 4H, H_5, H_8); 2.08 (s, 3H, CH_3C=O); 2.21–2.29 (m, 1H, H_4); 3.35–3.60 (m, 5H, H_6, H_7 and H_2); 3.84 (dd, 1H, H_2 ^2J = 14.5 and ^3J = 8.2 Hz); 4.57–4.69 (m, 2H, –C<u>H</u>$_2$–Ph); 4.80–5.10 (m, 1H, H_3); 7.52–7.57 (m, 5H, Ph). ^{13}C NMR (DMSO-d$_6$) δ/ppm: 17.94 (C_5); 20.56 (C_8); 28.87 (C_4); 24.14 (<u>C</u>H_3C=O); 52.27 (C_6); 53.72 (C_7); 59.62 (C_2); 65.74 (–<u>C</u>H_2–Ph); 67.28 (C_3); 127.63 (C_1 Ph); 129.05 (C_3, C_5 Ph); 130.27 (C_4 Ph); 133.19 (C_2, C_6 Ph); 170.10 (C=O).

Anal. Calcd. for $C_{16}H_{22}BrNO_2$ (M_r = 340.26): C 56.48, H 6.52, N 4.12; found: C 57.11, H 6.34, N 4.22 %.

Enzyme Sources

Sources of AChE and BChE were native human erythrocytes and native human plasma, respectively. Blood was taken from two healthy donors with the usual plasma BChE phenotype, which was determined according to the standard phenotyping procedure.[12] Blood was taken into tubes containing heparin, centrifuged for 20 min at 2500 r.p.m. and plasma was then separated from erythrocytes. For substrate hydrolysis experiments, erythrocytes were washed twice and diluted with 0.9 % sodium chloride to the original blood volume. Erythrocytes were then haemolysed with an equal volume of distilled water and frozen. For inhibition experiments, erythrocytes were washed twice and diluted with phosphate buffer to the original blood volume.

Substrate Hydrolysis

Enzyme hydrolysis of the (*R*)- and (*S*)-enantiomers of QA, MeQA and BzQA, and of acetylcholine was measured by an adapted pH-stat method originally described by Jensen-Holm *et al.*[13] The principle of the method is following. The acetic acid, the product of the enzyme hydrolysis of an acetate is titrated at constant pH = 7.4 with sodium hydroxide of known concentration. Volume of the delivered NaOH should be recorded during the reaction. The amount of the delivered NaOH is equal to the amount of acetic acid produced and that is equall to the amount of the hydrolysed acetate used as substrate. Our measurements were performed on a 718 STAT Titrino (Metrohm) with a combined pH glass LL microelectrode. The titration solution was 5.0×10^{-3} M NaOH; 0.2 M NaOH was prepared from Titrival NaOH (Kemika, Croatia) and then diluted to 5.0×10^{-3} M NaOH with distilled water free of CO_2. All measurements and solutions were prepared in 0.9 % NaCl solution. When the temperature equilibration (37 °C) was achieved, the reaction medium, containing erythrocytes or plasma was adjusted to pH = 7.4. Erythrocytes were finally diluted 40–240-fold (usually 240-fold) with 0.9 % NaCl. The final dilution for plasma BChE during the enzyme assay was 30- to 300-fold (usually 150-fold). The reaction was started by adding the substrate (0.25–1.0 mL). The total volume of the reaction mixture was 6.0 mL. Reading of the delivered NaOH per time was stopped after 5 min. Activities were calculated from the volume of 5.0×10^{-3} M sodium hydroxide delivered per minute. Activity was expressed as the amount of substrate hydrolised per minute (μmol min^{-1}) per millilitre of blood (for erythrocytes) or per millilitre of plasma. All activities were corrected for spontaneous hydrolysis of the substrates. All solutions were degassed before experiments.

Enzyme Inhibition

Inhibition of the enzymes by alcohols and acetates was measured with acetylthiocholine (ATCh) as substrate. All experiments were done in 0.1 M phosphate buffer, pH = 7.4, at 37 °C. The enzyme activity towards ATCh was measured by the Ellman spectrophotometric method using DTNB as the thiol reagent.[14] The increase of absorbance was followed at 412 nm. Measurements were done on a spectrophotometer (Cary III, Varian Inc., Australia) with an on-line computer provided with the software Cary Win UV Kinetics Application, Varian Australia Pty Ltd, Sax Software Corp, Software version 02.00(26). The following procedure was applied: suspensions of washed erythrocytes diluted to the original blood volume or plasma (0.3 mL and 0.1 mL, respectively) diluted in buffer were mixed with 0.10 mL inhibitor and DTNB (final concentration 0.33 mmol dm^{-3}). ATCh (0.1 mL) was added to the mixture and the absorbance was recorded for 3 min starting 30 s after the reagents were thoroughly mixed. Control samples contained buffer instead of inhibitor. Suspension of erythrocytes was diluted 600-fold and measurements were done against a blank that contained the erythrocyte suspension and DTNB in buffer. Final dilution of plasma BChE during the enzyme assay was 300-fold, and a solution of DTNB in buffer was used as a blank. All activities were corrected for spontaneous hydrolysis of ATCh. Final ATCh concentrations were from 0.05 to 10 mmol dm^{-3}. Concentrations of alcohols and acetates were 0.075–40 mmol dm^{-3} and 0.04–20 mmol dm^{-3}, respectively.

Catalytic Constants

The p*S*-curves of AChE and BChE were analyzed by applying the following equation, from which catalytic constants were calculated.[15]

$$v = \frac{V_{max}}{1 + \frac{K_M}{[S]} + \frac{[S]}{K_{SS}}} \quad (1)$$

where v is the enzyme activity at the given substrate concentration [S], V_{max} is the maximum rate of substrate hydrolysis, K_M is the Michaelis constant, and K_{ss} is the substrate inhibition constant.

Enzyme-inhibitor Dissociation Constants

The constants were evaluated from the kinetics of competition between ATCh and inhibitors for AChE or 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]). For each substrate concentration, the apparent dissociation constant (K_{app}) was calculated. The enzyme-inhibitor dissociation constants were evaluated from the Hunter-Downs equation using linear regression analysis.[16]

$$K_{app} = \frac{v_I \cdot [I]}{v_0 - v_I} = K_{(I)} + \frac{K_{(I)}}{K_{(S)}} \cdot [S] \quad (2)$$

If the relationship between K_{app} *vs.* [S] is linear, the intercept on the ordinate $K_{(I)}$ is either the enzyme-inhibitor dissociation constant for the catalytic site (K_a) or the dissociation constant for the peripheral site (K_i). The distinction between K_a and K_i was based on the numerical values of $K_{(S)}$, which is the intercept of the line on the abscissa of the Hunter-Downs plot (Eq. 2). The $K_{(S)}$ constants in the Hunter-Downs equation correspond either to the dissociation constants of the enzyme-substrate Michaelis complex that could be approximated to the Michaelis constant (K_M) or dissociation constants of the enzyme-substrate complex when two molecules of substrate are bound to the enzyme (K_{ss}).

If an inhibitor binds to both sites of an enzyme, K_{app} is a non-linear function of the substrate concentration. In this case, K_a can be calculated from K_{app} values determined at low substrate concentrations, and K_i from K_{app} values determined at higher substrate concentrations. At low substrate concentrations, inhibitors are expected to compete for the catalytic site (K_a), and at higher substrate concentrations for the peripheral site (K_i), the corresponding $K_{(S)}$ values being K_M and K_{ss}, respectively.[16] The peripheral anionic site of cholinesterases is located at the rim of the active site gorge and is crucial for dictating the specificity of ligands. Occupation at this site may affect the conformation of the active centre.

RESULTS

Synthesis of Chiral Quinuclidinium Derivatives

Enantiomers of QOH were prepared in three steps. In the first step, the racemic QOH was esterified with acetic anhydride to obtain the racemic acetate, which was then transformed to diastereomeric mixtures using L- or D-tartaric acids.[10] Diastereoisomers were separated by recrystallization and then hydrolyzed with aqueous sodium hydroxide to obtain (*R*)- and (*S*)-QOH. (*R*)- and (*S*)-QA were then prepared from (*R*)- and (*S*)-QOH using acetic anhydride as an esterification agent. Quaternary (*R*)- and (*S*)-MeQOH were obtained from the same alcohols by a previously described quaternization procedure using methyl iodide as a quaternization agent.[2,10] Quaternary (*R*)-BzQOH and (*S*)-BzQOH were also prepared from (*R*)- and (*S*)-QOH following the quaternization procedure reported for the synthesis of racemic BzQOH.[11] Enantiomers of BzQA were obtained from (*R*)- and (*S*)-QA by using benzyl bromide as a quaternization agent.

Synthesized compounds were characterized, identified, and their purity was established by ^{1}H NMR, ^{13}C NMR and MS spectroscopies, elemental analyses and by determining the melting points and optical rotation values.

Hydrolysis of the Acetates

All (*R*)-derivatives were hydrolyzed by both AChE and BChE while (*S*)-acetates were not hydrolyzed. The only exception was (*S*)-MeQA, which was a very poor substrate of AChE. The activities of AChE were measured with substrate concentrations between 0.5 and 20 mmol dm^{-3} and those of BChE between 1.0 and 10 mmol dm^{-3} (Figure 2). The activity measurement at lower substrate concentrations was not reliable, so only approximate values of some constants could be determined (Table I). The best substrate for both enzymes was (*R*)-MeQA. Maximum activities of AChE and BChE for this substrate were about 70 % and 60 %, respectively, compared to the maximum activities obtained with ACh as substrate. All K_M and K_{ss} values of AChE were higher for quinu-

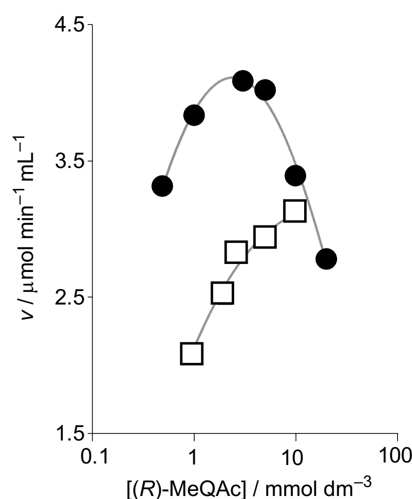

Figure 2. Concentration dependence of (*R*)-MeQA hydrolysis (v) by AChE (●) and BChE (□). Points represent the mean values of three measurements on average.

TABLE I. Catalytic constants for the hydrolysis of quinuclidine acetates, acetylcholine and acetylthiocholine catalyzed by AChE and BChE[(a)]

Compound	Acetylcholinesterase			Butyrylcholinesterase	
	K_M ± SD / mmol dm^{-3}	K_{ss} ± SD / mmol dm^{-3}	V_{max} ± SD / μmol min^{-1} mL^{-1}	K_M ± SD / mmol dm^{-3}	V_{max} ± SD / μmol min^{-1} mL^{-1}
(*R*)-QA	0.80 ± 0.10	>20[(b)]	3.0 ± 0.1	6.5 ± 1.1	1.1 ± 0.1
(*S*)-QA	–	–	–	–	–
(*R*)-MeQA	0.2[(b)]	39 ± 7	4.4 ± 0.2	0.57 ± 0.06	3.3 ± 0.0
(*S*)-MeQA	0.5[(b)]	–	0.3[(b)]	–	–
(*R*)-BzQA	<0.5[(b)]	34 ± 7	2.6 ± 0.1	0.2[(b)]	0.6[(b)]
(*S*)-BzQA	–	–	–	–	–
ACh	0.1[(b)]	24 ± 3	6.0 ± 0.2	1.3 ± 0.4	5.7 ± 0.4
ATCh	0.05 ± 0.02	13 ± 2	6.2 ± 0.4	0.38 ± 0.09	5.5 ± 0.1

[(a)] Activities of AChE are expressed per mL of whole blood and those of BChE per mL of plasma.
[(b)] Approximately estimated values.

clidinium compounds than for ACh. K_M values of BChE were lower for (*R*)-MeQA and (*R*)-BzQA than for ACh.

The k_{cat} for acetates were calculated from V_{max} values obtained for the acetates in this paper, and the concentration of AChE in human blood (3 nmol dm^{-3}) and BChE in serum (60 nmol dm^{-3}) reported in the literature.[17] For the best substrate, (*R*)-MeQA, k_{cat} values are 1.5×10^6 min^{-1} and 5.5×10^4 min^{-1} for AChE and BChE, respectively.

Inhibition of the ATCh Hydrolysis by Chiral Quinuclidin-3-ol Derivatives

The Hunter-Downs plots of the reversible inhibition of AChE and BChE by the enantiomers of quinuclidin-3-ol derivatives displayed curves for both enzymes that indicated competition between the inhibitors and ATCh at two sites of the enzymes (Figure 3). A linear relationship between the apparent dissociation constants and the substrate was obtained only for AChE and (*R*)-BzQOH. We considered two phases of competition: one at lower ATCh concentrations (up to 1 mmol dm^{-3}) and the other at higher concentrations. K_a, K_i, K_M and K_{ss} constants were determined for the substrate concentration ranges denoted (Table II).

The K_a or K_i constants for enantiomeric pairs do not differ much for any of the alcohols (Table II). For most compounds, however, the K_{app} values for the enantiomers differ significantly at higher ATCh concentrations, as it was shown for BChE and the (*R*)- and (*S*)-BzQOH (Figure 3). The strongest inhibitors (with the lowest dissociation constants) for both enzymes were both BzQOH enantiomers, with a slightly higher affinity of (*S*)- than of (*R*)-enantiomer for enzymes (Table II).

K_M and K_{ss} values obtained from the Hunter-Downs plot (Table II) are not the same but tend to the values of the respective constants obtained in the absence of an inhibitor (Table I).

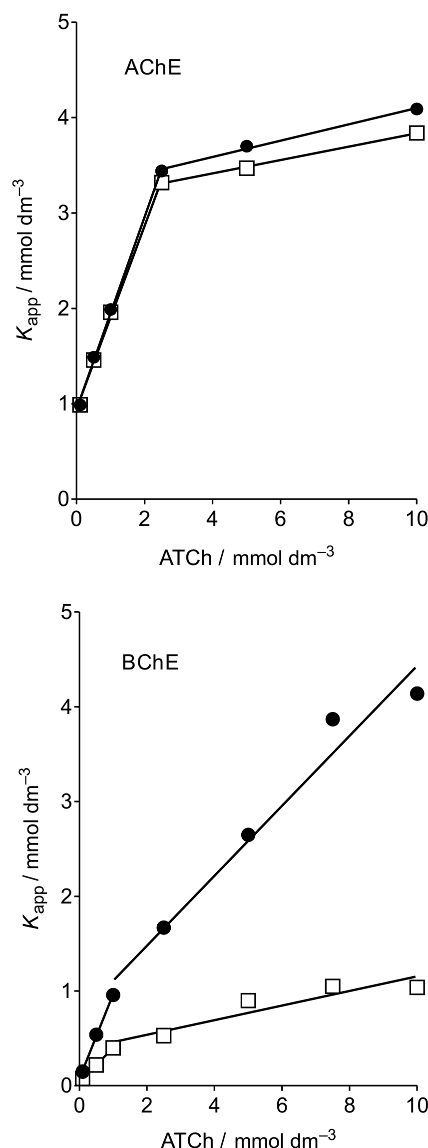

Figure 3. Inhibition of AChE by (*R*)-MeQOH (●) and (*S*)-MeQOH (□) and BChE by (*R*)-BzQOH (●), (*S*)-BzQOH (□). Activities were measured with acetythiocholine as substrate. Points represent the K_{app} values of six measurements on average.

TABLE II. Inhibition of AChE and BChE by quinuclidine alchohols[a]

Compound	K_a[b] / mmol dm^{-3}	K_M[b] / mmol dm^{-3}	K_i[c] / mmol dm^{-3}	K_{ss}[c] / mmol dm^{-3}
		Acetylcholinesterase		
(*R*)-QOH	2.6	0.31	8.3	2.5
(*S*)-QOH	4.8	0.69	8.5	4.1
(*R*)-MeQOH	0.90	0.81	3.3	38
(*S*)-MeQOH	0.90	0.83	3.1	45
(*R*)-BzQOH	–	–	0.70	2.9
(*S*)-BzQOH	0.26	0.61	0.54	3.6
		Butyrylcholinesterase		
(*R*)-QOH	3.3	0.17	17	2.2
(*S*)-QOH	2.9	0.11	18	1.5
(*R*)-MeQOH	4.4	0.48	11	3.2
(*S*)-MeQOH	1.3	0.40	4.9	3.5
(*R*)-BzQOH	0.07	0.08	0.74	2.0
(*S*)-BzQOH	0.04	0.12	0.38	5.0

[a] Dissociation constants of enzyme-inhibitor complexes K_a and K_i and catalytic constants K_M and K_{ss} were obtained from six experiments on average in the listed range of acetylthiocholine (ATCh) concentrations. Relative standard deviations of the K_a and K_i values were 19 % and those of K_M and K_{ss}, 23 %.

[b] ATCh = 0.10–1.0 mmol dm^{-3}.

[c] ATCh = 1.0–10 mmol dm^{-3}.

Inhibition of the ATCh Hydrolysis by (R)- and (S)-Quinuclidine Acetates

Inhibition by the acetate enantiomers proceeded in the same way as the inhibition by alcohols. The competitive inhibition presented in the Hunter-Downs plot gave straight lines in the range of ATCh concentration between 0.1 to 1.0 mmol dm^{-3} (Table III). The acetates were poor inhibitors of the acetylthiocholine hydrolysis catalyzed by AChE and BChE. The stereoselectivity of the enzymes was not manifested in the inhibition by the acetate enantiomers.

DISCUSSION

It was shown earlier that the stereoselectivity of hydrolysis catalyzed by AChE from *Electrophorus electricus* was in favour of the (*R*)-enantiomer in the case of (*R*)- and (*S*)-quinuclidin-3-yl acetates and their *N*-methyl derivatives.[18] BChE was used for the resolution of racemic quiniclidin-3-yl butyrate.[7] Although BChE-induced hydrolysis was also in favour of the (*R*)-enantiomer, the difference in hydrolysis rates was not sufficient to achieve complete resolution of the enantiomers. Furthermore, BChE from horse serum can be employed in stereoselective hydrolyses of quiniclidin-3-yl-benzoates and their *N*-methyl and *N*-benzyl derivatives.[19,20]

Our results do not show a qualitative difference between AChE and BChE in their reactions with the quinuclidine acetates or with their alcohol derivatives. Both AChE and BChE revealed in hydrolysis exclusive preference for the (*R*)- over (*S*)-acetates. The (*R*)-compounds evidently fit better into the active site of both enzymes than (*S*)-enantiomers. In general, all (*R*)-compounds were better substrates of AChE than BChE. (*R*)-*N*-methyl acetate was the best substrate among the tested quinuclidinium acetates for both enzymes.

Primožič *et al.*[19–21] studied the hydrolysis of the enantiomers of tertiary and quaternary *N*-methyl- and *N*-benzylquinuclidin-3-yl benzoates catalyzed by the horse serum BChE. They also found the preference for the hydrolysis of (*R*)- over that of (*S*)-enantiomers. The authors calculated the relative energies of the species involved in the hydrolytic process and analyzed their geometries in the constructed model of the active site of human BChE. These results indicate that hydrolysis is affected, to an appreciable extent, by a proper geometric orientation of substrates at the choline subsite of the enzyme, which serves as a recognition site of the substrate's quaternary ammonium groups. The main difference in binding of quinuclidinium and choline esters was found in the ammonium electrostatic region, which includes the cation-π-interaction of the ammonium moiety of substrates with the indole ring of Trp84. The important cation-π-interaction with Trp84 was lower in the case of the (*S*)-enantiomer of *N*-benzylquinuclidinium benzoate and that, as well as the steric limitations (accommodation of the benzyl group), could be the main explanation for the slower rate of the (*S*)-enantiomer hydrolysis.[20] In

TABLE III. Inhibition of AChE and BChE by quinuclidine acetates[a]

Compound	ATCh / mmol dm^{-3}	K_a / mmol dm^{-3}	K_M / mmol dm^{-3}
	Acetylcholinesterase		
(*S*)-QA	0.10 – 5.0	8.8[b]	2.2[b]
(*S*)-MeQA	0.10 – 1.0	0.61	0.22
(*S*)-MeQA	1.0 – 10	2.8[b]	2.8[b]
(*S*)-BzQA	1.0 – 10	0.14	0.63
(*R*)-MeQA[c]	0.10 – 1.0	0.3	0.1
(*R*)-BzQA[c]	0.10 – 1.0	0.4	0.4
	Butyrylcholinesterase		
(*S*)-QA	0.10 – 1.0	2.8	0.18
(*S*)-MeQA	0.10 – 1.0	1.8	0.10
(*S*)-BzQA	1.0 – 10	0.07	0.30
(*R*)-MeQA[c]	0.10 – 1.0	0.8	0.1
(*R*)-BzQA[c]	0.10 – 1.0	0.1	0.1

[a] Dissociation constants of enzyme inhibitor complexes K_a and K_i and catalytic constants K_M and K_{ss} were obtained on average from three experiments in the listed range of acetylthiocholine (ATCh) concentrations. Relative standard deviations of the constants for (*S*)-compounds were 33 %.

[b] The values refer to K_i and K_{ss}, respectively.

[c] Approximately estimated values.

the case of the tertiary (*S*)-enantiomers, it was shown that no strong interaction with the choline domain existed in the orientation of the complex necessary for the reaction.[19,21] Therefore, it can be assumed that the acylation step is additionally rate limited by the free energy spent on the pre-organization of (*S*)-ligands. The same explanations for the difference in the interaction of (*R*)- and (*S*)-enantiomers could be assumed for the derivatives of the quinuclidine acetates studied in this paper.

All tested quinuclidinium alcohols and acetates are competitive inhibitors of both AChE and BChE. The Hunter-Downs plot shows that alcohols bind to two sites of the enzymes, catalytic and peripheral sites. The affinities of both enzymes for the alcohols increase in the order QOH < MeQOH < BzQOH.

Contrary to the hydrolysis of quinuclidine acetates, there is no enantioselectivity or it is hardly manifested in the reversible inhibition of the enzymes by the quinuclidinium alcohols and acetates. Demands for steric accommodation of acylating cholinesterase substrates are more restrictive than those for the inhibition by reversible inhibitors. Acylating substrates or inhibitors, such as organophosphorus compounds, should be favourably accommodated in the active centre acyl pocket considered to be the main component of the enzyme active centre asymmetry.[8,9,22] Reversible inhibitors have more freedom to rotate within the active site gorge, and probably for this reason the enantioselectivity of the cholinesterases toward certain reversible inhibitors was found to be much lower than that found for covalent chiral inhibitors.[23]

In this paper, both human native AChE and BChE were shown to be very strongly stereoselective in the reaction with quinuclidine acetates. Therefore, both enzymes could be used in the stereoselective preparation of this class of compounds as well as for preparation of enantiomers of other esters of quinuclidin-3-ols.

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SAŽETAK

Enantiomeri kinuklidin-3-ol derivata: Razdvajanje enantiomera i interakcija s ljudskim kolinesterazama

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Priređeni su (*R*)- i (*S*)-enantiomeri kinuklidin-3-ola i kinuklidin-3-il-acetata te odgovarajući kvaterni *N*-metilni i *N*-benzilni derivati kako bi se proučila njihova interakcija s ljudskom eritrocitnom acetilkolinesterazom (EC 3.1.1.7) i butirilkolinesterazom iz plazme (EC 3.1.1.8). Spojevi su studirani kao supstrati i inhibitori tih enzima. Obje kolinesteraze pokazuju visoku stereoselektivnost pri hidrolizi kinuklidin-3-il acetata preferirajući (*R*)- u odnosu na (*S*)-enantiomere. Nasuprot hidrolizi enantiomera acetatnih derivata, inhibicija acetilkolinesteraze i butirilkolinesteraze s (*R*)- i (*S*)-enantiomerima kinuklidin-3-ola i kinuklidin-3-il-acetata te njihovih *N*-metilnih i *N*-benzilnih derivata, ne pokazuje stereoselektivnost tih enzima. Kao najbolji supstrat za oba enzima pokazao se (*R*)-*N*-metilkinuklidinijev acetat, s $k_{cat} = 1{,}5 \times 10^{6}$ min^{-1} za acetilkolinesterazu, odnosno $k_{cat} = 5{,}5 \times 10^{4}$ min^{-1} za butirilkolinesterazu. (*R*)- i (*S*)-*N*-benzilkinuklidinijevi derivati bili su najjači inhibitori za te enzime.