Design, Synthesis and Evaluation of Difunctionalized 4-Hydroxybenzaldehyde Derivatives as Novel Cholinesterase Inhibitors

Liang Yu, Rihui Cao, Wei Yi, Qin Yan, Zhiyong Chen, Lin Ma, and Huacan Song*

School of Chemistry and Chemical Engineering, Sun Yat-sen University; 135 Xin Gang West Road, Guangzhou 510275, China. Received February 11, 2010; accepted May 24, 2010; published online June 16, 2010

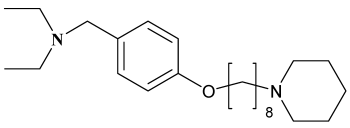
A series of difunctionalized 4-hydroxybenzaldehyde derivatives were designed, synthesized and evaluated as cholinesterase (acetylcholinesterase (AChE) and butyrylcholinesterase (BChE)) inhibitors. The results demonstrated that all the compounds had more potent AChE and BChE inhibitory activities than galanthamine-HBr, one of the best cholinesterase inhibitors known so far. The inhibition mechanism revealed that the best active compound 4e displayed a mix-type mode of AChE and BChE by its dual-site interactions with the catalytic triad active center and the peripheral anionic site (PAS) of enzyme. All these data suggested that further development of such compounds may be of interest.

Key words synthesis; 4-hydroxybenzaldehyde derivative; cholinesterase inhibitor; inhibition mechanism

It is well-known that two forms of cholinesterases coexist ubiquitously throughout the body, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). They can catalyze the degradation of the neurotransmitter acetylcholine, leading to the termination of cholinergic neurotransmission.[1,2] Cholinesterases inhibitors (ChEI) represented the treatment of choice for Alzheimer's disease (AD).[3,4] Currently, AchE has a well established esterase activity, while the pharmacological role of BChE is not yet completely understood. BChE may have a compensatory role in the modulation of the hydrolysis of acetylcholine (ACh) in brain with degenerative changes. Consequently, BChE may be additional target for increasing the cholinergic tone in AD patients.[5,6]

Many efforts have been spent in the search for potent AChE inhibitors, and a large number of naturally occurring and synthetic AChE inhibitors have already been reported.[7,8] Among them, four representative anti-AChE agents, tacrine, rivastigmine, donepezil, and galanthamine, have been approved by FDA for the treatment of AD.[9—11] Unfortunately, the potential effectiveness offered by these inhibitors is often limited mainly due to their some adverse effects. For example, recent clinical studies showed that tacrine had hepatotoxic liability.[12] Obviously, this is still needed to search and develop novel AChE inhibitors with better activities together with lower side effects.

4-Hydroxybenzaldehyde was originally isolated as one of the main active constituents from *Gastrodia elata*, a plant named tianma in China, which is a very important Chinese herbal medicine used for medical treatment for a long time in China, such as headaches, migraine and some neuralgic and nervous disorders.[13,14] On the basis of the above mentioned information and the crystallographic structure of AChE, our group had ever reported a series of helicid analogues and methyl 2-[2-(4-formylphenoxy)acetamido]-2-substituted acetate derivatives as potent AChE inhibitors.[15,16] In addition, some galanthamine derivatives were also found to exhibit significant inhibitory effects on AChE,[17] and these compounds exhibited the potent activity mainly due to its strong interaction with the active site gorge of AChE.[18] On the other hand, numerous investigations provided evidence that the introduction of amine cation moiety remarkably improved the AChE inhibitory effects by the electrostatic interaction with the peripheral anionic site (PAS) of enzyme.[19—21]



3a IC_{50}=0.092 μM (AChE); IC_{50}=0.0073 μM(BChE)

Fig. 1. Chemical Structure of Lead Compound

Recently, our group investigated the syntheses and the inhibitory effects on AChE of a variety of 4-hydroxybenzaldehyde derivatives. We found that compound **3a** having a piperidine moiety connected to the phenyl ring *via* eight CH_2 unit spacer (Fig. 1) was the most potent inhibitor with IC_{50} value lower than 92 nM and 7.3 nM against AChE and BChE, respectively. (These results will be published elsewhere.) In our continuing efforts for simple but efficient cholinesterase inhibitors (ChEI), we designed and synthesized a series of difunctionalized 4-hydroxybenzaldehyde derivatives by the replacement of diethylamino moieties with appropriate cyclic or dialkylamino group on the basis of the chemical structure of compound **3a**. These compounds were expected to exhibit more potent ChE inhibitory effects. To the best of our knowledge, this is the first time to report the inhibitory effects of such compounds on the AChE and BChE.

Experimental

Chemistry NMR spectra were recorded on a Varian Mercury-Plus 300 spectrometer in $CDCl_3$ or deuteriated dimethyl sulfoxide (DMSO)-d_6 at 25 °C. All chemical shifts (δ) are quoted in parts per million downfield from TMS and coupling constants (J) are given in Hertz. All reactions were monitored by TLC (Merck Kieselgel 60 F_{254}) and spots were visualized with UV light or iodine. Acetylcholinesterase (AChE, E.C. 3.1.1.7, from electric eel), butyrylcholinesterase (BChE, E.C. 3.1.1.8, from horse serum), 5,5′-dithiobis(2-nitrobenzoic acid) (Ellman's reagent, DTNB), butylthiocholine chloride (BTC) and acetylthiocholine chloride (ATC) were purchased from Sigma-Aldrich (Steinheim, Germany). Galanthamine hydrobromide was obtained from Sigma-Aldrich (Sintra, Portugal). All commercially available reagents and solvents were used without further purification.

Procedure for the Synthesis of 4-(8-Bromooctyloxy)benzaldehyde (1) A stirred suspension of 4-hydroxybenzaldehyde (20 mmol), 1,8-dibromooctane (40 mmol) and K_2CO_3 (5.52 g, 40 mmol) in dry acetone was refluxed for 24 h. The reaction was monitored by TLC. The hot reaction mixture was filtered and evaporated to dryness. The residue was purified by chromatography using CH_2Cl_2 as eluent to afford compound **1** (8.71 g, 70%) as colorless

* To whom correspondence should be addressed. e-mail: yjhxhc@mail.sysu.edu.cn.

oil. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ, ppm: 9.86 (s, 1H, CHO), 7.82 (d, J=8.8 Hz, 2H, ArH), 6.99 (d, J=8.7 Hz, 2H, ArH), 4.04 (t, J=6.5 Hz, 2H, H1), 3.20 (t, J=7.0 Hz, 2H, H8), 1.88—1.78 (m, 4H, H7, H2), 1.42—1.30 (m, 8H, H3, H4, H5, H6).

General Procedure for the Synthesis of Compounds 2a—h Compound **1** (2 mmol) and the corresponding cyclic or dialkylamine (2 mmol) were mixed in 1,2-dichloroethane (35 ml) and then treated with sodium triacetoxyborohydride (0.6 g, 14 mmol). The mixture was stirred at room temperature for 1.5 h. The reaction mixture was quenched by adding aqueous saturated $NaHCO_3$, and the product was extracted with EtOAc. The combined organic phase was dried ($MgSO_4$), and the solvent was evaporated to give **2a**—**h** in 95—98% yields.

N-[4-(8-Bromooctyloxy)benzyl]-*N*-ethylethanamine (**2a**): Compound **2a** was obtained as yellow solid (0.73 g, 96%). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ, ppm: 7.32 (d, J=8.8 Hz, 2H, ArH), 6.85 (d, J=8.8 Hz, 2H, ArH), 3.93 (t, J=6.6 Hz, 2H, H1), 3.72 (s, 2H, CH_2Ph), 3.18 (t, J=6.6 Hz, 2H, H8), 2.48 (q, J=7.2 Hz, 4H, NCH_2CH_3), 1.86—1.72 (m, 4H, H7, H2), 1.42—1.30 (m, 8H, H3, H4, H5, H6), 1.01 (t, 6H, J=7.1 Hz, NCH_2CH_3).

General Procedure for the Synthesis of Compounds 3a—h A mixture of compounds **2a**—**h** (2 mmol), KI (3 mmol) and piperidine (20 mmol) in anhydrous ethanol (50 ml) was refluxed for 5—10 h. After completion of the reaction as indicated by TLC, the solution was cooled and filtered, and then concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2, and then washed with saturated $NaHCO_3$ and brine, dried with anhydrous Na_2SO_4, and solvent was removed *in vacuo*. The crude product was purified by chromatography using CH_2Cl_2/MeOH/aqueous NH_3 (50 : 1 : 0.5) as eluent to afford **3a**—**h** in 80—95% yields.

N-Ethyl-*N*-{4-[8-(piperidin-1-yl)octyloxy]benzyl}ethanamine (**3a**): Compound **3a** was obtained as yellow oil (0.70 g, 95%). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ, ppm: 7.20 (d, J=8.8 Hz, 2H, ArH), 6.82 (d, J=8.8 Hz, 2H, ArH), 3.92 (t, J=6.6 Hz, 2H, H1), 3.49 (s, 2H, CH_2Ph), 2.49 (q, J=6.8 Hz, 4H, NCH_2CH_3), 2.42—2.30 (m, 4H, H_{pip}-2,6), 2.26 (t, J=6.4 Hz, 2H, H8), 1.80—1.71 (m, 2H, H2), 1.62—1.52 (m, 4H, H_{pip}-3,5), 1.50—1.40 (m, 6H, H7, H_{pip}-4, H3), 1.37—1.26 (m, 6H, H6, H5, H4), 1.04 (t, 6H, J=6.8 Hz, NCH_2CH_3). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 158.1 (C_{phenyl}-1), 131.4 (C_{phenyl}-4), 130.3 (C_{phenyl}-3,5), 114.3 (C_{phenyl}-2,6), 68.2 (H1), 60.0 (CH_2Ph), 57.0 (H8), 55.0 (C_{pip}-2,6), 46.7 ($2NCH_2CH_3$), 29.9 (C2), 29.7 (C4), 29.6 (C5), 28.1 (C7), 27.2 (C6), 26.4 (C3), 26.3 (C_{pip}-3,5), 24.8 (C_{pip}-4), 12.0 ($2NCH_2CH_3$); high resolution-mass spectra (HR-MS)-electron ionization (EI) Calcd for $C_{24}H_{42}O_1N_2$: 374.3292. Found: 374.3288.

N-{4-[8-(Piperidin-1-yl)octyloxy]benzyl}-*N*-propylpropan-1-amine (**3b**): Compound **3b** was obtained as yellow oil (0.73 g, 91%). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ, ppm: 7.20 (d, J=8.5 Hz, 2H, ArH), 6.80 (d, J=8.6 Hz, 2H, ArH), 3.93 (t, J=6.5 Hz, 2H, H1), 3,48 (s, 2H, CH_2Ph), 2.40—2.30 (m, 8H, H_{pip}-2,6, H_N1), 2.26 (t, J=6.4 Hz, 2H, H8), 1.81—1.72 (m, 2H, H2), 1.62—1.55 (m, 4H, H_{pip}-3,5), 1.52—1.39 (m, 10H, H7, H_N2, H_{pip}-4, H3), 1.39—1.23 (m, 6H, H6, H5, H4), 0.85 (t, 6H, J=7.3 Hz, NCH_2CH_3). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 158.1 (C_{phenyl}-1), 132.1 (C_{phenyl}-4), 130.1 (C_{phenyl}-3,5), 114.2 (C_{phenyl}-2,6), 68.2 (H1), 60.0 (CH_2Ph), 58.2 (H8), 56.0 (C_{pip}-2,6), 55.0 ($2H_N$1), 29.9 (C2), 29.7 (C4), 29.6 (C5), 28.1 (C7), 27.3 (C6), 26.4 (C3), 26.3 (C_{pip}3,5), 24.9 (C_{pip}-4), 20.5 ($2H_N$2), 12.0 ($2H_N$3); electrospray ionization (ESI)-MS m/z: 403 $[M+H]^+$.

N-Butyl-*N*-{4-[8-(piperidin-1-yl)octyloxy]benzyl}butan-1-amine (**3c**): Compound **3c** was obtained as yellow oil (0.79 g, 92%). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ, ppm: 7.20 (d, J=8.5 Hz, 2H, ArH), 6.82 (d, J=8.5 Hz, 2H, ArH), 3.93 (t, J=6.5 Hz, 2H, H1), 3,47 (s, 2H, CH_2Ph), 2.42—2.31 (m, H, H_N1, H_{pip}-2,6), 2.29—2.24 (m, 2H, H8), 1.81—1.72 (m, 2H, H2), 1.51—1.39 (m, 10H, H7, H3, H_N2, H_{pip}-3,5), 1.36—1.23 (m, 10H, H6, H5, H4, H_N3), 0.88 (t, 6H, J=7.2 Hz, NCH_2CH_3). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 158.0 (C_{phenyl}-1), 131.4 (C_{phenyl}-4), 130.3 (C_{phenyl}-3,5), 114.3 (C_{phenyl}-2,6), 68.2 (H1), 60.0 (CH_2Ph), 58.1 (H8), 55.0 (C_{pip}-2,6), 53.6 ($2C_N$1), 30.5 ($2C_N$2), 29.9 (C2), 29.7 (C4), 29.5 (C5), 28.1 (C7), 27.3 (C6), 26.4 (C3), 26.3 (C_{pip}-3,5), 24.9 (C_{pip}-4), 21.0 ($2C_N$3), 14.5 ($2C_N$4); ESI-MS m/z: 431 $[M+H]^+$.

4-{4-[8-(Piperidin-1-yl)octyloxy]benzyl}morpholine (**3d**): Compound **3d** was obtained as yellow oil (0.69 g, 90%). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ, ppm: 7.20 (d, J=8.4 Hz, 2H, ArH), 6.83 (d, J=8.5 Hz, 2H, ArH), 3.92 (t, J=6.5 Hz, 2H, H1), 3.72—3.65 (m, 4H, H_{mpl}3,5) 3,42 (s, 2H, CH_2Ph), 2.46—2.39 (m, 4H, H_{mpl}2,4), 2.39—2.30 (m, 4H, H_{pip}-2,6), 2.29—2.24 (m, 2H, H8), 1.81—1.72 (m, 2H, H2), 1.60—1.52 (m, 4H, H_{pip}-3,5), 1.52—1.39 (m, 6H, H7, H_{pip}-4, H3), 1.37—1.23 (m, 6H, H6, H5, H4). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 158.5 (C_{phenyl}-1), 130.5 (C_{phenyl}-3,5), 129.6 (C_{phenyl}-4), 114.4 (C_{phenyl}-2,6), 68.2 (H1), 67.3 (C_{mpl}3,5), 63.2 (CH_2Ph), 60.0 (H8), 55.0 (C_{pip}-2,6), 53.8 (C_{mpl}2,4), 29.9 (C2), 29.7 (C4), 29.5 (C5), 28.1 (C7), 27.3 (C6), 26.3 (C3), 26.2 (C_{pip}-3,5), 24.9 (C_{pip}-4); ESI-MS m/z: 389 $[M+H]^+$.

1-{8-[4-(Pyrrolidin-1-ylmethyl)phenoxy]octyl}piperidine (**3e**): Compound **3e** was obtained as yellow oil (0.65 g, 88%). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ, ppm: 7.20 (d, J=8.5 Hz, 2H, ArH), 6.80 (d, J=8.6 Hz, 2H, ArH), 3.92 (t, J=6.6 Hz, 2H, H1), 3.53 (s, 2H, CH_2Ph), 2.52—2.45 (m, 4H, H_{pyr}-2,5), 2.40—2.30 (m, 4H, H_{pip}-2,6), 2.29—2.26 (m, 2H, H8), 1.83—1.71 (m, 6H, H2, H_{pyr}-3,4), 1.62—1.55 (m, 4H, H_{pip}-3,5), 1.51—1.40 (m, 6H, H7, H_{pip}-4, H3), 1.37—1.25 (m, 6H, H6, H5, H4). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 158.3 (C_{phenyl}-1), 131.3 (C_{phenyl}-4), 130.3 (C_{phenyl}-3,5), 114.4 (C_{phenyl}-2,6), 68.2 (C1), 60.3 (CH_2Ph), 59.9 (H8), 54.9 (C_{pyr}-2,5), 54.3 (C_{pip}-2,6), 29.9 (C2), 29.7 (C4), 29.5 (C5), 28.1 (C7), 27.2 (C6), 26.4 (C3), 26.3 (C_{pip}-3,5), 24.8 (C_{pip}-4), 23.7 (C_{pyr}-3,4); HR-MS (EI) Calcd for $C_{24}H_{40}O_1N_2$: 372.3135. Found: 372.3137.

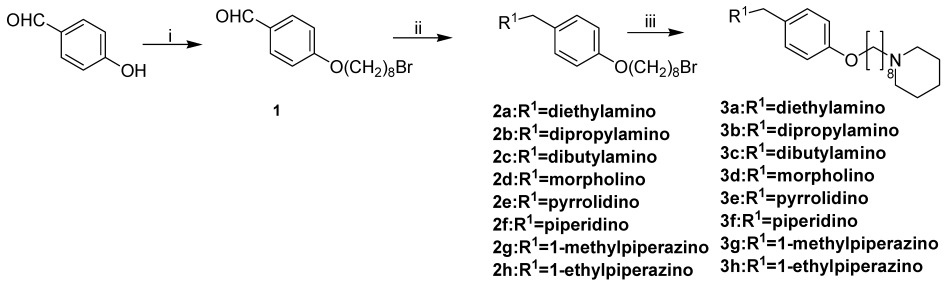
1-{4-[8-(Piperidin-1-yl)octyloxy]benzyl}piperidine (**3f**): Compound **3f** was obtained as yellow oil (0.66 g, 86%). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ, ppm: 7.19 (d, J=8.8 Hz, 2H, ArH), 6.80 (d, J=8.8 Hz, 2H, ArH), 3.92 (t, J=6.6 Hz, 2H, H1), 3,40 (s, 2H, CH_2Ph), 2.44—2.30 (m, 8H, H_{pip}-2,6, H′$_{pip}$-2,6), 2.29—2.24 (m, 2H, H8), 1.81—1.71 (m, 2H, H2), 1.62—1.52 (m, 8H, H_{pip}-3,5 , H′$_{pip}$-3,5), 1.50—1.38 (m, 8H, H7, H_{pip}-4, H′$_{pip}$-4, H3), 1.37—1.30 (m, 6H, H6, H5, H4). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 158.3 (C_{phenyl}-1), 130.6 (C_{phenyl}-3,5), 130.3 (C_{phenyl}-4), 114.2 (C_{phenyl}-2,6), 68.2 (H1), 63.6 (CH_2Ph), 60.0 (H8), 55.0 (C_{pip}-2,6), 54.6 (C′$_{pip}$-2,6), 29.9 (C2), 29.7 (C4), 29.5 (C5), 28.1 (C7), 27.3 (C6), 26.4 (C3), 26.3 (C_{pip}-3,5 , C′$_{pip}$-3,5), 24.9 (C_{pip}-4), 24.8 (C′$_{pip}$-4); HR-MS (EI) Calcd for $C_{25}H_{42}O_1N_2$: 386.3292. Found: 386.3294.

1-Methyl-4-{4-[8-(piperidin-1-yl)octyloxy]benzyl}piperazine (**3g**): Compound **3g** was obtained as yellow oil (0.71 g, 89%). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ, ppm: 7.18 (d, J=8.5 Hz, 2H, ArH), 6.80 (d, J=8.5 Hz, 2H, ArH), 3.91 (t, J=6.5 Hz, 2H, H1), 3,42 (s, 2H, CH_2Ph), 2.56—2.29 (m, 12H, H_{ppz}-2,3,5,6, H_{pip}-2,6), 2.29—2.22 (m, 2H, H8), 2.26 (s, 3H, H_{ppz}-NCH_3), 1.79—1.70 (m, 2H, H2), 1.61—1.53 (m, 4H, H_{pip}-3,5), 1.50—1.38 (m, 6H, H7, H_{pip}-4, H3), 1.37—1.25 (m, 6H, H6, H5, H4). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 158.4 (C_{phenyl}-1), 130.5 (C_{phenyl}-3,5), 130.1 (C_{phenyl}-4), 114.3 (C_{phenyl}-2,6), 68.2 (H1), 62.8 (CH_2Ph), 60.0 (H8), 55.4 (C_{ppz}-3,5), 55.0 (C_{pip}-2,6), 53.3 (C_{ppz}-2,6), 46.4 (C_{pip}-NCH_3), 29.9 (C2), 29.6 (C4), 29.6 (C5), 28.1 (C7), 27.3 (C6), 26.3 (C3), 26.3 (C_{pip}-3,5), 24.8 (C_{pip}-4); ESI-MS m/z: 402 $[M+H]^+$.

1-Ethyl-4-{4-[8-(piperidin-1-yl)octyloxy]benzyl}piperazine (**3h**): Compound **3h** was obtained as yellow oil (0.72 g, 87%). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ, ppm: 7.19 (d, J=8.5 Hz, 2H, ArH), 6.81 (d, J=8.5 Hz, 2H, ArH), 3.91 (t, J=6.5 Hz, 2H, H1), 3,43 (s, 2H, CH_2Ph), 2.69—2.30 (m, 14H, H_N1, H_{ppz}-2,3,5,6, H_{pip}-2,6), 2.29—2.23 (m, 2H, H8), 1.80—1.71 (m, 2H, H2), 1.61—1.53 (m, 4H, H_{pip}-3,5), 1.51—1.38 (m, 6H, H7, H_{pip}-4, H3), 1.37—1.24 (m, 6H, H6, H5, H4), 1.07 (t, 3H, J=7.2Hz, H_N2). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 158.4 (C_{phenyl}-1), 130.6 (C_{phenyl}-3,5), 130.0 (C_{phenyl}-4), 114.3 (C_{phenyl}-2,6), 68.2 (H1), 62.8 (CH_2Ph), 60.0 (H8), 55.0 (C_{pip}-2,6), 53.3 (C_{ppz}-3,5), 53.1 (C_{ppz}-2,6), 52,6 (C_N1), 29.9 (C2), 29.7 (C4), 29.7 (C5), 28.1 (C7), 27.3 (C6), 26.4 (C3), 26.3 (C_{pip}-3,5), 24.9 (C_{pip}-4), 12.4(C_N2); ESI-MS m/z: 416 $[M+H]^+$.

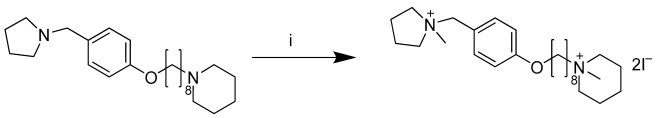
1-Methyl-1-(8-{4-[(1-methylpyrrolidinium-1-yl)methyl]phenoxy}octyl) piperidinium iodide (**4e**): Compound **3e** (0.40 g, 1 mmol) was dissolved in 15 ml $CHCl_3$, and 1 ml CH_3I was added to the solution. The mixture was refluxed for 24 h. After completion of the reaction as indicated by TLC, the solution was concentrated under reduced pressure to obtain **4e** (0.61 g, 95%) as colorless oil. ^{1}H-NMR (DMSO-d_6, 300 MHz) δ, ppm: 7.17 (d, J=7.8 Hz, 2H, ArH), 6.80 (d, J=8.0 Hz, 2H, ArH), 4.47 (s, 2H, CH_2Ph), 3.98 (t, J=6.6 Hz, 2H, H1), 3.40—3.32 (m, 2H, H8), 3.32—3.21 (m, 8H, H_{pyr}-2,5, H_{pip}-2,6), 2.96 (s, 3H, H_{pyr}-NCH_3), 2.85 (s, 3H, H_{pip}-NCH_3), 2.18—2.00 (m, 4H, H_{pyr}-3,4), 1.88—1.58 (m, 8H, H2, H7, H_{pip}-3,5), 1.57—1.46 (m, 2H, H_{pip}-4), 1.42—1.20 (m, 8H, H3, H4, H5, H6). ^{13}C-NMR (DMSO-d_6, 75 MHz) δ: 160.5 (C_{phenyl}-1), 134.5 (C_{phenyl}-3,5), 121.2 (C_{phenyl}-4), 115.4 (C_{phenyl}-2,6), 68.4 (C_{pyr}-2,5), 65.6 (H1), 63.3 (CH_2Ph), 63.1 (C_{pip}-2,6), 60.8 (H8), 48.0 (C_{pip}-NCH_3), 47.8 (C_{pyr}-NCH_3), 29.4 (C2), 29.4 (C4), 29.3 (C5), 26.6 (C6), 26.2 (C7), 26.2 (C3), 21.6 (C_{pyr}-3,4), 21.5 (C_{pip}-4), 20.1 (C_{pip}-3,5); ESI-MS m/z: 402 $[M]^+$.

Assay of the AChE and BChE Inhibitory Activity The assay was performed as described in the following procedure.[22] Five different concentration of each compound were measured at 412 nm for 1 min, each con centration in triplicate. For buffer preparation, 0.1 M dipotassium hydrogen phosphate was adjusted to pH=8.0 with 1 M potassium dihydrogen phosphate. Enzyme solutions were prepared to give 2.5 units/ml in 1.5 ml aliquots. Furthermore, 0.01 M DTNB solution, 0.075 M ATC and BTC solutions, respectively, were used. A cuvette containing 880 μl of phosphate buffer, 10 μl of the respective enzyme, 50 μl of DTNB and 20 μl of the test com-



Reagents and conditions: (i) $Br(CH_2)_8Br$, K_2CO_3 reflux, 24 h; (ii) the corresponding cyclic or dialkylamine, $NaBH(OAc)_3$, DCE, rt 4 h; (iii) piperidine, KI, anhydrous ethanol, reflux 15 h.

Chart 1. Synthesis of Compounds **3a**—**h**



Reagents and conditions: (i) CH_3I, $CHCl_3$, 24 h.

Chart 2. Synthesis of Compound **4e**

pound solution was allowed to pre-incubate for 15 min at 37 °C, and the reaction was started by addition of 40 μl of the substrate solution (ATC/BTC). The reaction was monitored by measuring the absorbance at 412 nm. For the reference value, 20 μl of dimethyl sulfoxide (DMSO) replaced the test compound solution. For determining the blank value, additionally 10 μl of buffer replaced the enzyme solution.

Assay of Kinetic Characterization of AChE and BChE Inhibition Kinetic characterization of AChE was performed using a reported method.[23] Seven different concentrations of AChE and inhibitors were mixed in the assay buffer (pH=8.0), containing 40 mM of 5,5′-dithio-bis(2-nitrobenzoic acid), 0.035 units/ml AChE. Test compound was added into the assay solution and pre-incubated with the enzyme at 37 °C for 15 min, followed by the addition of substrate with various concentrations. Kinetic characterization of the hydrolysis of acetylthiocholine catalyzed by AChE was done spectrometrically at 412 nm. A parallel control with no inhibitor in the mixture, allowed adjusting activities to be measured at various times.

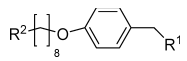
Results and Discussion

Synthetic Chemistry The synthetic routes of compounds **3a**—**h** were outlined in Chart 1. The 4-hydroxybenzaldehyde was treated with 1,8-dibromooctane in the presence of K_2CO_3 to afford compound **1**.[24] Compound **1** was reacted with the selected cyclic or dialkylamines in 1,2-dichloroethane (DCE), the resulting product was reduced with $NaBH(OAc)_3$ to afford compounds **2a**—**h**.[25] Compounds **2a**—**h** were reacted with piperidine to give the final compounds **3a**—**h**. The synthetic routes of compound **4e** was outlined in Chart 2. The compound **3e** was treated with iodomethane in $CHCl_3$ to generate the compound **4e**. The chemical structures of all the synthesized compounds were characterized by ^{1}H- and ^{13}C-NMR spectra.

Inhibitory Activity The inhibitory activities of the newly synthesized compounds against AChE and BChE were investigated by determining the rate of hydrolysis of acetylthiocholine (ATCh) and butyrylthiocholine (BuTCh) in comparison with reference compound galanthamine-HBr (one of the best cholinesterase inhibitors known so far), using the slightly modified method of Ellmann *et al.*[22] The IC_{50} value of all compounds investigated was summarized in Table 1.

As shown in Table 1, most of compounds displayed more potent activities against AChE and BchE than the reference inhibitor galanthamine-HBr. Particularly, compounds **3e** and **3f**, bearing pyrrolidino and piperidino substituents, respectively, demonstrated more potent anti-AChE activities than lead compound **3a** (Fig. 1). However, replacement of the diethylamino moiety of compound **3a** with the dipropylamino, dibutylamino, morpholino, 1-methylpiperazino and 1-ethylpiperazino, respectively, to provide compounds **3b**—**d** and **3g**—**h**, which caused the decreasing anti-AChE and anti-BChE activities. Of compounds **3a**—**h**, compound **3e** having pyrrolidino connected with the benzyl group exhibited the most potent anti-AChE activity with IC_{50} value of 0.048 μM.

Table 1. Chemical Structure and Inhibitory Activity against Isolated AChE[a] and BChE[b] of Compounds **3a**—**h** and **4e**, and Resulting Selectivities Expressed as the Ratio of IC_{50} Values



Compd.	R^1	R^2	Yield (%)	ChE inhibition IC_{50} (μM)		Selectivity
				AChE[c]	BChE[c]	(AChE/BChE)
3a	N	N	95	0.092	0.0073	12.50
3b	N	N	91	0.21	0.39	0.54
3c	N	N	92	0.39	0.19	2.05
3d	N O	N	90	0.47	1.42	0.33
3e	N	N	88	0.048	0.0088	5.45
3f	N	N	86	0.081	0.023	3.52
3g	N N—	N	89	0.20	0.19	1.05
3h	N N	N	87	1.17	0.48	2.44
4e	—N+	—N+	93	0.022	0.0086	2.56
	Galanthamine-HBr[d]			0.67	1.52	0.044

a) AChE, E.C. 3.1.1.7, from electric eel. *b*) BChE, E.C. 3.1.1.8, from horse serum. *c*) IC_{50} values are means of three different experiments. *d*) IC_{50} values of AChE reported in the literature: 0.3—0.8 μM.

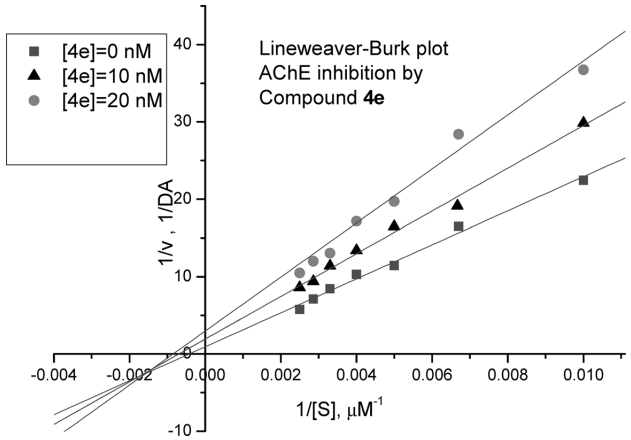


Fig. 2. Lineweaver–Burk Plots Resulting from Substrate–Velocity Curves of AChE Activity with Different Substrate Concentrations (100—400 μM) in the Absence and Presence of Compound **4e** with Concentration of 10 and 20 nM

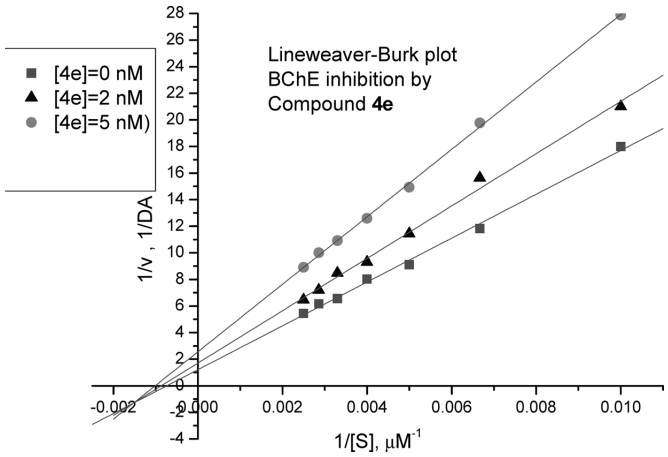


Fig. 3. Lineweaver–Burk Plots Resulting from Substrate–Velocity Curves of BChE Activity with Different Substrate Concentrations (100—400 μM) in the Absence and Presence of Compound **4e** with Concentration of 2 and 5 nM

Interestingly, compound **4e**, a cationic compound with quaternary nitrogen derived from compound **3e**, showed twofold anti-AChE activities higher than compound **3e**. These results suggested that (1) the nature and size of dialkylamino moiety connected with benzyl group significantly influenced the cholinesterase inhibitory potencies, and the small size amine moieties were more preferable to their inhibitory activities; (2) the quaternary ammonium salt derivative facilitated their inhibitory effects on AChE.

We also investigated the inhibitory effect and the selectivity of various dialkylamino moieties on AChE and BChE. As shown in Table 1, compound **3a** presented the best BChE inhibitory potencies with an IC_{50} value of 0.0073 μM and showed a surprising selectivity to BChE. Other compounds also showed moderate to strong BChE inhibiting property, but with decreased selectivity.

Inhibitory Mechanism Analysis Compound **4e** was selected for kinetic measurements because it showed the highest inhibitory activity against AChE and BChE. The mechanism of inhibition was analyzed by recording substrate–velocity curves in the absence and the presence of compound **4e** at different concentrations. Substrate concentration was varied from 100 to 400 μM. For AChE, 0 nM, 10 nM and 20 nM concentrations of compound **4e** were applied. For BChE, 0 nM, 2 nM and 5 nM concentrations of compound **4e** were used. Figure 2 showed the Lineweaver–Burk plots, which are reciprocal rates *versus* reciprocal substrate concentrations for the different inhibitor concentrations resulting from the substrate–velocity curves for AChE. The results showed that the plots of 1/V *versus* 1/[S] gave a family of straight lines with different slopes but they intersected one another in the third quadrant. Similar results were obtained for BChE (Fig. 3). The inhibitory behavior of compound **4e**, as deduced from Fig. 2, is virtually the same as that of some reported compounds which could bind simultaneously at the catalytic site and at the PAS of AChE and could be characterized by a linear mix-type of enzyme inhibition.[26] The reason for the unexpected inhibitory potency of compound **4e** might be that, to a certain extent, the interaction between compound **4e** and enzyme was formed, and the pyrrolidine moiety connected with benzyl group of compound **4e** bound with the catalytic triad active centre of enzyme and the piperidine moiety interacted with the PAS of enzyme. Therefore, we concluded that compound **4e** caused a mix-type of inhibition, that is, compound **4e** could interact with the active site and the second distal site of enzyme at the same time.

Conclusion

In conclusion, a series of difunctionalized 4-hydroxybenzaldehyde derivatives bearing various cyclic or dialkylamino moiety connected with benzyl group described in this paper were proved to have significant inhibitory activity against AChE and BChE. Structure–activity relationships analysis indicated that the nature and size of dialkylamine moiety which connects with benzyl group obviously influenced the cholinesterase inhibitory potency, and the small size amine moiety was more favorable. In addition, the quaternary ammonium salt derivative showed higher inhibitory effects on AChE, but not obviously on BChE. The inhibition kinetics analyzed by Lineweaver–Burk plots revealed that such compounds were mix-type inhibitors. All these results suggested that such compounds might be utilized for the development of new candidates for treatment of Alzheimer's disease.

Acknowledgment This work was supported by the Science and Technology Project of Guangdong Province, China (2009B060700048) and the Natural Science Foundation of Guangdong Province, China (2004B30101007).

References

1) Silman I., Sussman J. L., *Curr. Opin. Pharmacol.*, **5**, 293—302 (2005).
2) Nachon F., Nicolet Y., Masson P., *Ann. Pharm. Fr.*, **63**, 194—206 (2005).
3) Shen Q., Peng Q., Shao J., Liu X. Huang Z., Pu X., Ma L., Li Y. M., Chan A. S., Gu L. Q., *Eur. J. Med. Chem.*, **20**, 1307—1315 (2005).
4) Ridrugyez-Franco M. I., Fernandez-Bachiller M. I., Perez C., Castro A., Martinez A., *Bioorg. Med. Chem.*, **13**, 6795—6802 (2005).
5) Greig N. H., Utsuki T., Yu Q., Zhu X., Holloway H. W., Perry T., Lee B., Ingram D. K., Lahiri D. K., *Curr. Med. Res. Opin.*, **17**, 159—165 (2001).
6) Giacobini E., Spiegel R., Enz A., Veroff A. E., Cutler N. R., *J. Neural Trans.*, **109**, 1053—1065 (2002).
7) Savini L., Gaeta A., Fattorusso C., Catalanotti B., Campiani G., Chiasserini L., Pellerano C., Novellino E., Mckissic D., Saxena A., *J. Med. Chem.*, **46**, 1—4 (2003).

8) Bolognesi M. L., Andrisano V., Bartolini M., Banzi R., Carlo Melchiorre C., *J. Med. Chem.*, **48**, 24—27 (2005).
9) Allen N. H. P., Burns A., *J. Psychopharmacol.*, **9**, 43—56 (1995).
10) Francis P. T., Palmer A. M., Snap M., Wilcock G. K., *J. Neurol. Neurosurg. Psychiatry*, **66**, 137—147 (1999).
11) Ibach B., Haen E., *Curr. Pharm. Des.*, **10**, 231—251 (2004).
12) Galisteo M., Rissel M., Sergent O., Chevanne M., Cillard J., Guilouzo A., Lagadic-Gossmann D., *J. Pharmacol. Exp. Ther.*, **294**, 160—167 (2000).
13) Tang W., Eisenbrand G., "Chinese Drugs of Plant Origin. Chemistry, Pharmacology, and Use in Traditional and Modern Medicine," Springer Verlag, Berlin, 1992, pp. 545—547.
14) Hayashi J., Sekine T., Deguchi S., Lin Q., Horie S., Tsuchiya S., Yano S., Watanabe K., Ikegami F., *Phytochemistry*, **59**, 513—519 (2002).
15) Wen H., Lin C. L., Que L., Peng W. L., Wang Z. H., Song H. C., *Eur. J. Med. Chem.*, **43**, 166—173 (2008).
16) Wen H., Zhou Y. Y., Lin C. L., Song H. C., *Bioorg. Med. Chem. Lett.*, **17**, 2123—2125 (2007).
17) Jia P., Sheng R., Zhang J., Fang L., He Q. J., Yang B., Hu Y. Z., *Eur. J. Med. Chem.*, **44**, 772—784 (2009).
18) Zhou X., Wang X. B., Wang T., Kong L. Y., *Bioorg. Med. Chem.*, **16**, 8011—8021 (2008).
19) Tang H., Wei Y. B., Zhang C., Ning F. X., Qiao W., Huang S. L., Ma L., Huang Z. S., Gu L. Q., *Eur. J. Med. Chem.*, **44**, 2523—2532 (2009).
20) Tang H., Ning F. X., Wei Y. B., Huang S. L., Huang Z. S., Chan A. S. C., Gu L.Q., *Bioorg. Med. Chem. Lett.*, **17**, 3765—3768 (2007).
21) Markmee S., Ruchirawat S., Prachyawarakorn V., Ingkaninan K., Khorana N., *Bioorg. Med. Chem. Lett.*, **16**, 2170—2172 (2006).
22) Ellman G. L., Courtney D. K., Andres V., Featherstone R. M., *Biochem. Pharmacol.*, **7**, 88—92 (1961).
23) Massoulie J., Bon S., Silman I., *Prog. Brain Res.*, **98**, 139—146 (1993).
24) Piazzi L., Belluti F., Bisi A., Gobbi S., Rampa A., *Bioorg. Med. Chem.*, **15**, 575—585 (2007).
25) Abdel-Magid A., Carson K., Harris B., *J. Org. Chem.*, **61**, 3849—3862 (1996).
26) Pang Y. P., Quiram P., Jelacic T., Hong F., Brimijoin S., *J. Biol. Chem.*, **271**, 23646—23649 (1996).