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RESEARCH ARTICLE

Tertiary amine derivatives of chlorochalcone as acetylcholinesterase (AChE) and buthylcholinesterase (BuChE) inhibitors: the influence of chlorine, alkyl amine side chain and α,β-unsaturated ketone group

Xiao-hui Gao[a], Chao Zhou[b], Hao-ran Liu[b], Lin-bo Liu[b], Jing-jing Tang[b] and Xin-hua Xia[a]

[a]College of Pharmacy, Hu'nan University of Chinese Medicine, Changsha, PR China; [b]College of Chemistry and Chemical Engineering, Hu'nan University, Changsha, PR China

ABSTRACT

A new series of tertiary amine derivatives of chlorochalcone (**4a~4l**) were designed, synthesized and evaluated for the effect on acetylcholinesterase (AChE) and buthylcholinesterase (BuChE). The results indicated that all compounds revealed moderate or potent inhibitory activity against AChE, and some possessed high selectivity for AChE over BuChE. The structure–activity investigation showed that the substituted position of chlorine significantly influenced the activity and selectivity. The alteration of tertiary amine group also leads to obvious change in bioactivity. Among them, IC_{50} of compound **4l** against AChE was 0.17 ± 0.06 μmol/L, and the selectivity was 667.2 fold for AChE over BuChE. Molecular docking and enzyme kinetic study on compound **4l** suggested that it simultaneously binds to the catalytic active site (CAS) and peripheral anionic site (PAS) of AChE. Further study showed that the pyrazoline derivatives synthesized from chlorochalcones had weaker activity and lower selectivity in inhibiting AChE compared to that of chlorochalcone derivatives.

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Acetylcholinesterase inhibitors; Alzheimer's disease; chlorochalcones; pyrazoline; structure–activity relationship

Introduction

Alzheimer's disease (AD), as one of most common diseases in the elderly population, is a chronic and progressive neurodegenerative disorder[1–3]. Although the precise etiology of AD is not elucidated enough, acetylcholinesterase (AChE) inhibitors were confirmed to be as the primary drugs to slow down the progression of AD in the present[4,5].

In recent, a lot of tertiary amine derivatives originated from natural products were synthesized and evaluated as AChE inhibitors[6–9]. The previous investigations in our laboratory suggested that chalcones with tertiary amine side chain, such as dimethylamine, diethylamine, dihydropyrrole or piperidine had more potent effects than other nitrogen-containing chalcones on inhibiting AChE[10–12].

In order to explore the influence of substituent on inhibiting AChE in chalcone derivatives, halogen atoms were considered to introduce into the chalcone scaffold. Chlorine was selected to modify the chalcone derivatives, considering chlorine exist in a lot of drugs in clinic application, such as chlorphenamine, chlorpromazine, chloroquine, etc. In the investigations searching for AChE inhibitors, some chlorine-containing compounds revealed AChE inhibition properties[13,14].

In this study, a series of chlorochalcones with tertiary amine side chain were synthesized and structure–activity relationship investigation was performed to explore the influence of chlorine in inhibiting AChE. In addition, four pyrazoline compounds were synthesized from chalcones and evaluated for the bioactivity to explore the influence of α,β-unsaturated ketone group. Compound **4l**, which had the most potent inhibitory activity against AChE, was selected to perform the kinetic and molecular docking studies for exploring the binding mode or mechanism to AChE.

Materials and methods

Chemistry

All chemicals and reagents were of analytical reagent grade and used without further purification. The melting points were measured on a WRS-IA melting point detector[1]. H NMR spectra were recorded on a Bruker 400 MHz instrument in $CDCl_3$ with TMS as the internal reference. Mass spectra were obtained on Finnigan LCQ advantage MAX by electrospray ionization (ESI-MS). Infrared spectrum (IR) was obtained on Shimadzu Infinity-1 infrared spectrometer. The purity of compounds was checked by Shimadzu LC-20A high-performance liquid chromatography. Elemental analysis was performed by elemental analyzer.

General method for synthesis of 4-hydroxy-chlorochalcones (3a–3c)

Compounds **3a–3c** were synthesized according to the relative references[15,16]. 4-hydroxyacetophenone (1.36 g, 10 mmol) and ethanol (20 mL) were mixed and stirred in the flask for 10 min until the solid was dissolved. Then 30% NaOH (3 mL) was dropped into the mixture, followed by stirring for 30 min in ice bath. Then chlorine benzaldehyde (11 mmol) dissolving in ethanol (5 mL) was dropped into the mixture, and the mixture continued to stir for about 36 h at 25 °C monitoring by TLC until the reaction was completed. Then the pH of the mixture was adjusted to 2–3 by HCl (1 mol/L).The mixture was kept in ice bath for 2 h, followed by the appearance of precipitation. The precipitation was filtered and washed by distilled water, and then dried at 50 °C, refined by the recrystallization in anhydrous ethanol. In result, compounds **3a–3c** were gained.

CONTACT Hao-ran Liu pharmaliu@126.com College of Chemistry and Chemical Engineering, Hu'nan University, Changsha, PR China; Xin-hua Xia xiaxinhua001@163.com College of Pharmacy, Hu'nan University of Chinese Medicine, Changsha, PR China

Supplemental data for this article can be accessed here

4'-Hydroxy-2-chlorochalcone (3a)

Light yellow solid was gained with yield of 80.7%. It was a known compound which was reported to have anticancer activity[17].

4'-Hydroxy-3-chlorochalcone (3b)

Light yellow solid, yield: 73.5%; m.p: 1 2 0 ~ 122 °C; ^{1}H NMR (400 MHz, $CDCl_3$, ppm): 6.96–6.98 (2H, d, J = 8.0 Hz, 3′-H and 5′-H), 7.26–7.34 (2H, m, 4-H and 5-H), 7.54–7.81 (3H, m, α-H, 2-H and 6-H), 8.10–8.16 (3H, m, β-H, 2′-H and 6′-H), 11.10 (1H, s, OH). MS *m/z* (ESI): $[M+H]^+$ 259. IR (KBr) ν/cm^{-1}: 3426, 3016, 1667.2, 1617, 1583, 1446, 1340, 1288, 1215, 1172, 839 and 732. Anal. calcd for $C_{15}H_{11}ClO_2$: C, 69.64; H, 4.29; O, 12.37; found C, 69.52; H, 4.27; N, 12.42%.

4'-Hydroxy -4-chlorochalcone (3c)

Light yellow solid was gained with yield of 85.6%. It is a known compound which was reported to inhibiting monoamine oxidases[18].

General method for synthesis of 4-amino alkyl-chlorochalcones (4a–4l)

Compounds **4a–4l** were synthesized according to the relative references[19]. 4′-hydroxy chlorochalcones (0.2620 g, 1 mmol) and potassium carbonate (0.4140 g, 3 mmol) were mixed in acetone (25 mL) in oil bath with continuous stirring at 56 °C for 30 min, then chloroethyldimethylamine hydrochloride, chloroethyldiethylamine hydrochloride, chloroethylpiperidine hydrochloride or chloroethylpyrrolidine hydrochloride (3 mmol) and sodium iodide (0.075 g, 0.5 mmol) were added into it. The mixture was refluxed overnight, filtered and concentrated. The concentrate was extracted with CH_2Cl_2 (3 × 30 mL), The CH_2Cl_2 phase was washed by saturated $NaHCO_3$ (2 × 30 mL), saturated salt water (3 × 30 mL), dried with sodium sulfate anhydrous, followed by concentrating in vacuum and then the residue was purified by silica gel to gain the product with dichloromethane/methanol (80:1) as the eluent.

(E)-3–(2-chlorophenyl)-1–(4-(2-(dimethylamino)ethoxy)phenyl)prop-2-en-1-one (4a)

Light yellow solid, yield: 81.6%; m.p: 7 6 ~ 77 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.09 (6H, s, 2 × $NC\underline{H}_3$), 2.92 (2H, t, J = 12.0 Hz, $OCH_2C\underline{H}_2$), 4.14 (2H, t, J = 12.0 Hz, $OC\underline{H}_2CH_2$), 6.98–7.00 (2H, m, 3′-H and 5′-H), 7.27–7.34 (2H, m, 3-H and 6-H), 7.43–7.77 (3H, m, α-H and 4-H and 5-H), 8.02–8.05 (2H, m, 2′-H and 6′-H), 8.15–8.19 (1H, d, J = 16.0 Hz, β-H). MS *m/z* (ESI): $[M+H]^+$ 330. IR (KBr) ν/cm^{-1}: 2806, 2769, 1661, 1611, 1576, 1458, 1261, 1227, 1176, 1020 and 730. Anal. calcd for $C_{19}H_{20}ClNO_2$: C, 69.19; H, 6.11; N, 4.25; O, 9.70; found C, 68.28; H, 6.05; N, 4.21; O, 9.63.

(E)-3–(3-chlorophenyl)-1–(4-(2-(dimethylamino)ethoxy)phenyl)prop-2-en-1-one (4b)

Light white solid, yield: 82.6%; m.p: 6 2 ~ 63 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.79 (6H, s, 2 × $NC\underline{H}_3$), 3.31 (2H, t, J = 12.0 Hz, $OCH_2C\underline{H}_2$), 4.51 (2H, t, J = 12.0 Hz, $OC\underline{H}_2CH_2$), 6.96–6.98 (2H, d, J = 8.0 Hz, 3′-H and 5′-H), 7.26–7.35 (2H, m, 4-H and 5-H), 7.54–7.81 (3H, m, α-H, 2-H and 6-H), 8.10–8.16 (3H, m, β-H, 2′-H and 6′-H), MS *m/z* (ESI): $[M+H]^+$ 330. IR (KBr) ν/cm^{-1}: 2805, 2774, 1678, 1626, 1576, 1458, 1246, 1227, 1175, 1020 and 722. Anal. calcd for $C_{19}H_{20}ClNO_2$: C, 69.19; H, 6.11; N, 4.25; O, 9.70; found C, 69.32; H, 6.04; N, 4.19; O, 9.74.

(E)-3–(4-chlorophenyl)-1–(4-(2-(dimethylamino)ethoxy)phenyl)prop-2-en-1-one (4c)

A white solid, yield: 75.6%; m.p: 2 3 3 ~ 234 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.37 (6H, s, 2 × $NC\underline{H}_3$), 2.79 (2H, t, J = 12.0 Hz, $OCH_2C\underline{H}_2$), 4.14 (2H, t, J = 12.0 Hz, $OC\underline{H}_2CH_2$), 7.00–7.02 (2H, m, 3′-H and 5′-H), 7.38–7.40 (2H, m, 2-H and 6-H), 7.51 (1H, d, J = 16.0 Hz, α-H), 7.55–7.59 (2H, m, 3-H and 5-H), 7.73–7.77 (1H, d, J = 16.0 Hz, β-H), 8.02–8.04 (2H, m, 2′-H and 6′-H). MS *m/z* (ESI): $[M+H]^+$ 330. IR (KBr) ν/cm^{-1}: 2805, 2774, 2359, 1658, 1595, 1558, 1456, 1246, 1227, 1175, 1020 and 731. Anal. calcd for $C_{19}H_{20}ClNO_2$: C, 69.19; H, 6.11; N, 4.25; O, 9.70; found C, 68.98; H, 6.07; N, 4.28; O, 9.72.

(E)-3–(2-chlorophenyl)-1–(4-(2-(diethylamino)ethoxy)phenyl)prop-2-en-1-one (4d)

Yellow viscous liquid was gained with yield of 78.1%. It is a known compound with no reports about bioactivity[20].

(E)-3–(3-chlorophenyl)-1–(4-(2-(diethylamino)ethoxy)phenyl)prop-2-en-1-one (4e)

Yellow viscous liquid, yield: 73.1%; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.12 (6H, t, J = 12.0 Hz, 2 × $NCH_2C\underline{H}_3$), 2.68–2.74 (4H, m, 2 × $NC\underline{H}_2CH_3$), 2.96 (2H, t, J = 12.0 Hz, $OCH_2C\underline{H}_2$), 4.17 (2H, t, J = 12.0 Hz, $OC\underline{H}_2CH_2$), 6.96–6.98 (2H, d, J = 8.0 Hz, 3′-H and 5′-H), 7.26–7.35 (2H, m, 4-H and 5-H), 7.54–7.81 (3H, m, α-H, 2-H and 6-H), 8.10–8.16 (3H, m, β-H, 2′-H and 6′-H). MS *m/z* (ESI): $[M+H]^+$ 358. IR (KBr) ν/cm^{-1}: 2970, 2810, 1661, 1508, 1337, 1246, 1173, 1024, 833 and 735. Anal. calcd for $C_{21}H_{24}ClNO_2$: C, 70.48; H, 6.76; N, 3.91; O, 8.94; found: C, 70.63; H, 6.62; N, 3.85; O, 8.78.

(E)-3–(4-chlorophenyl)-1–(4-(2-(diethylamino)ethoxy)phenyl)prop-2-en-1-one (4f)

Yellow viscous liquid was gained with yield of 72.4%. It is a known compound with no reports about bioactivity[21].

(E)-3–(2-chlorophenyl)-1–(4-(2-(piperidin-1-yl)ethoxy)phenyl)prop-2-en-1-one (4g)

Light white solid, yield: 83.7%; m.p: 5 5 ~ 56 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.24–1.28 (2H, m, piperidine-H), 1.22–1.36 (4H, m, piperidine-H), 2.35–2.43 (4H, m, piperidine-H), 2.73(2H, t, J = 12.0 Hz, $OCH_2C\underline{H}_2$), 4.12 (2H, t, J = 12.0 Hz, $OC\underline{H}_2CH_2$), 6.98–7.00 (2H, m, 3′-H and 5′-H), 7.27–7.34 (2H, m, 3-H and 6-H), 7.43–7.77 (3H, m, α-H and 4-H and 5-H), 8.02–8.05 (2H, m, 2′-H and 6′-H), 8.15–8.19 (1H, d, J = 16.0 Hz, β-H). MS *m/z* (ESI): $[M+H]^+$ 370. IR (KBr) ν/cm^{-1}: 3904, 3853, 3838, 3821, 3802, 3726, 3725, 3649, 2960, 2930, 2360, 1608, 1558, 1508, 1458 and 730. Anal. calcd for $C_{22}H_{24}ClNO_2$: C, 71.44; H, 6.54; N, 3.79; O, 8.65 found C, 71.58; H, 6.41; N, 3.65; O, 8.72.

(E)-3–(3-chlorophenyl)-1–(4-(2-(piperidin-1-yl)ethoxy)phenyl)prop-2-en-1-one (4h)

Light yellow solid, yield: 85.7%; m.p: 7 5 ~ 76 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.28–1.29 (2H, m, piperidine-H), 1.35–1.39 (4H, m, piperidine-H), 2.33–2.43 (4H, m, piperidine-H), 2.77 (2H, t, $J=12.0$ Hz, OCH_2CH_2), 4.15 (2H, t, $J=12.0$ Hz, OCH_2CH_2), 6.98–7.00 (2H, m, 3′-H and 5′-H), 7.27–7.34 (2H, m, 3-H and 6-H), 7.43–7.77 (3H, m, α-H and 4-H and 5-H), 8.02–8.05 (2H, m, 2′-H and 6′-H), 8.15–8.19 (1H, d, $J=16.0$ Hz, β-H). MS *m/z* (ESI): $[M+H]^+$ 370. IR (KBr) ν/cm^{-1}: 3903, 3853, 3838, 3802, 3736, 3649, 3566, 2960, 2934, 2359, 1659, 1611, 1578, 1236, 1175, 1026 and 730. Anal. calcd for $C_{22}H_{24}ClNO_2$: C, 71.44; H, 6.54; N, 3.79; O, 8.65 found C, 71.52; H, 6.48; N, 3.71; O, 8.62.

(E)-3–(4-chlorophenyl)-1–(4-(2-(piperidin-1-yl)ethoxy)phenyl)prop-2-en-1-one (4i)

White solid was gained with the yield: 82.7%. It is a known compound with anticancer effect in a recent investigation[22].

(E)-3–(2-chlorophenyl)-1–(4-(2-(pyrrolidin-1-yl)ethoxy)phenyl)prop-2-en-1-one (4j)

Yellow solid was gained with the yield of 80.7%. It is a known compound with anticancer effect in a recent investigation[22].

(E)-3–(3-chlorophenyl)-1–(4-(2-(pyrrolidin-1-yl)ethoxy)phenyl)prop-2-en-1-one (4k)

White solid, yield: 80.7%; m.p: 9 7∼98 °C; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.72–1.75 (4H, m, pyrrolidine-H), 2.56–2.58 (4H, m, pyrrolidine-H), 2.76 (2H, t, $J=12.0$ Hz, OCH_2CH_2), 4.01 (2H, t, $J=12.0$ Hz, OCH_2CH_2), 6.98–7.00 (2H, m, 3′-H and 5′-H), 7.27–7.34 (2H, m, 3-H and 6-H), 7.43–7.77 (3H, m, α-H and 4-H and 5-H), 8.02–8.05 (2H, m, 2′-H and 6′-H), 8.15–8.19 (1H, d, $J=16.0$ Hz, β-H). MS *m/z* (ESI): $[M+H]^+$ 356. IR (KBr) ν/cm^{-1}: 2956, 2781, 1657, 1604, 1593, 1510, 1479, 1339, 1269, 1211, 1175, 1020, 980 and 735. Anal. calcd for $C_{21}H_{22}ClNO_2$: C, 70.88; H, 6.23; N, 3.94; O, 8.99; found C, 70.95; H, 6.27; N, 3.88; O, 8.91.

(E)-3–(4-chlorophenyl)-1–(4-(2-(pyrrolidin-1-yl)ethoxy)phenyl)prop-2-en-1-one (4l)

Yellow solid was gained with the yield of 81.7%. It is a known compound with anticancer effect in a recent investigation[22].

General procedure of synthesis of compounds 5a–5d

Compounds **4i, 4j, 4k** or **4l** (1 mmol), 80% hydrazine hydrate (0.2 mL, 4 mmol) and acetic acid (5 mL) were mixed and refluxed[23]. When the reaction was completed, the mixture was cooled to room temperature and 30 mL CH_2Cl_2 was added into it. The CH_2Cl_2 phase was washed by saturated K_2CO_3 solution, and dried by anhydrous sodium sulfate. After the solution was filtered and the solvent was evaporated under reduced pressure, the crude product was gained. The refined product was achieved by the silica column chromatography with the eluent (methanol: methylene chloride =1:20).

1–(5-(4-Chlorophenyl)-3–(4-(2-(piperidin-1-yl)ethoxy)phenyl)-4,5-dihydro-1H-pyrazol-1-yl) ethan-1-one (5a)

Yellow viscous liquid, yield: 70.6%; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.84–1.86 (6H, m, piperidine-H), 2.43 (3H, s, $COCH_3$), 2.54–2.57 (4H, m, piperidine-H), 2.86 (2H, t, $J=12.0$ Hz, OCH_2CH_2), 3.07 (1H, dd, $J=4.0$ Hz, 16.0 Hz, CH_2-H^a), 3.64 (1H, dd, $J=12.0$ Hz, 16.0 Hz, CH_2-H^b), 4.20 (2H, t, $J=12.0$ Hz, OCH_2CH_2), 5.79–5.83 (1H, m, NCH), 6.92–6.95 (2H, m, 3′-H and 5′-H), 7.03–7.28 (4H, m, 1-H and 2-H and 4-H and 5-H), 7.65–7.67 (2H, m, 2′-H and 6′-H). MS *m/z* (ESI): $[M+H]^+$ 426. IR (KBr) ν/cm^{-1}: 3442, 3419, 3062, 3030, 2933, 2853, 2787, 1653, 1608, 1510, 1422, 1251, 1223, 1175, 1163, 1028, 988 and 814. Anal. calcd for $C_{24}H_{28}ClN_3O_2$: C, 67.67; H, 6.63; N, 9.87; O, 7.51; found C, 67.78; H, 6.52; N, 9.81; O, 7.46.

1–(5-(2-Chlorophenyl)-3–(4-(2-(pyrrolidin-1-yl)ethoxy)phenyl)-4,5-dihydro-1H-pyrazol-1-yl) ethan-1-one (5b)

Yellow viscous liquid, yield:74.2%; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.84–1.87 (6H, m, piperidine-H), 2.69–2.73 (3H, s, $COCH_3$), 2.96–2.99 (4H, m, piperidine-H), 3.09 (2H, t, $J=12.0$ Hz, OCH_2CH_2), 3.12 (1H, dd, $J=4.0$ Hz, 16.0 Hz, CH_2-H^a), 3.76 (1H, dd, $J=12.0$ Hz, 16.0 Hz, CH_2-H^b), 4.20 (2H, t, $J=12.0$ Hz, OCH_2CH_2), 5.76–5.80 (1H, m, NCH), 6.90–6.93 (2H, m, 3′-H and 5′-H), 7.13–7.35 (4H, m, 3-H and 4-H and 5-H and 6-H), 7.63–7.67 (2H, m, 2′-H and 6′-H). MS *m/z* (ESI): $[M+H]^+$ 412. IR (KBr) ν/cm^{-1}: 3446, 3421, 3063, 3032, 2935, 2858, 2779, 1642, 1590, 1512, 1339, 1243, 1219, 1173, 1020, 980 and 726. Anal. calcd for $C_{23}H_{26}ClN_3O_2$: C, 67.06; H, 6.36; N, 10.20; O, 7.77; found C, 66.91; H, 6.48; N, 10.09; O, 7.84.

1–(5-(3-Chlorophenyl)-3–(4-(2-(pyrrolidin-1-yl)ethoxy)phenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one (5c)

Yellow viscous liquid, yield:77.6%; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.84–1.87 (6H, m, piperidine-H), 2.69–2.73 (3H, s, $COCH_3$), 2.96–2.99 (4H, m, piperidine-H), 3.09 (2H, t, $J=12.0$ Hz, OCH_2CH_2), 3.13 (1H, dd, $J=4.0$ Hz, 16.0 Hz, CH_2-H^a), 3.76 (1H, dd, $J=12.0$ Hz, 16.0 Hz, CH_2-H^b), 4.20 (2H, t, $J=12.0$ Hz, OCH_2CH_2), 5.76–5.80 (1H, m, NCH), 6.83–6.85 (2H, m, 3′-H and 5′-H), 7.04–7.29 (4H, m, 2-H and 4-H and 5-H and 6-H), 7.66–7.70 (2H, m, 2′-H and 6′-H). MS *m/z* (ESI): $[M+H]^+$ 412. IR (KBr) ν/cm^{-1}: 3443, 3417, 3062, 3032, 2936, 2881, 1645, 1583, 1510, 1479, 1339, 1269, 1243, 1211, 1175, 1020, 980 and 735. Anal. calcd for $C_{23}H_{26}ClN_3O_2$: C, 67.06; H, 6.36; N, 10.20; O, 7.77; found C, 66.89; H, 6.45; N, 10.34; O, 7.61.

1–(5-(4-Chlorophenyl)-3–(4-(2-(pyrrolidin-1-yl)ethoxy)phenyl)-4,5-dihydro-1H-pyrazol-1-yl) ethan-1-one (5d)

Yellow viscous liquid, yield: 73.2%; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.84–1.87 (6H, m, piperidine-H), 2.69–2.73 (3H, s, $COCH_3$), 2.96–2.99 (4H, m, piperidine-H), 3.09 (2H, t, $J=12.0$ Hz, OCH_2CH_2), 3.13 (1H, dd, $J=4.0$ Hz, 16.0 Hz, CH_2-H^a), 3.76 (1H, dd, $J=12.0$ Hz, 16.0 Hz, CH_2-H^b), 4.20 (2H, t, $J=12.0$ Hz, OCH_2CH_2), 5.76–5.80 (1H, m, NCH), 6.92–6.95 (2H, m, 3′-H and 5′-H), 7.03–7.28 (4H, m, 1-H and 2-H and 4-H and 5-H), 7.65–7.67 (2H, m, 2′-H and 6′-H). MS *m/z* (ESI): $[M+H]^+$ 412. IR (KBr) ν/cm^{-1}: 3442, 3419, 3060, 3031, 2930, 2883, 1640, 1585, 1510, 1307, 1263, 1230, 1221, 1175, 1032, 978 and 738. Anal. calcd for $C_{23}H_{26}ClN_3O_2$: C, 67.06; H, 6.36; N, 10.20; O, 7.77; found C, 67.24; H, 6.18; N, 10.12; O, 7.65.

Log p *measurement*

Log *p*, defined as the logarithm of octanol-water partition coefficient is an important parameter to evaluate the lipophilicify of compounds. It can be calculated by determining the concentration of compound in octanol phase and water phase until the partition equilibrium was completed. In present investigation, log *p* of compounds **4a∼4l** was measured by the shake flask method with slight modification, and PBS (pH =7.4) was used as the water phase[24]. The mobile phase was methanol: 0.1% triethylamine

(TEA)/80:20 (v/v), at a flow rate of 1.0 mL.min^{-1} through a C_{18} column (250 nm ×4.6 mm, 5 μm) at 32 °C with detect wavelength 318 nm. Experiments were conducted in triplicate and log *p* values were calculated.

AChE and BuChE inhibition assay

AChE/BuChE activity assays were conducted by *Ellman* method with light modification[25]. The brain and serum of Sprague–Dawley rat was used as the resource of AChE and BuChE, respectively. Each compound was dissolved in Tween 80 and diluted with water to various concentrations immediately before use. The reaction mixture containing 40 μL AChE/BuChE, 100 μL acetylthiocholine iodide/S-Butyrylthiocholine iodide, 2.76 mL Na_2HPO_4/NaH_2PO_4 buffer (pH 8.0, 0.1 mol/L), and 100 μL different concentrations of test compounds were incubated at 30 °C for 25 min. Then the reaction was terminated via adding 100 μL 20% sodium dodecyl sulfate (SDS), then 100 μL 10 mmol/L 5, 5'-Dithiobis-(2-nitrobenzoic acid) (DTNB) was added to generate the yellow anion 5-thio-2-nitro-benzoic acid. The absorbance of each assay mixture was measured at 412 nm by UV spectroscopy. The IC_{50} values were calculated by Bliss method and expressed as mean ± SD of the replicates. Rivastigmine was used as the control drug.

Kinetic studies

Kinetic experiment was conducted by a reported method with some modifications[26]. Compound **4l** was added into the assay solution and pre-incubated with the enzyme at 30 °C, followed by the addition of 100-μL acetylthiocholine iodide including five concentrations. The assay solution contained 100 μL compound **4l**, 100 μL DTNB, 2.76 mL 0.1 mol/L Na_2HPO_4/NaH_2PO_4 buffer (pH 8.0). Kinetic profile of AChE was determined by UV spectrophotometer at 412 nm. Additionally, the blank control experiment was conducted with the vehicle to replace compound **4l** solution.

Molecular docking

Molecular docking was performed by molecular operating environment (MOE) software package (Chemical Computing Group Inc., Montréal, Canada). The X-ray crystallographic structures of AChE (PDB code: 1EVE) and BuChE (PDB code: 1P0I) were gained from protein data bank. 3D structure of compound **4l** as the strongest AChE inhibitor in the present investigation was established by virtue of the builder interface of MOE program, and docked into the active site of the protein after energy being minimized. The dock scoring in MOE software was done by ASE scoring function.

Results and discussion

Chemistry

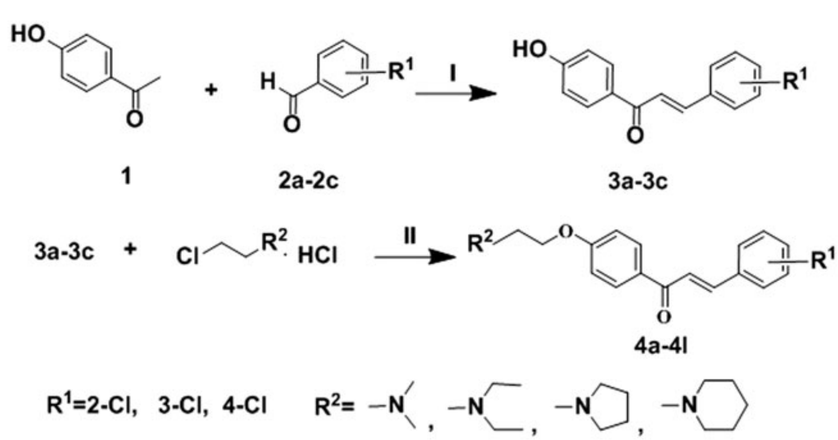
Three different chlorobenzaldehydes (compounds **2a–2c**) reacted with 4-hydroxyl chalcone in ethanol with sodium hydroxide as catalyst to generate three 4'-hydroxy–chlorochalcones (compounds **3a–3c**). Then compounds **3a–3c** reacted with four different alkyl amines to gain a series of amine alkyl – substituted chlorochalcone derivatives (compounds **4a–4l**) in the presence of potassium carbonate, acetone and sodium iodide. The total synthetic route is shown in Scheme 1.

For the synthesis of 4′-hydroxy–chlorochalcones, the concentration of sodium hydroxide dramatically influences the yield and purity of the products. As a result, 30% sodium hydroxide was chosen as the catalyst. For the synthesis of compounds **4a–4l**, the solvent for the reaction is important. In this reaction, acetone was selected as the solvent for its easy operation and low toxicity. In addition, sodium iodide was used as the catalyst to enhance the reaction activity and accelerate the reaction process. For the synthesis of different tertiary amine substituted chlorochalcones, the reaction time ranged from 5 to 8 h. Pyrazoline compounds were synthesized from chlorochalcones, hydrazine and acetic acid (Scheme 2). High-yield desired compounds were gained via this reaction.

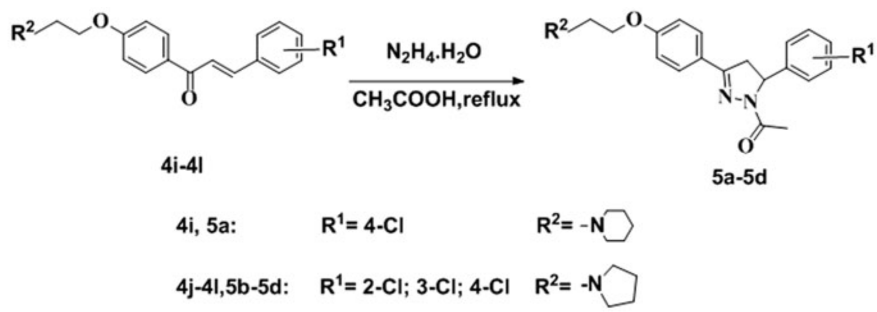
New synthetic compounds were characterized by proton nuclear magnetic resonance spectroscopy (^{1}H NMR), IR and mass spectrometry (MS) and elemental analysis. The purities of all synthesized compounds were confirmed to be higher than 98% by HPLC.

Bioactivity evaluation

Tertiary amine group, which is thought as a versatile pharmacophore appeared in the structures of many drugs in clinic practice, such as local anesthetics lidocaine, estrogen receptor modulator, tamoxifen, antipsychotic drug chlorpromazine and antimalarial chloroquine. Interestingly, in this investigation, some of synthesized compounds are known compounds. Compounds **3a** and **3c** were reported to be as anticancer agent and monoamine oxidase Inhibitor, respectively[17,18]. Compounds **4d** and **4f** were patent protected compounds three decades ago (US Patent 4342782, US Patent 2668813), but there are no reports on the bioactivity of

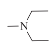
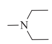
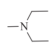
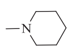
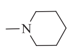
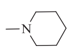
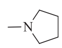
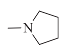
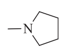
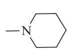
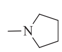
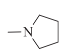
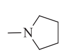


Scheme 1. The synthesis of compounds **4a–4l** (I) 30% NaOH, EtOH, 25 °C, stirring 36 h; (II) K2CO3, acetone, NaI, 56 °C, reflux.



Scheme 2. The synthesis of compounds **5a–5d**.

Table 1. AChE and BuChE inhibitory activity and log *p* of chlorochalcone derivatives.

			IC_{50} (μmol/L)*			
Compound	R^1	R^2	AChE	BuChE	Selectivity†	Log *p*
3a	2-Cl	–	>500	>500	–	–
3b	3-Cl	–	>500	>500	–	–
3c	4-Cl	–	>500	>500	–	–
4a	2-Cl		2.11 ± 0.38	41.12 ± 2.31	19.5	1.75
4b	3-Cl		3.76 ± 0.26	32.14 ± 7.16	8.5	1.83
4c	4-Cl		1.55 ± 0.16	43.62 ± 6.94	28.1	1.61
4d	2-Cl		5.42 ± 0.20	63.83 ± 9.21	11.8	1.63
4e	3-Cl		5.83 ± 0.42	80.21 ± 7.18	13.8	1.62
4f	4-Cl		3.78 ± 0.39	101.03 ± 17.23	26.7	1.69
4g	2-Cl		1.80 ± 0.48	35.62 ± 7.31	19.8	1.75
4h	3-Cl		1.31 ± 0.17	24.51 ± 6.14	18.7	1.72
4i	4-Cl		0.91 ± 0.09	114.21 ± 26.15	125.5	1.79
4j	2-Cl		0.81 ± 0.03	32.08 ± 5.71	39.6	1.85
4k	3-Cl		0.28 ± 0.05	31.06 ± 3.41	110.9	1.81
4l	4-Cl		0.17 ± 0.06	113.43 ± 18.22	667.2	1.83
5a	4-Cl		5.94 ± 0.27	97.64 ± 3.78	16.4	–
5b	2-Cl		6.31 ± 0.16	29.91 ± 1.25	4.74	–
5c	3-Cl		2.41 ± 0.34	27.62 ± 2.58	11.5	–
5d	4-Cl		2.04 ± 0.26	108.03 ± 5.56	52.96	–
Rivastigmine‡	–	–	10.54 ± 0.86	0.26 ± 0.08	0.025	–

*IC_{50}: 50% inhibitory concentration (means ± SD of three experiments).
†Selectivity for AChE is defined as IC_{50} (BuChE)/IC_{50} (AChE).
‡Used for positive control.

them up to now[20,21]. Compounds **4i**, **4j** and **4l** were reported as anticancer agents in pharmacology experiments[22].

Although some known compounds contained in the present investigation, the bioactivity of them in inhibiting AChE was not be reported. As shown in Table 1, the data indicated that all amino alkyl substituted chlorochalcones exhibited better inhibitory activities against AChE than the precursor compounds **3a–3c** (IC_{50} >500 μmol/L). Among them, compounds **4i**, **4j**, **4k** and **4l** with IC_{50} values of 0.91, 0.81, 0.28 and 0.17 μmol/L respectively, showed 11–62 folds inhibitory activity as that of Rivastigmine (IC_{50} = 10.54 μmol/L). In addition, compound **4l** possessed high selectivity for AChE (Ratio: 667.2).

Based on the data in Table 1, it seemed that the variation of amino-alkyl side chain markedly influence the inhibitory potency of compounds against AChE. Generally, the order of the inhibitory potency of these derivatives was as followed: pyrrolidine group > piperidine group > dimethylamine group > diethylamine group. Compounds **4j**, **4k** and **4l**, which were substituted by pyrrolidine group, exhibited potent inhibitory activity with IC_{50} values less than 1.0 μmol/L. On other hand, the substituted position of chlorine atom in chalcone scaffold was very important for the inhibitory and selectivity for AChE. For dimethylamine or diethylamine substituted chlorochalcone derivatives the order of inhibitory potency against AChE was: *Para* > *Meta* > *Ortho*, while for piperidine or pyrrolidine substituted chlorochalcone derivatives the order was: *Para* > *Orto* > *Meta*. In addition, all *Para*-substituted chlorochalcone derivatives had the highest selectivity in inhibiting AChE over BuChE. Among them, compound **4l** had the highest selectivity as 667 fold. Compared with the tertiary amine derivatives of fluorochalcone in our previous report, compound **4l** showed higher selectivity against AChE than that of fluorochalcone derivatives[27].

Compound **4l** was selected for enzyme kinetic studies. The linear Lineweaver–Burk equation was applied to evaluate the inhibition profile. The graphical analysis of the steady-state inhibition data of compound **4l** was shown in Supplement data: Figure 1. According to the analysis, compound **4l** presented a mixed-type inhibition for that K_m increased and V_{max} decreased with the increasing of the concentration of compound **4l**. The competitive inhibition constant (K_i) and the noncompetitive constant (K_i') are 0.38 and 2.95 μmol/L, respectively (Supplement data: Table 2).

To explore the possible interacting mode between the chlorochalcone derivatives and AChE, molecular docking was performed for compound **4l** with software MOE2008. As shown in Supplement data: Figure 2, this compound exhibited a multiple points-binding mode with AChE (Supplement data: Figure 2A). In the top of the gorge, the aromatic moiety adopted an appropriate orientation for its binding to peripheral anionic site (PAS), via the π–π stacking interaction with Trp279. The side chain interacted with Tyr334 in the mid-gorge zone. In the bottom of the gorge, the nitrogen of pyrrolidine ring binds to Trp84 via a cation–π in catalytic active site (CAS). Compared to the interaction between compound **4l** and AChE mentioned above, compound **4l** can only bind with BuChE via Tyr257 and Gly311 that were not the important amino acids in the catalytic process of BuChE (Supplement data: Figure 2B). These results may partially explain its potent inhibition and high selectivity for AChE. As a potential compound for treatment of AD, log *p* was thought as an important physical chemistry parameter to valuate or predict the ability to cross blood brain barrier (BBB). It was reported that the log *p* with optimum central nervous system (CNS) penetration was around 2 ± 0.7[28]. As shown in Table 1, log *p* values of new synthesized compounds ranged from 1.61 to 1.83, which indicated that all the compounds had sufficiently lipophilicify to pass the BBB *in vivo*.

In further study to explore the influence of chalcone scaffold on bioactivity, pyrazoline derivatives were synthesized from chlorochalcones. The bioactivity evaluation showed that the inhibition potency of pyrazoline derivatives against AChE dramatically decreased compared to that of chlorochalcone derivatives (Table 1). It indicated that α,β-unsaturated ketone group in chalcone possibly pay an important role for the inhibitory activity against AChE. In addition, the selectivity of pyrazoline derivatives to inhibiting AChE over BuChE also decreased significantly.

Conclusion

In the present investigation, a series of chlorochalcones and pyrazoline derivatives were synthesized and evaluated in inhibiting AChE and BuChE. The result showed that the substituted position of chlorine significantly influenced the activity and selectivity of compounds in inhibiting AChE. For those compounds with the same amine alkyl side chain, *Para*-substituted chlorochalcone had the highest activity and selectivity. The pyrazoline derivatives synthesized from chlorochalcones with the cyclization of α,β-unsaturated ketone group had weaker activity and lower selectivity in inhibiting AChE compared to that of chlorochalcone derivatives, suggesting that α,β-unsaturated ketone group was important for inhibiting AChE. Among them, compound **4l** revealed the strongest AChE inhibitory activity (IC_{50}: 0.17 μmol/L) and highest selectivity (Ratio: 667.2). Enzyme kinetic study suggested that compound **4l** was a mixed-type inhibitor. Molecular docking supported the mixed-type inhibition mechanism. Compound **4l** might serve as a potential agent for the treatment of AD.

Disclosure statement

The authors confirm that this article content has no conflict of interest.

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