JPP Journal of Pharmacy and Pharmacology

JPP 2003, 55: 1397–1404

Received January 6, 2003
Accepted June 12, 2003
DOI 10.1211/0022357021855
ISSN 0022-3573

Synthesis and biological activity of pyridinium-type acetylcholinesterase inhibitors

Vildan Alptüzün, Petra Kapková, Knut Baumann, Ercin Erciyas and Ulrike Holzgrabe

Abstract

A novel series of bispyridinium-type acetylcholinesterase (AChE) inhibitors derived from obidoxime, being active in the lower micromolar range, has been reported recently. According to the hypothesis that shorter pyridinium compounds should exhibit higher activity, a new series of compounds was synthesized that has 2,6-dichlorobenzyl, 2-chlorobenzyl and phthalimidomethyl moieties, respectively, at one end of the molecule and that are systematically shortened from the contralateral end. The concentration inhibiting the AChE and butyrylcholinesterase (BChE) by 50% (IC50) was evaluated by means of Ellman's test. Compounds characterized by a phenylpropyl residue at the contralateral end (**3**) were found to have IC50 values comparable with tacrine. In addition, the affinity of **3c** toward the BChE was lower, indicating a lower degree of side effects.

Introduction

The physiological role of acetylcholinesterase (AChE) in the transmission of the peripheral nervous system has been known since the early 1900s (Giacobini 2000). However, the enzyme is still in the focus of drug development, not only in the therapy of victims of organophosphate-type insecticide and nerve-agent intoxication, such as paraoxone, soman, sarin or tabun, but also in the treatment of myasthenia gravis, glaucoma and Alzheimer's disease. In addition, AChE inhibitors are used in anaesthetic practice to rescind the skeletal muscle relaxation induced by non-depolarizing neuromuscular blocking agents (Bevan et al 1992).

A lot of dimeric compounds have been reported to show better inhibitory potency as their respective monomers (e.g. bis-galantamine (Guillou et al 2000), bis-tacrine (Carlier et al 1999a; Hu et al 2002), a huperzine A-tacrine hybrid (Carlier et al 1999b) and 1-n-bispyridiniumalkanes (Bunyapaiboonsri et al 2001). Recently, we have investigated, and systematically varied, a series of bisbenzyl ethers of the bispyridinium-type compound TMB-4, which is related to the reactivator obidoxime (see Figure 1, Kapková et al (2003)). Compounds having a 2,6-dichloro- and a 2-chlorobenzyl moiety at both ends of the molecule were found to be active in the submicromolar range of concentration.

To explain the differences in activity, the entire series of the bispyridinium compounds was docked into the AChE. Almost all of the compounds docked display a general binding mode into the gorge of the AChE. The interactions found after docking consist of π–π stacking of the benzyl residue with Trp 84 and a face-to-face contact (cation–π and π–π contacts) of the pyridinium with amino acid residues Phe 331 or Tyr 334 of the anionic substrate binding site and the peripheral anionic binding site (Trp 279) (Figure 2).

However, not the whole molecule is able to interact with amino acid residues of the enzyme. The compounds are too long, which is likely to be the reason for their reduced activity as compared with other AChE inhibitors, such as tacrine, donepezil or galantamine. This problem of the ligands has already been addressed by extensive molecular dynamics studies (Shen et al 2002). It was shown that the primary entrance to the gorge of AChE is a sort of bottleneck, especially for greater ligands. Longer ligands may

Faculty of Pharmacy, Ege-University, 35100 Bornova-Izmir, Turkey

Vildan Alptüzün, Ercin Erciyas

Institute of Pharmacy and Food Chemistry, University of Würzburg, Am Hubland, 97074 Würzburg, Germany

Petra Kapková, Knut Baumann, Ulrike Holzgrabe

Correspondence: U. Holzgrabe, Institute of Pharmacy and Food Chemistry, University of Würzburg, Am Hubland, 97074 Würzburg, Germany. E-mail: U.Holzgrabe@pharmazie.uni-wuerzburg.de

Acknowledgement and funding: Thanks are due to the DFG (Deutsche Forschungsgemeinschaft) and TUBITAK (Turkish scientific and technical research council) for a grant given to VA and the Fonds der Chemischen Industrie for financial support. The authors are grateful to Nikolaus Stiefl for the provision of Figure 1.

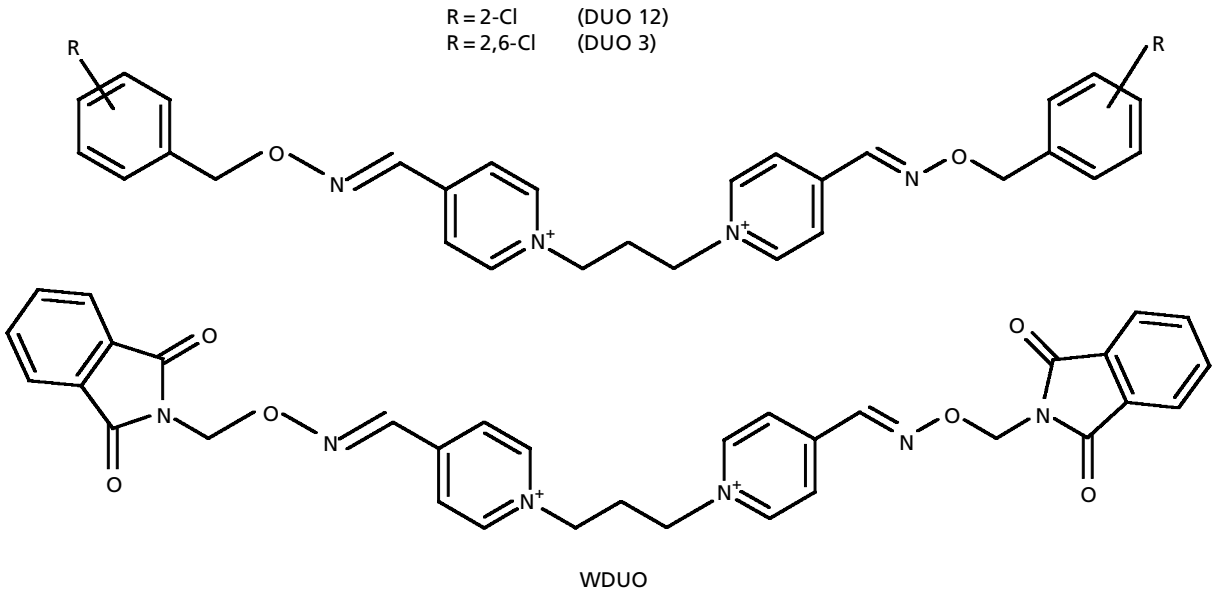


Figure 1 General structural formula of the bispyridinium-type inhibitors of AChE.

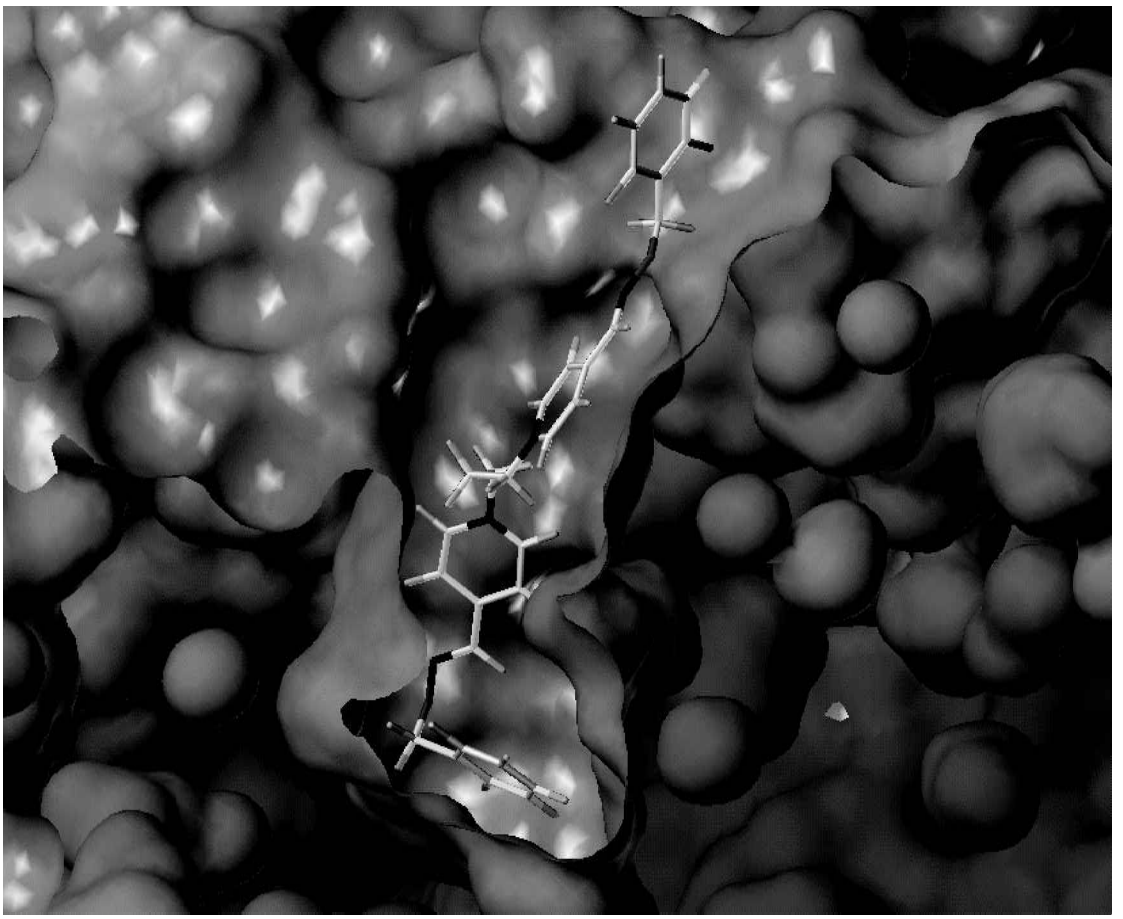


Figure 2 Bisbenzylether substituted bispyridinium-type compound DUO 3 docked into AChE using the X-ray structure of the AChE liganded with donepezil (X-ray cf. Kryger et al 1998).

escape by diffusion before fluctuations open the bottleneck wide enough to allow binding. Therefore, the purpose of this study was to synthesize compounds related to the bispyridinium-type ligands, which were systematically shortened from one end of the molecule. The most active compounds characterized by chlorobenzyl-substituted residues at the ends were taken as lead compounds for the study. In addition, the dichlorobenzyl substituent was replaced with the isoelectronic phthalimidomethyl moiety (Bejeuhr et al 1992).

Materials and Methods

Melting points were determined with a Gallenkamp and a Dr Tottoli melting point apparatus (Büchi, Switzerland) and were not corrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV 400 instrument (^{1}H 400.132 MHz; ^{13}C 100.613 MHz). Abbreviations for data quoted are: s, singlet; d, doublet; t, triplet; quin, quintet; dd, doublet of doublets; m, multiplet; br, broad. The centres of the peaks of $CDCl_3$, MeOH-d_4 and DMSO-d_6 were used as internal references. Infrared spectra were obtained using a Biorad PharmalyzIR FT-IR spectrometer. Dry solvents were used throughout. The electron impact ionisation (EI) (electron energy, 70eV; ion source temperature, 220 °C) and chemical ionisation (CI) (ion polarity, positive; reagent gas, ammonia or isobutane; electron energy, 150eV; ion source temperature, 150 °C) mass spectra were obtained on a Micromass VG Platform-II and Finnigan MAT spectrometer, respectively. The electrospray ionisation (ESI) mass spectra were measured on an Agilent 1100 LC/MSD Trap. The conditions of the spray chamber were as follows: ion polarity, positive; drying gas temperature, 300 °C; nebulizer pressure, 10 psi; drying gas flow, 5.00 L min^{-1}. Chemicals were of analytical grade. The *N*-(bromomethyl)-phthalimide and tetraoctylammonium bromide were purchased from Acros (Schwerte, Germany). The pyridine-4-carboxaldehyde-(*E*)-oxime; 2,6-dichlorobenzyl bromide and 1,3-dibromopropane were purchased from Aldrich (Steinheim, Germany). Thin-layer chromatographies were done on pre-coated silica gel 60 F_{254} plates (Merck, Darmstadt, Germany). The column chromatography was performed on silica gel 60 30–70 mesh (Merck, Darmstadt, Germany).

Benzyl and phthalimidomethyl oxime ethers were synthesized according to Kapková et al (2003); Bejeuhr et al (1992) and Botero Cid et al (1994).

General procedure for synthesis of the final compounds 1a–6c

Corresponding benzyl or phthalimidomethyl oxime ethers (2 mmol) and corresponding bromopropyl derivatives (6 mmol) in acetonitrile (60 mL) were heated at reflux for 80 h. In case of incomplete conversion, remaining oxime ethers can be removed by addition of water, extraction with diethyl ether (2×20 mL) and evaporation of water. Otherwise, after the mixture was cooled to room temperature, the solvent was removed in-vacuo and the obtained oil or solid was crystallised from solvent mixture given below.

*1-[3-(Pyridinium-1-yl)propyl]-4-[([(2-chlorophenyl)methoxy]imino)methyl]pyridinium dibromide (**1a**)*

Following the general procedure reported above, a yellowish solid was obtained and recrystallised from ethanol–ether (yield 0.45 g, 43%), mp 180–182 °C. IR ν (cm^{-1}) 1642, 1598, 1466, 846, 682. ^{1}H NMR (DMSO-d_6): δ (ppm) 2.78 (2H, *quin*, $J = 7.4$ Hz, N^+-CH_2-$\underline{CH_2}$), 4.85 (4H, *dd*, $J = 7.1/7.3$ Hz, N^+-CH_2), 5.59 (2H, *s*, OCH_2), 7.55 (2H, *m*, H-4′, H-5′), 7.70 (2H, *m*, H-3′, H-6′), 8.33 (2H, *t*, $J = 6.6$ Hz, H-3″, H-5″), 8.41 (2H, *d*, $J = 6.8$ Hz, H-3, H-5), 8.77 (1H, *t*, $J = 7.6$ Hz, H-4″), 8.78 (1H, *s*, N═CH), 9.24–9.22 (4H, *m*, H-2″, H-6″, H-2, H-6). ^{13}C NMR (DMSO-d_6): δ (ppm) 31.95 (t), 57.61 (t), 57.81 (t), 74.78 (t), 125.12 (d), 125.77 (d), 128.58 (d), 129.86 (d), 130.73 (d), 131.46 (d), 133.39 (s), 134.29 (s),145.33 (d), 145.83 (d), 146.22 (d), 146.68 (s). EI-MS m/z (% relative intensity): 246.1 [2-Cl-(C_6H_4)-CH_2-O-N═CH-(C_5H_4-N)]$^+$ (15.2), 141.1 [2-Cl-(C_6H_4)-CH_2-O]$^+$ (33.5), 139.1 (75.3), 125 (100), 111.1 (34.8), 89 (16.3), 107 (35.4), 77.1 (59.3), 51.1 (29). CI (NH_3)-MS: 247.1 (23.2), 264.1 (29.9), 175.0 (15.1), 141 (7.3), 97 (100). CI (isobutane)-MS: 247.1 (6.4), 141 (100.0), 181.1 (14.8). ESI-MS m/z: 183.5 (M^+).

*1-[3-(p-Methoxyphenyl)propyl]-4-[([(2-chlorophenyl)methoxy]imino)methyl]pyridinium bromide (**2a**)*

Following the general procedure reported above, a yellowish solid was obtained (yield 0.59 g, 62%), mp 66 °C. IR (KBr) ν (cm^{-1}) 1638, 1602, 1510, 1459, 1015, 768. ^{1}H NMR (MeOH-d_4): δ (ppm) 2.24 (2H, *quin*, $J = 7.3$ Hz, Ar-CH_2-$\underline{CH_2}$), 2.61 (2H, *t*, $J = 7.3$ Hz, Ar-CH_2), 3.64 (3H, *s*, OCH_3), 4.49 (2H, *t*, $J = 7.3$ Hz, N^+-CH_2), 5.39 (2H, *s*, OCH_2), 6.71 (2H, *d*, $J = 8.6$ Hz, H-3″, H-5″), 7.01 (2H, *d*, $J = 8.6$ Hz, H-2″, H-6″), 7.24 (2H, *m*, H-4′, H-5′), 7.34 (1H, *m*, H-6′), 7.43 (1H, *m*, H-3′), 8.06 (2H, *d*, $J = 6.8$ Hz, H-3, H-5), 8.33 (1H, *s*, N═CH), 8.78 (2H, *d*, $J = 6.8$ Hz, H-2, H-6). ^{13}C NMR (MeOH-d_4): δ (ppm) 31.81 (t), 32.91 (t), 55.03 (q), 62.83 (t), 76.62 (t), 114.48 (d), 125.24 (d), 127.56 (d), 129.71 (d), 130.01 (d), 130.50 (d), 131.36 (d), 132.35 (d), 134.37 (s), 134.86 (s), 145.52 (d), 145.74 (d), 149.07 (s), 159.17 (s). EI-MS m/z (% relative intensity): 246 (8.1), 142 (42.3), 141 (14.4), 139 (26.8), 125 (100), 107 (42.8), 77 (62.8), 51 (19.8). ESI-MS m/z: 395.2 (M^+).

*1-(3-Phenylpropyl)-4-[([(2-chlorophenyl)methoxy]imino)methyl]pyridinium bromide (**3a**)*

Following the general procedure reported above, a yellowish oil was obtained and recrystallised from acetone–ether (yield 0.77 g, 87%), mp 81–82 °C. IR (KBr) ν (cm^{-1}) 1638, 1597, 1458, 1055, 920, 751, 698. ^{1}H NMR ($CDCl_3$): δ (ppm) 2.34 (2H, *quin*, $J = 7.5$ Hz, N^+-CH_2-$\underline{CH_2}$-CH_2-Ar), 2.72 (2H, *t*, $J = 7.3$ Hz, Ar-CH_2), 4.93 (2H, *t*, $J = 7.1$ Hz, N^+-CH_2), 5.38 (2H, *s*, OCH_2), 7.09 (2H, *m*, H-4′, H-5′), 7.14–7.24 (5H, *m*, Ar-H), 7.35 (2H, *m*, H-3′, H-6′), 8.01 (2H, *d*, $J = 6.3$ Hz, H-3, H-5), 8.19 (1H, *s*,

N=CH), 9.27 (2H, *d*, $J = 6.5$ Hz, H-2, H-6). ^{13}C NMR ($CDCl_3$): δ (ppm) 32.54 (t), 33.33 (t), 61.56 (t), 72.25 (t), 125.07 (d), 126.95 (d), 127.44 (d), 128.78 (d), 129.16 (d), 130.11 (d), 130.40 (d), 131.01 (d), 134.04 (s), 134.33 (s), 139.76 (d), 144.34 (d), 145.56 (d), 148.39 (s). EI-MS m/z (% relative intensity): 246 (4.7), 142 (38.8), 141 (13.2), 139 (19.2), 125 (100), 107 (42.6), 89.1 (22.5), 77 (71.4), 51.1 (19.6). ESI-MS m/z: 365.2 (M^+).

*1-(3-Hydroxypropyl)-4-[([(2-chlorophenyl) methoxy]imino)methyl]pyridinium bromide (**4a**)*

Following the general procedure reported above, a yellowish oil was obtained (yield 0.20 g, 26%). IR (KBr) ν (cm^{-1}) 1641, 1599, 1464, 931, 846, 762. ^{1}H NMR (MeOH-d_4): δ (ppm) 2.11 (2H, *quin*, $J = 6.8$ Hz, N^+CH_2-$\underline{CH_2}$), 3.50 (2H, *t*, $J = 5.6$ Hz, N^+CH_2), 4.63 (2H, *t*, $J = 6.9$ Hz, HO-CH_2), 5.39 (2H, *s*, OCH_2), 7.24 (2H, *m*, H-4′, H-5′), 7.34 (1H, *m*, H-6′), 7.43 (1H, *m*, H-3′), 8.13 (2H, *d*, $J = 6.8$ Hz, H-3, H-5), 8.37 (1H, *s*, N=CH), 8.86 (2H, *d*, $J = 6.8$ Hz, H-2, H-6). ^{13}C NMR (MeOH-d_4): δ (ppm) 34.77 (t), 59.26 (t), 60.67 (t), 76.60 (t), 126.30 (d), 126.53 (d), 128.63 (d), 131.05 (d), 131.54 (d), 132.42 (d), 135.39 (s), 135.93 (s), 146.87 (d), 150.14 (s). EI-MS m/z (% relative intensity): 246 (2.6), 142 (50.6), 125 (100), 113 (16.2), 107 (56.9), 89.1 (23.2), 79 (63.0), 77 (82.4), 51 (22.2). ESI-MS m/z: 305.1 (M^+).

*1-(Propyl)-4-[([(2-chlorophenyl)methoxy] imino)methyl]pyridinium bromide (**5a**)*

Following the general procedure reported above, a white solid was obtained and recrystallised from acetone–ether (yield 0.60 g, 82%), mp 43–45 °C. IR (KBr) ν (cm^{-1}) 1640, 1596, 1460, 991, 847, 753. ^{1}H NMR (DMSO-d_6): δ (ppm) 0.93 (3H, *t*, $J = 7.3$ Hz, CH_3), 1.98 (2H, *sextet*, $J = 7.3$ Hz, N^+-CH_2-$\underline{CH_2}$), 4.61 (2H, *t*, $J = 7.3$ Hz, N^+-CH_2), 5.51 (2H, *s*, OCH_2), 7.43–7.50 (2H, *m*, H-4′, H-5′), 7.57–7.64 (2H, *m*, H-3′, H-6′), 8.29 (2H, *d*, $J = 6.8$ Hz, H-3, H-5), 8.67 (1H, *s*, N=CH), 9.11 (2H, *d*, $J = 6.6$ Hz , H-2, H-6). ^{13}C NMR (DMSO-d_6): δ (ppm) 11.11 (q), 24.97 (t), 62.73 (t), 75.28 (t), 125.50 (d), 128.33 (d), 130.50 (d), 131.26 (d), 132.07 (d), 133.96 (s), 134.86 (s), 146.15 (d), 147.21 (d), 147.76 (s). EI-MS m/z (% relative intensity): 246 (8.9), 142 (36.1), 139 (23.4), 125 (100), 111 (12.7), 107 (34.8), 89.1 (17.6), 79 (55.6), 79 (36.7), 63.1 (9.3), 51.1 (17.2). ESI-MS m/z: 289.1 (M^+).

*1-(Ethyl)-4-[([(2-chlorophenyl)methoxy] imino)methyl]pyridinium bromide (**6a**)*

Following the general procedure reported above, a yellowish oil was obtained (yield 0.31 g, 44%). IR (KBr) ν (cm^{-1}) 1641, 1599, 1464, 930, 847, 762. ^{1}H NMR (MeOH-d_4): δ (ppm) 1.68 (3H, *t*, $J = 7.3$ Hz, CH_3), 4.69 (2H, *q*, $J = 7.3$ Hz, N^+-CH_2), 5.51 (2H, *s*, OCH_2), 7.36 (2H, *m*, H-4′, H-5′), 7.44–7.56 (2H, *m*, H-3′, H-6′), 8.26 (2H, *d*, $J = 6.8$ Hz, H-3, H-5), 8.49 (1H, *s*, N=CH), 9.00 (2H, *d*, $J = 6.8$ Hz , H-2, H-6). ^{13}C NMR (MeOH-d_4): δ (ppm) 17.05 (q), 58.56 (t), 76.58 (t), 126.46 (d), 128.64 (d), 131.05 (d), 131.55 (d), 132.43 (d), 135.39 (s), 135.93 (s), 146.36 (d), 146.87 (d), 150.08 (s). EI-MS m/z (% relative intensity): 246.1 (7.5), 142.1 (23), 125 (100), 111 (7.7), 107 (26.1), 89 (18.6), 79 (31.2), 77 (49.8), 51.1 (14.8). ESI-MS m/z: 275.1 (M^+).

*1-[3-(Pyridinium-1-yl)propyl]-4-[([(2,6-dichlorophenyl)methoxy]imino) methyl]pyridinium dibromide (**1b**), 1-[3-(p-Methoxyphenyl)propyl)]-4-[([(2,6-dichlorophenyl)methoxy]imino)methyl] pyridinium bromide (**2b**) and 1-(3-phenylpropyl)-4-[([(2,6-dichlorophenyl) methoxy] imino) methyl]pyridinium bromide (**3b**)*

Compounds **1b**, **2b** and **3b** were synthesized according to Botero Cid et al (1994).

*1-(3-Hydroxypropyl)-4-[([(2,6-dichlorophenyl) methoxy]imino)methyl]pyridinium bromide (**4b**)*

Following the general procedure reported above, an extremely hygroscopic white solid was obtained (yield 0.32 g, 39%), mp 38–39 °C. IR (KBr) ν (cm^{-1}) 1640, 1601, 1437, 932, 768. ^{1}H NMR (DMSO-d_6): δ (ppm) 2.13 (2H, *quin*, $J = 6.3$ Hz, N^+CH_2-$\underline{CH_2}$) 3.51 (2H, *t*, $J = 6.8$ Hz N^+CH_2), 4.71 (2H, *t*, $J = 6.8$ Hz, HO-CH_2), 5.64 (2H, *s*, OCH_2), 7.53 (1H, *dd*, $J = 7.0/7.1$ Hz, H-4′), 7.63 (2H, *d*, $J = 7.0$ Hz, H-3′, H-5′), 8.24 (2H, *d*, $J = 6.8$ Hz, H-3, H-5), 8.63 (1H, *s*, N=CH), 9.13 (2H, *d*, $J = 6.8$ Hz, H-2, H-6). ^{13}C NMR (MeOH-d_4): δ (ppm) 33.26 (t), 57.30 (t), 58.65 (t), 71.68 (t), 124.56 (d), 128.97 (d), 131.40 (s), 131.98 (d), 136.43 (s), 145.63 (d), 146.40 (d), 146.84 (s). EI-MS m/z (% relative intensity): 280.1 [2,6-Cl-(C_6H_3)-CH_2-O-N = CH-(C_5H_4-N)]$^+$ (9.2), 176 (28.4),159 (100), 139 (13.4), 123 (13.3), 113 (29), 111 (22), 104 (19.9), 89 (9.7), 77.1 (48.8), 63 (10.3), 51.1 (11.1). ESI-MS m/z: 339.1 (M^+).

*1-(Propyl)-4-[([(2,6-dichlorophenyl)methoxy] imino)methyl]pyridinium bromide (**5b**)*

Compound **5b** was synthesized according to Botero Cid et al (1994).

*1-(Ethyl)-4-[([(2,6-dichlorophenyl) methoxy]imino)methyl]pyridinium bromide (**6b**)*

Following the general procedure reported above, a white solid was obtained (yield 0.38 g, 49%), mp 87.3 °C. IR (KBr) ν (cm^{-1}) 1641, 1599, 1462, 956, 766. ^{1}H NMR (MeOH-d_4): δ (ppm) 1.68 (3H, *t*, $J = 7.5$ Hz, CH_3), 4.69 (2H, *q*, $J = 7.3$ Hz, N^+-CH_2), 5.69 (2H, *s*, OCH_2), 7.38 (1H, *dd*, $J = 7.1/7.1$ Hz, H-4′), 7.47 (2H, *d*, $J = 7.4$ Hz, H-3′, H-5′), 8.24 (2H, *d*, $J = 6.8$ Hz, H-3, H-5), 8.42 (1H, *s*, N=CH), 9.00 (2H, *d*, $J = 6.8$ Hz , H-2, H-6). ^{13}C NMR (MeOH-d_4): δ (ppm) 17.03 (q), 58.56 (t), 73.46 (t), 126.40 (d), 130.20 (d), 132.80 (d), 133.37 (s), 138.73 (s), 146.39 (d), 146.74 (d), 150.06 (s). EI-MS m/z (% relative intensity): 280 (13.7), 178 (13.3), 159 (100), 141 (26.8), 123 (11.9), 113 (24.3), 111 (15.8), 89 (10.2), 77.1 (46), 51.1 (10.9). ESI-MS m/z: 309.1 (M^+).

*1-[3-(Pyridinium-1yl)propyl]-4-[([phthalimidomethoxy]imino)methyl] pyridinium dibromide (**1c**)*

Following the general procedure reported above, a yellowish solid was obtained and recrystallised from methanol–ether (yield 0.45 g, 40%), mp 238–239 °C. IR (KBr) ν (cm^{-1}) 1775, 1720 1425, 1185, 775, 715. ^{1}H NMR (MeOH-d_4): δ (ppm) 2.71 (2H, *quin*, $J = 7.9$ Hz, N^+-CH_2-$\underline{CH_2}$), 4.78 (4H, *m* , 2(N^+-CH_2), 5.73 (2H, *s*, OCH_2), 7.77 (2H, *m*, H-4′, H-5′), 7.82 (2H, *m*, H-3′, H-6′), 8.07 (2H, *t*, $J = 6.9$ Hz, H-3″, H-5″), 8.17 (2H, *d*, $J = 6.7$ Hz, H-3, H-5), 8.36 (1H, *s*, N═CH), 8.54 (1H, *t*, $J = 7.9$ Hz, H-4″), 9.01 (2H, *d*, $J = 6.9$ Hz, H-2″, H-6″*), 9.04 (2H, *d*, $J = 6.6$ Hz, H-2, H-6*) *exchangeable. ^{13}C NMR (MeOH-d_4): δ (ppm) 33.97 (t), 59.44 (t), 59.69 (t), 72.16 (t), 125.17 (d), 126.90 (d), 130.18 (d), 133.44 (s), 136.50 (s), 146.68 (d), 147.01 (d), 147.86 (d), 148.24 (s), 168.88 (s). EI-MS m/z (% relative intensity): 160 [$C_9H_6NO_2$] $^+$ (44),147 (15), 130 (12), 105 (24), 104 (48), 103 (33), 79 (38), 90 (12), 80 (10), 78 (14), 77 (42), 76 (100), 74 (47), 64 (12), 63 (20), 60 (10) , 52 (13), 51 (41), 50 (98), 44 (27), 43 (44), 39 (24), 38 (25), 37 (24), 32 (30), 31 (29), 30 (12). ESI-MS m/z: 201.0 (M^+).

*1-[3-(p-Methoxyphenyl)propyl)]-4-[([phthalimidomethoxy]imino)methyl] pyridinium bromide (**2c**)*

Following the general procedure reported above, a yellowish oil was obtained and recrystallised from methanol–ether (yield 0.37 g, 37%), mp 65 °C. IR (KBr) ν (cm^{-1}) 1775, 1720 1425, 1185, 775, 715. ^{1}H NMR (DMSO-d_6): δ (ppm) 2.23 (2H, *m*, Ar-CH_2-CH_2), 2.57 (2H, *t*, $J = 7.1$ Hz, Ar-CH_2), 3.69 (3H, *s*, OCH_3), 4.60 (2H, *t*, $J = 7.2$ Hz, N^+-CH_2), 5.75 (2H, *s*, OCH_2), 6.82 (2H, *d*, $J = 8.5$ Hz, H-3″, H-5″), 7.11 (2H, *d*, $J = 8.5$ Hz, H-2″, H-6″), 7.92 (2H, *m*, H-3′, H-6′), 7.98 (2H, *m*, H-4′, H-5′), 8.14 (2H, *d*, $J = 6.6$ Hz, H-3, H-5), 8.61 (1H, *s*, N = CH), 9.09 (2H, *d*, $J = 6.6$ Hz, H-2, H-6). ^{13}C NMR (DMSO-d_6): δ (ppm) 31.52 (t), 32.94 (t), 55.83 (q), 61.33 (t), 71.26 (t), 114.69 (d), 124.62 (d), 125.56 (d), 126.88 (d), 128.95 (s), 130.06 (d), 132.08 (d), 132.87 (s), 136.04 (d), 146.27 (d), 147.32 (s), 148.40 (d), 158.50 (s), 167.72 (s). EI-MS m/z (% relative intensity): 160 (8), 105 (23), 104 (25), 103 (17), 79 (76), 90 (10), 82 (58), 81 (48), 80 (61), 79 (78), 77 (50), 76 (66), 75 (25), 74 (56), 65 (27), 64 (17), 63 (20), 53 (11), 52 (17), 51 (43), 50 (100), 47 (46), 44 (26), 43 (41), 42 (18), 41 (17), 40 (21), 39 (28), 38 (27), 37 (25), 32 (67). ESI-MS m/z: 430.2 (M^+).

*1-(3-Phenylpropyl)-4-[([phthalimidomethoxy]imino)methyl] pyridinium dibromide (**3c**)*

Following the general procedure reported above, a yellowish solid was obtained and recrystallised from methanol–ether (yield 0.39 g, 41%), mp 120 °C. IR (KBr) ν (cm^{-1}) 1780, 1730, 1470, 1190, 760, 725. ^{1}H NMR ($CDCl_3$): δ (ppm) 2.38 (2H, *quin*, $J = 7.3$ Hz, N^+-CH_2-$\underline{CH_2}$-CH_2-Ar), 2.77 (2H, *t*, $J = 7.8$ Hz, Ar-CH_2), 5.03 (2H, *t*, $J = 7.3$ Hz, N^+-CH_2), 5.78 (2H, *s*, OCH_2), 7.10–7.19 (5H, *m*, Ar-H), 7.77 (2H, *m*, H-4′, H-5′), 7.91 (2H, *m*, H-3′, H-6′), 8.13 (2H, *d*, $J = 6.6$ Hz, H-3, H-5), 8.31 (1H, *s*, N═CH), 9.39 (2H, *d*, $J = 6.6$ Hz , H-2, H-6). ^{13}C NMR ($CDCl_3$): δ (ppm) 32.50 (t), 33.41 (t), 61.61 (t), 70.84 (t), 124.48 (d), 125.51 (d), 126.88 (d), 128.79 (d), 129.11 (d), 131.99 (s), 135.23 (d), 139.88 (s), 145.76 (d), 146.06 (d), 147.75 (s), 167.34 (s). EI-MS m/z (% relative intensity): 160 (9), 103 (12), 91 (100), 89 (17), 79 (13), 78 (15), 77 (16), 76 (12), 74 (11), 65 (31), 64 (10), 63 (18), 62 (15), 52 (11), 51 (30), 50 (35), 44 (12), 43 (13), 41 (13), 39 (36), 38(14), 32 (13). ESI-MS m/z: 400.2 (M^+).

*1-(3-Hydroxypropyl)-4-[([phthalimidomethoxy]imino)methyl] pyridinium dibromide (**4c**)*

Following the general procedure reported above, a white solid was obtained and recrystallised from ethanol–ether (yield 0.41 g, 49%), mp 172 °C. IR (KBr) ν (cm^{-1}) 3375, 1775, 1730, 1430, 1190, 730. ^{1}H NMR ($CDCl_3$ + MeOH-d_4): δ (ppm) 2.19–2.24 (2H, *m*, N^+CH_2-$\underline{CH_2}$), 3.62 (2H, *t*, $J = 5.7$ Hz, HO-CH_2), 4.79 (2H, *t*, $J = 7.1$ Hz, N^+-CH_2), 5.82 (2H, *s*, OCH_2 *Z*), 7.80 (2H, *m*, H-4′, H-5′), 7.91 (2H, *m*, H-3′, H-6′), 8.18 (2H, *d*, $J = 6.7$ Hz, H-3, H-5), 8.31 (1H, *s*, N═CH), 9.05 (2H, *d*, $J = 6.8$ Hz, H-2, H-6). ^{13}C NMR ($CDCl_3$ + MeOH-d_4): δ (ppm) 33.70 (t), 57.95 (t), 60.00 (t), 71.10 (t), 124.35 (d), 124.38 (d), 125.58 (d), 129.03 (d), 131.90 (s), 135.30 (s), 135.32 (d), 142.94 (d), 145.68 (d), 145.96 (d), 146.30 (d), 148.04 (s), 167.57 (s). EI-MS m/z (% relative intensity): 160 (5), 105 (15), 104 (31), 103 (25), 79 (8), 77 (26), 76 (61), 75 (25), 74 (52), 63 (11), 61(11), 52 (13), 50 (100), 44 (13), 43 (48), 41 (12), 40 (10), 39 (22), 38 (27), 37 (32), 32 (39), 31 (26), 30 (16). ESI-MS m/z: 340.1 (M^+).

*1-(Propyl)-4-[([phthalimidomethoxy]imino)methyl]pyridinium bromide (**5c**)*

Following the general procedure reported above, a white solid was obtained and recrystallised from methanol–ether (yield 0.46 g, 58%), mp 207–208 °C. IR (KBr) ν (cm^{-1}) 1775, 1730, 1435, 1190, 730. ^{1}H NMR ($CDCl_3$): δ (ppm) 1.03 (3H, *t*, $J = 7.4$ Hz, CH_3), 2.10 (2H, *m*, N^+-CH_2-$\underline{CH_2}$), 5.00 (2H, *t*, $J = 7.4$ Hz, N^+-CH_2), 5.83 (2H, *s*, OCH_2), 7.82 (2H, *m*, H-4′, H-5′), 7.95 (2H, *m*, H-3′, H-6′), 8.25 (2H, *d*, $J = 6.6$ Hz, H-3, H-5), 8.37 (1H, *s*, N═CH), 9.50 (2H, *d*, $J = 6.6$ Hz , H-2, H-6). ^{13}C NMR ($CDCl_3$): δ (ppm) 10.93 (q), 25.68 (t), 63.44 (t), 70.86 (t), 124.50 (d), 125.56 (d), 132.01 (s), 135.22 (d), 145.85 (d), 146.05 (d), 147.78 (s), 167.35 (s). EI-MS m/z (% relative intensity): 160 (8), 147 (11), 105 (12), 104 (42), 103 (35), 77 (34), 76 (100), 74 (47), 60 (11), 51 (21), 50 (73), 45 (11), 43 (55), 41(19), 40 (11), 39 (26), 38 (25), 37 (27), 32 (14), 31 (15). ESI-MS m/z: 324.1 (M^+).

*1-(Ethyl)-4-[([phthalimidomethoxy]imino)methyl]pyridinium bromide (**6c**)*

Following the general procedure reported above, a white solid was obtained and recrystallised from methanol–ether (yield 0.47 g, 61%), mp 185 °C. IR (KBr) ν (cm^{-1}) 1775, 1730, 1435, 1190, 730, 710. ^{1}H NMR ($CDCl_3$): δ ppm 1.73 (3H, *t*, $J = 7.4$ Hz, CH_3), 5.08 (2H, *q*, $J = 7.4$ Hz, N^+-CH_2), 5.83 (2H, *s*, OCH_2), 7.82 (2H, *m*, H-4′, H-5′), 7.95–7.96 (2H, *m*, H-3′, H-6′), 8.24 (2H, *d*, $J = 6.6$ Hz, H-3, H-5), 8.36 (1H, *s*, N═CH), 9.48 (2H, *d*, $J = 6.6$ Hz, H-2, H-6). ^{13}C NMR ($CDCl_3$): δ ppm 17.12 (q), 57.30 (t), 70.43 (t),

124.07 (d), 125.29 (d), 131.58 (s) 134.79 (d), 145.28 (d), 145.60 (d), 147.7 (s), 166.93 (s). EI-MS m/z (% relative intensity): 160 (16) ,147 (24), 142 (13), 130 (9), 105 (15), 104 (56), 103 (39), 79 (9), 77 (24), 76 (100), 74 (43), 63 (9), 52 (8), 51 (14), 50 (78), 43 (23), 39 (9), 38 (14), 37 (15), 32 (21), 31 (15), 30 (15). ESI-MS m/z: 310.1 (M^+).

Biological assay

The inhibitory potency towards AChE and butyrylcholinesterase (BChE) was evaluated according to Ellman et al (1961) – details of the procedure are given in Kapková et al (2003).

Data analysis

Results for the IC50 values are shown as the means ± s.e.m. Six replicates were taken for the control group (first line in Table 1; starting point of the optimization), whereas the newly synthesized compounds were measured in triplicate. The nonparametric Kruskal-Wallis test was carried out for each series (each column in Table1) to check whether there is a significant influence of the varying substituents. If a statistically significant change in activity was detected with the Kruskal-Wallis test, a post-hoc multiple comparison procedure with the control was carried out within each series to figure out which compound was more active than the control (one-sided test). This was accomplished with the nonparametric Steel-procedure (Miller 1981). Since the significance value for the Steel-test was not tabulated for the number of replicates and the number of compounds synthesized in this study, the respective value was simulated according to Critchlow & Fligner (1991) with the obvious modifications for the Steel-test. A statistical hypothesis test was deemed significant when $P < 0.10$. Since a multiple comparison with the control within each series was used, no statement about statistical significance between the series can be made. The correlation between the three series was computed as the nonparametric Spearman rank correlation coefficient.

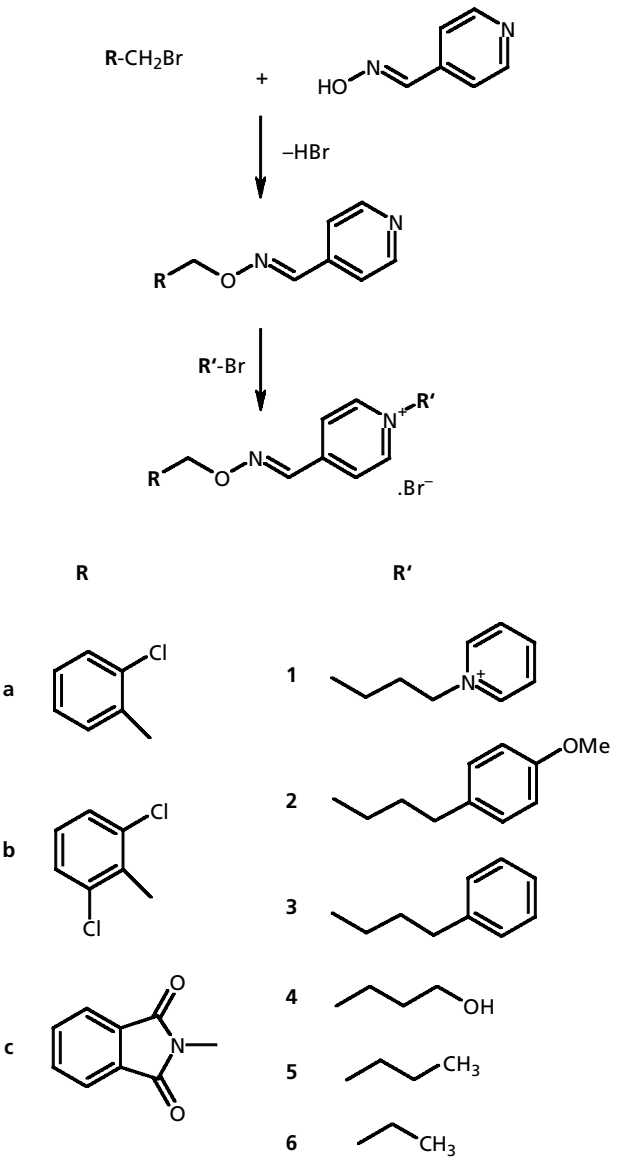


Figure 3 Synthesis pathway of the compounds studied.

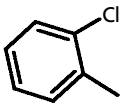
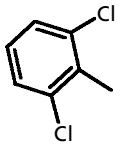
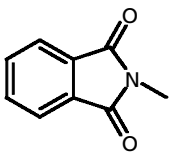
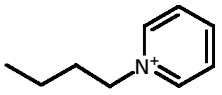
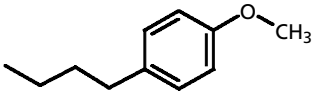
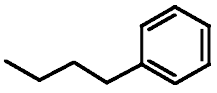
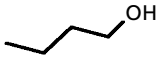
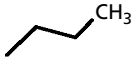
Results and Discussion

Benzyl and phthalimidomethyl oxime ethers were synthesized according to Kapková et al (2003) and Botero Cid et al (1994). *N*-Alkylation of the pyridinium ring was achieved by conversion with the corresponding bromo compound (i.e. 3-pyridiniumpropyl bromide, 3-(*p*-methoxyphenyl) propylbromide, phenylpropylbromide, 3-hydroxypropylbromide, propylbromide and ethylbromide) in refluxing acetonitrile (Figure 3). The WDUO and DUO 3 were synthesized according to Bejeuhr et al (1992).

The affinity to AChE was evaluated by means of the Ellman's test (Ellman et al 1961). The data reported in Table 1 and visualized in Figure 4 will be discussed following the step-wise cutting off of the moieties at one end of the molecule. Comparing the inhibition activity of both chlorobenzyl-substituted DUO compounds and the phthalimido compound WDUO, it was revealed that shortening the molecules in a step-wise manner results in similar changes in activity (e.g. the highest activity is attained with the same substituent; see Table 1). Spearman rank correlation coefficients (r_S) for the three series **a**, **b** and **c** (including the ditopic compounds) underline this finding. The respective correlation coefficients are $r_S(a,b) = 0.78$, $r_S(a,c) = 0.54$, and $r_S(b,c) = 0.60$. It can be seen that series **a** and **b** are more similar to each other than their respective similarity to series **c**. However, all three correlation coefficients are significant at the 5% level (type I error).

Checking whether the newly synthesized compounds varied significantly in activity was done with the help of the Kruskal-Wallis test within each series. The respective test statistics were significant for each series. The following structure–activity relationships could be observed.

Table 1 AChE inhibitory activity of compounds **1a**–**6c**.

No.	R/R′	IC50 (μM)		
		a 	**b** 	**c** 
	Ditopic compounds	0.58 ± 0.18* (DUO 12)	0.34 ± 0.05* (DUO 3)	4.57 ± 0.19 (WDUO)
1		7.16 ± 0.73	1.44 ± 0.09	13.66 ± 2.0
2		1.53 ± 0.12	0.27 ± 0.044	1.72 ± 0.36
3		0.48 ± 0.006	0.18 ± 0.007	0.073 ± 0.02
4		6.19 ± 0.66	2.26 ± 0.007	11.09 ± 1.9
5		6.70 ± 1.83	2.39 ± 0.36	3.78 ± 0.68
6		13.10 ± 2.08	4.85 ± 0.41	6.02 ± 0.61

The pharmacological data are the mean ± s.e.m., n = 3–6 experiments. IC50, concentration inhibiting activity by 50%. *Kapková et al (2003).

Cutting off the benzyl- and phthalimidomethyloxime ether (**1a**–**c**), respectively, from the ditopic compounds DUO 12, DUO 3, and WDUO resulted in a loss in affinity. Replacing the positively charged pyridinium ring with the neutral *p*-methoxyphenyl rings (**2a**–**c**) retained the affinity again to the range of concentration of the ditopic compounds. Omitting the methoxy group on the phenyl ring (**3a**–**c**) gave the compounds with the highest activity in each series. For series **b** and **c** these compounds are significantly more active than the respective ditopic compound (one-sided Steel-test; see data analysis). For series **a**, the difference was not significant owing to the large spread of the control measurements. Interestingly, the phthalimido compound **3c** is the most active compound found in this study, which is somewhat surprising because the WDUO was the less active lead compound of the three series. The activity of **3c** is in the same range of concentration as tacrine (IC50 = 0.044 ± 0.04 μM) (Kapková et al 2003).

Further reduction of the molecules (**4a**–**c**, **5a**–**c**, **6a**–**c**) substantially decreased the activity, indicating the importance of the phenylpropane moiety attached to the pyridinium nitrogen. Previous docking experiments (Kapková et al 2003) support this experimental result because a π–π stacking of the aromatic pyridine of the DUO3 compound with Trp279 was found corresponding to a π–π stacking of the lateral phenyl ring with Trp279 of compounds **3c**, which may increase the affinity.

However, the variations in affinity to the AChE are different in the three series of compounds. Whereas the affinity in the two chlorobenzyl series was found to differ with a factor of 20–30, the differences in the phthalimidopropyl series is about ×100, indicating that the structural

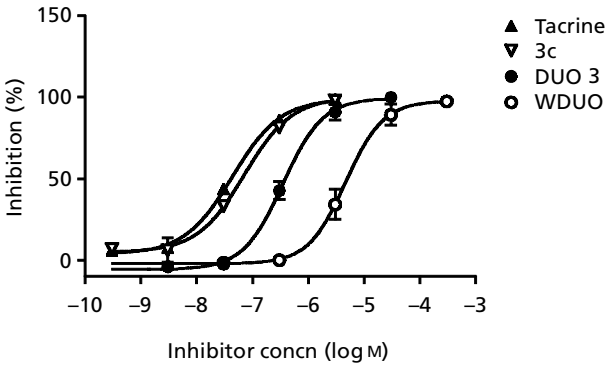


Figure 4 Concentration–response curves for inhibition of AChE by tacrine, **3c** and ditopic compounds DUO 3 and WDUO. Data are expressed as the mean ± s.e.m., n = 3–6. Where the error bars of data points are not visible the s.e.m. is very small.

variations in the latter series of compounds is more critical. This might be due to stronger interactions between the phthalimide group and the bottom of the AChE gorge in comparison with the chlorobenzyl group. The previous docking study, considering the ditopic bisbenzyl-substituted bispyridinium compounds, revealed a π–π stacking between the benzyl group and Trp84. In addition, this part of the AChE gorge is characterized by a number of serine and tyrosine residues (Ser200/122 and Tyr130/334) that are able to form hydrogen bonds with the carbonyl groups of the phthalimide moiety of compounds **1c**–**6c** in addition to the π–π stacking between the phenyl ring of the phthalimido group and Trp84. These strong interactions may reduce the flexibility within the AChE gorge in a way that the variations in the structure at the other end of molecule result in a great difference in affinity to the enzyme.

As already known, many AChE inhibitors showed higher potency toward BChE than to AChE (Radić et al 1993). Due to this fact, these drugs produce, in many cases, side effects (e.g. tacrine's beneficial effect is associated with hepatotoxicity, which has been mainly attributed to the BChE activity) (Radić et al 1993; Giacobini 2000). Thus, the most potent compound presented in this paper (**3c**) was additionally tested toward BChE. The IC50 value was found to be rather high (2.49 ± 0.18 μM) compared to the IC50 value found toward AChE (0.073 ± 0.02 μM).

Conclusion

Using the hypothesis of the previous study on ditopic bisbenzylethers of bispyridinium, that compounds were too long to tightly fit into the gorge of AChE, shorter compounds of higher affinity were found within the frame of this study. For series **b** and **c** it could be shown that the best compounds show significantly higher affinity than the ditopic compounds, which were the starting point for this study. Additionally, the most active compound (**3c**) exhibited a very low affinity to BChE, which might reduce the incidence of side effects often observed with non-selective cholinesterase inhibitors.

For high affinity to AChE, inhibitors should carry a phenylpropyl substituent attached to the pyridinium nitrogen. As can be seen from compounds **2a**, **2b** and **2c**, substituents on the phenyl ring seem to strongly influence the affinity. Thus, further investigations focussing on the substitution pattern on the phenyl ring are in progress.

References

Bejeuhr, G., Holzgrabe, U., Mohr, K., Sürig, U., Petersenn, A. (1992) Molecular modeling and synthesis of potent stabilizers of antagonist binding to M-cholinoceptors. *Pharm. Pharmacol. Lett.* **2**: 100–103

Bevan, D. R., Donati, F., Kopman, A. F. (1992) Reversal of neuromuscular blockade. *Anesthesiology* **77**: 785

Botero Cid, M. H., Holzgrabe, U., Kostenis, E., Mohr, K., Tränkle, C. (1994) Search for the pharmacophore of bispyridinium-type allosteric modulators of muscarinic receptors. *J. Med. Chem.* **37**: 1439–1445

Bunyapaiboonsri, T., Ramström, O., Lohmann, S., Lehn, J.-M., Peng, L., Goeldner, M. (2001) Dynamic deconvolution of a pre-equilibrated dynamic combinatorial library of acetylcholinesterase inhibitors. *Chembiochem.* **2**: 438–444

Carlier, P. R., Han, Y. F., Chow, E. S.-H., Li, C. P.-L., Wang, H., Lieu, T. X., Wong, H. S., Pang, Y.-P. (1999a) Evaluation of short-tether Bis-THA AChE inhibitors. A further test of the dual binding site hypothesis. *Bioorg. Med. Chem.* **7**: 351–357

Carlier, P. R., Du, D.-M., Han, Y., Liu, J., Pang, Y.-P. (1999b) Potent, easily synthesized huperzine A-tacrine hybrid acetylcholinesterase inhibitors. *Bioorg. Med. Chem. Lett.* **9**: 2335–2338

Critchlow, D. E., Fligner, M. A. (1991) On distribution-free multiple comparisons in the one-way analysis of variance. *Commun. Statist.-Theory Meth.* **20**: 127–139

Ellman, G. L., Coutney, K. D., Andres, V., Featherstone, R. M. (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.* **7**: 88–95

Giacobini, E. (2000) *Cholinesterases and cholinesterase inhibitors.* Martin Dunitz, London, pp 27, 180

Guillou, C., Mary, A., Renko, D. Z., Gras, E., Thal, C. (2000) Potent acetylcholinesterase inhibitors: design, synthesis and structure-activity relationships of alkene linked bis-galanthamine and galanthamine-galanthamine salts. *Bioorg. Med. Chem. Lett.* **10**: 637–639

Hu, M.-L., Wu, L.-J., Hsiao, G., Yen, M.-H. (2002) Homodimeric tacrine congeners as acetylcholinesterase inhibitors. *J. Med. Chem.* **45**: 2277–2282

Kapková, P., Stiefl, N., Sürig, U., Engels, E., Baumann, K., Holzgrabe, U. (2003) Synthesis, biological activity and docking studies of new acetylcholinesterase inhibitors of the bispyridinium type. *Arch. Pharm. Pharm. Med. Chem.* In press

Kryger, G., Silman, I., Sussman, J. L. (1998) Three-dimensional structure of a complex of E2020 with acetylcholinesterase from Torpedo californica. *J. Physiol. Paris* **92**: 191–194

Miller, R. G. (1981) *Simultaneous statistical inference.* 2nd Edn, Springer-Verlag, New York, pp 143–157

Radić, Z., Pickering, A. N., Vellom, C. D., Camp, S., Taylor P. (1993) Three distinct domains in the cholinesterase molecule confer selectivity for acetyl- and butyrylcholinesterase inhibitors. *Biochemistry* **32**: 12074–12084

Shen, T., Tai, K., Henchman, R. H., McCammon, J. A. (2002) Molecular dynamics of acetylcholinesterase. *Acc. Chem. Res.* **35**: 332–340