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Novel potent and selective acetylcholinesterase inhibitors as potential drugs for the treatment of Alzheimer's disease: Synthesis, pharmacological evaluation, and molecular modeling of amino alkyl substituted fluoro-chalcones derivatives

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Abstract: A new series of fluoro-chalcones substituted amino alkyl derivatives (**3a~3l**) were designed, synthesized, characterized and evaluated for the inhibitory activity against acetylcholinesterase (AChE) and butylcholinesterase (BuChE). The results showed that the alteration of fluorine atom position and amino alkyl groups markedly influenced the activity and the selectivity of chalcone derivates in inhibiting AChE and BuChE. Among them, compound **3l** posses the most potent inhibitory against AChE (IC_{50}= 0.21±0.03 μmol/L), and the highest selectivity for AChE over BuChE(IC_{50} (BuChE)/IC_{50} (AChE)=65.0). Molecular modeling and enzyme kinetic study on compound **3l** supported its dual AChE inhibitory profile, simultaneously binding at the catalytic active and peripheral anionic site of the enzyme.

Keywords: *Fluorine; Chalcone; Selectivity; AChE inhibitors; Molecular modeling*

In recent years, a lot of attention were thrown to natural products for the therapy of Alzheimer's disease (AD) which was usually treated with acetylcholinesterase(AChE) inhibitors (1-5). By screening AChE inhibitors in clinical application, such as tacrine, donepezil, galanthamine and rivastigmine, we thought that amino substituent was possibly important pharmacophore of them. Thereby, a series chalcone derivates containing amino substituents were designed and synthesized in our recent investigations(6,7). The previous results suggested that dimethylamine, diethylamine, dipropylamine, pyrrolidine-containing chalcones had more potent effects in inhibiting AChE compared with other nitrogen-containing chalcones.

In order to develop chalcone derivates as AChE inhibitors in further, we considered to introduce halogen atoms such as fluorine into nitrogen-containing chalcones since fluorine seemed important to enhance bioactivities(8-10). So far hundreds of fluorine-containing drugs had been applied in clinical practice, such as antidepressant Fluoxetine，antibacterial Ofloxacin, anti-cancer agent 5-Fluorouracil, and antilipemic agent Atorvastatin calcium, etc (**Figure 1**).

In the present study, 2-fluorobenzaldehyde, 3-fluorobenzaldehyde and 4-fluorobenzaldehyde (**1a-1c**) were used for the synthesis of 4-hydroxy-fluoro chalcones (**2a-2c**) and fluoro-chalcones substituted amino alkyl derivatives (**3a-3l**). Followed by the synthesis of these compounds, biological activity evaluation, kinetic assay and molecular modeling were conducted.

Materials and Method

Chemistry

Melting points were measured on a WRS-IA melting point detector. The ^{1}H NMR spectra were recorded on a spectrometer operating at 500 MHz. Mass spectra (MS) were recorded on a ZAB-HS mass spectrometer using ESI (electrospray ionization) model. Ultraviolet spectrum (UV) was determined by Shimadzu UV1800 UV-Vis spectrophotometer. Infrared spectrum (IR) was obtained from Shimadzu Infinity-1

infrared spectrometer. The purity of the compounds was checked by Shimadzu LC-20A high performance liquid chromatograph. All reagents were reagent grade and used directly as obtained commercially.

General method for synthesis of 4-hydroxy-fluochalcones (2a-2c)

4-hydroxyacetophenone (1.36 g, 0.01 mol) and fluo-benzaldehyde (0.01 mol) were mixed in ethanol (20 mL) in a three-necked flask placed in an ice bath. Then sodium hydroxide solution (10 mL, 10%) was added drop-wise into it with stirring for 30 min. The mixing was continued for 24 h at room temperature. The mixture was kept in a refrigerator overnight when it became quite thick. Then it was diluted with ice-cold distilled water (30 mL), filtered, washed with cold water, dried in air and re-crystallized from rectified methanol.

(E)-1-(2-fluorophenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one (2a)

Light white powder solid (1.961 g, 81%); mp: 155-156 □; ^{1}H NMR (500 MHz, DMSO): δ=6.910-6.927 (m, 2H), 7.304-7.347 (m, 2H), 7.493-7.808 (m, 3H), 7.970 (d, J=16.0 Hz, 1H), 8.067-8.133 (m, 2H), 10.483 ppm (s, 1H); IR (KBr): v=3147, 3026, 1651, 1608, 1573, 1440, 1342, 1286, 1219, 1167, 839, 761 cm^{-1}; MS (ESI) m/z: 242 $[M+H]^+$.

(E)-1-(3-fluorophenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one (2b)

Light white powder solid (2.008 g, 83%); mp: 142-143 □; ^{1}H NMR (500 MHz, DMSO): δ=6.905 (d, J=8.5 Hz, 2H), 7.254-7.498 (m, 2H), 7.514-7.847 (m, 3H), 7.984 (d, J=15.5 Hz, 1H), 8.097 (d, J=8.5 Hz, 2H), 10.452 ppm (1H, s); IR (KBr): v=3134, 3026, 1649, 1607, 1583, 1446, 1340, 1288, 1215, 1172, 839, 779 cm^{-1}; MS (ESI) m/z: 242 $[M+H]^+$.

(E)-1-(4-fluorophenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one (2c)

Bright yellow powder solid (2.057 g, 85%); mp: 192-193 □; ^{1}H NMR (500 MHz, DMSO): δ=6.898 (d, J=8.5 Hz, 2H), 7.280-7.316 (m, 2H), 7.680 (d, J=15.5 Hz, 1H), 7.885 (d, J=15.5 Hz, 1H), 7.941-7.969 (m, 2H), 8.076 (d, J=8.5 Hz, 2H), 10.424 ppm (s,1H); IR (KBr): v=3134, 3026, 1649, 1610, 1571, 1438, 1342, 1286, 1217, 1167, 823, 763 cm^{-1}; MS (ESI) m/z: 242 $[M+H]^+$.

General method for synthesis of 4-aminomethoxy- fluochalcones (3a-3l)

4-hydroxy-fluochalcones (0.242 g, 1 mmol) and potassium carbonate (0.414 g, 3 mmol) were mixed in acetone (25 mL) with continuous stirring at 56 □ in an oil bath for 30 min, then 2-chloro-N-substituted ethanamine hydrochloride (2 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 salt water (3 × 30 mL), dried with sodium sulphate anhydrous, followed by concentrating in vacuum, then the residue was purified with silica gel column chromatography (eluting with 60:1 dichloromethane/ methyl alcohol) to gain the product.

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

Light yellow powder solids (234 mg, 75%); mp: 86-87 ℃; ^{1}H NMR (500 MHz, $CDCl_3$): *δ*=2.417 (s, 6H), 2.844 (t, *J*=11.0 Hz, 2H), 4.195 (t, *J*=11.0 Hz, 2H), 6.994-7.023 (m, 2H), 7.111-7.151 (m, 1H), 7.179-7.211 (m, 1H), 7.352-7.675 (m, 3H), 7.888 (d, *J*=16.5, 1H), 8.023-8.046 ppm (m, 2H); IR (KBr): *v*=2806, 2769, 1661, 1611, 1576, 1458, 1261, 1227, 1176, 1020, 760 cm^{-1}; MS (ESI) m/z: 313 $[M+H]^+$.

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

White powder solids (250 mg, 80%); mp: 62-63 ℃; ^{1}H NMR (500 MHz, $CDCl_3$): *δ*=2.791 (s, 6H), 3.306 (t, *J*=9.5 Hz, 2H), 4.507 (t, *J*=9.5 Hz, 2H), 7.010-7.028 (m, 2H), 7.094-7.134 (m, 1H), 7.336-7.422 (m, 3H), 7.518 (d, *J*=15.5 Hz, 1H), 7.753 (d, *J*=15.5 Hz, 1H), 8.029-8.058 ppm (m, 2H); IR (KBr): *v*=2805, 2774, 1658, 1606, 1576, 1458, 1246, 1227, 1175, 1020, 730 cm^{-1}; MS (ESI) m/z: 313 $[M+H]^+$.

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

White powder solids (244 mg, 78%); mp: 233-234 ℃; ^{1}H NMR (500 MHz, $CDCl_3$): *δ*=2.385 (s, 6H), 2.803 (t, *J*=11.0 Hz, 2H), 4.176 (t, *J*=11.0 Hz, 2H), 7.000-7.018 (m, 2H), 7.092-7.127 (m, 2H), 7.475 (d, *J*=16.0 Hz, 1H), 7.621-7.650 (m, 2H), 7.766 (d, *J*=15.5 Hz, 1H), 8.020-8.043 ppm (m, 2H); IR (KBr): *v*=2805, 2774, 2359, 1658, 1595,

1558, 1456, 1246, 1227, 1175, 1020, 730 cm^{-1}; MS (ESI) m/z: 313 $[M+H]^+$.

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

Yellow viscous oily liquid (262 mg, 77%); ^1H NMR (500 MHz, $CDCl_3$): δ=1.100 (t, J=14.5 Hz, 6H), 2.659-2.702 (m, 4H), 2.931 (t, J=12.0 Hz, 2H), 4.154 (t, J=12.0 Hz, 2H), 6.981-7.004 (m, 2H), 7.110-7.150 (m, 1H), 7.178-7.210 (m, 1H), 7.267-7.678 (m, 3H), 7.964 (d, J =16.0, 1H), 8.034 ppm (m, 2H); IR (KBr): v=2810, 2771, 1663, 1606, 1574, 1458, 1335, 1260, 1219, 1169, 1020, 983, 833, 760 cm^{-1}; MS (ESI) m/z: 341 $[M+H]^+$.

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

Yellow viscous oily liquid (279 mg, 82%); ^1H NMR (500 MHz, $CDCl_3$): δ=1.090 (t, J=14.5 Hz, 6H), 2.644-2.686 (m, 4H), 2.916 (t, J=12.5 Hz, 2H), 4.141 (t, J=12.5 Hz, 2H), 6.984-6.998 (m, 2H), 7.002-7.121 (m, 1H), 7.329-7.412 (m, 3H), 7.537 (d, J=16.0 Hz, 1H), 7.746 (d, J =15.5 Hz, 1H), 8.016-8.045 ppm (m, 2H); IR (KBr): v=2970, 2810, 1661, 1508, 1337, 1246, 1173, 1024, 833, 785 cm^{-1}; MS (ESI) m/z: 341 $[M+H]^+$.

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

Yellow viscous oily liquid (259 mg, 76%); ^1H NMR (500 MHz, $CDCl_3$): δ=1.132 (t, J=13.5 Hz, 6H), 2.732-2.751 (m, 4H), 2.977 (t, J=11.5 Hz, 2H), 4.197 (t, J=12.0 Hz, 2H), 6.982-6.999 (m, 2H), 7.093-7.127 (m, 2H), 7.474 (d, J=15.5 Hz, 1H), 7.633-7.656 (m, 2H), 7.766 (d, J=15.5 Hz, 1H), 8.018-8.036 ppm (m, 2H); IR (KBr): v=2802, 1660, 1601, 1576, 1456, 1339, 1261, 1223, 1173, 1026, 829, 760 cm^{-1}; MS (ESI) m/z: 341 $[M+H]^+$.

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

Light yellow powder solids (265 mg, 75%); mp: 45-46 □; ^1H NMR (500 MHz, $CDCl_3$): δ=1.442-1.476 (m, 2H), 1.616-1.661 (m, 4H), 2.551-2.627 (m, 4H), 2.832(t, J=12.0 Hz, 2H), 4.212 (t, J=11.5 Hz, 2H), 6.983-7.007 (m, 2H), 7.113-7.153 (m, 1H), 7.181-7.213 (m, 1H), 7.368-7.677 (m, 3H), 7.887 (d, J=16.0, 1H), 8.025-8.043 ppm (m, 2H); IR (KBr): v=3904, 3853, 3838, 3821, 3802, 3726, 3725, 3649, 2960, 2930, 2360, 1608, 1558, 1508, 1458 cm^{-1}; MS (ESI) m/z: 353 $[M+H]^+$.

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

Light yellow powder solids (289 mg, 82%); mp: 85-86 □; ^1H NMR (500 MHz, $CDCl_3$):

δ=1.482-1.494 (m, 2H), 1.648-1.693 (m, 4H), 2.527-2.627 (m, 4H), 2.874 (t, *J*=12.5 Hz, 2H), 4.246 (t, *J*=12.5 Hz, 2H), 6.981-6.991 (m, 2H), 7.001-7.123 (m, 1H), 7.331-7.414 (m, 3H), 7.533 (d, 1H), 7.747 (d, *J*=16.0 Hz, 1H), 8.016-8.040 ppm (m, 2H); IR (KBr): *v*=3903, 3853, 3838, 3802, 3736, 3649, 3566, 2960, 2934, 2359, 1659, 1611, 1578, 1236, 1175, 1026 cm^{-1}; MS (ESI) m/z: 353 $[M+H]^+$.

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

White powder solids (295 mg, 84%); mp: 123-124 □; ^1H NMR (500 MHz, $CDCl_3$): *δ*=1.484-1.493 (m, 2H), 1.652-1.685 (m, 4H), 2.581-2.627 (m, 4H), 2.876 (t, *J*=11.0 Hz, 2H), 4.247 (t, *J*=11.5 Hz, 2H), 6.982-7.000 (m, 2H), 7.092-7.127 (m, 2H), 7.472 (d, *J* = 15.5 Hz, 1H), 7.622-7.650 (m, 2H), 7.766 (d, *J*=16.0 Hz, 1H), 8.016-8.040 ppm (m, 2H); IR (KBr): *v*=3854, 3736, 3649, 3566, 2934, 2849, 2787, 1657, 1611, 1510, 1422, 1261, 1223, 1175, 1163, 1028, 988, 814 cm^{-1}; MS (ESI) m/z: 353 $[M+H]^+$.

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

Yellow powder solids (281 mg, 83%); mp: 87-88 □; ^1H NMR (500 MHz, $CDCl_3$): *δ*=1.823-1.850 (m , 4H), 2.658-2.682 (m, 4H), 2.960 (t, *J*=11.5 Hz, 2H), 4.213 (t, *J*=11.5 Hz, 2H), 6.997-7.020 (m, 2H), 7.130-7.212 (m, 2H), 7.363-7.678 (m, 3H), 7.887 (d, *J*=16.0 Hz, 1H), 8.027-8.041 ppm (m, 2H); IR (KBr): *v*=2955, 2928, 2779, 1659, 1610, 1512, 1339, 1219, 1173, 1020, 980, 780 cm^{-1}; MS (ESI) m/z: 339 $[M+H]^+$.

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

White powder solids (287 mg, 85%); mp: 87-88 □; ^1H NMR (500 MHz, $CDCl_3$): *δ*=1.823-1.850 (m , 4H), 2.660-2.670 (m, 4H), 2.961 (t, *J*=11.5 Hz, 2H), 4.214 (t, *J*=11.5 Hz, 2H), 7.000-7.018 (m, 2H), 7.081-7.122 (m, 1H), 7.330-7.412 (m, 3H), 7.534 (d, *J*=15.5 Hz, 1H), 7.746 (d, *J*=15.5 Hz, 1H), 8.023-8.041 ppm (m, 2H); IR (KBr): *v*=2956, 2781, 1657, 1604, 1593, 1510, 1479, 1339, 1269, 1211, 1175, 1020, 980, 785 cm^{-1}. MS (ESI) m/z: 339 $[M+H]^+$.

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

Yellow powder solids. (278 mg, 82%); mp: 208-209 □; ^1H NMR (500 MHz, $CDCl_3$):

δ=1.854-1.881 (m, 4H), 2.715-2.765 (m, 4H), 3.008 (t, *J*=11.5 Hz, 2H), 4.249 (t, *J*=11.5 Hz, 2H), 6.996-7.014 (m, 2H), 7.093-7.127 (m, 2H), 7.473 (d, *J*=15.5 Hz, 1H), 7.622-7.650 (m, 2H), 7.766 (d, *J*=16.0 Hz, 1H), 8.019-8.036 ppm (m, 2H); IR (KBr): *v*=2960, 2932, 2783, 1655, 1609, 1595, 1510, 1307, 1263, 1221, 1175, 1032, 978, 718 cm^{-1}; MS (ESI) m/z: 339 $[M+H]^+$.

Log P measurement

Octanol-water partition coefficients of compounds **3a-3l** and Rivastigmine were measured by the shake flask method with some modifications(11). The concentrations of compounds were assayed by HPLC. The chromatography method was as followed: the mobile phase: methanol: 0.1% triethanolamine(TEA)/85:15(v/v); flow rate: 1.0 $mL.min^{-1}$; chromatography column: C_{18} column (250mm×4.6mm, 5μm) ; column temperature: 32℃; detect wavelength: 318 nm (262 nm for Rivastigmine). The assay was conducted in triplicate and log P values were calculated.

Acetylcholinesterases (AChE) and butylcholinesterase (BuChE) Inhibition Assay

AChE（BuChE） activity assays were conducted utilizing acetylthiocholine iodide（ butyrylthiocholine iodide） as the substrate with modified *Ellman* method(12). The test compounds were dissolved in Tween 80 and diluted with water. Five different concentrations were tested for each compound in triplicate. The reaction mixture containing AChE or BuChE, acetylthiocholine iodide(butyrylthiocholine iodide), phosphoric acid buffer (pH 8.0), and the test compounds was incubated at 30℃ for 25 min. The reaction was terminated by adding 100 μL 20% sodium dodecyl sulfate (SDS) and followed by addition of 100 μL 10 mmol/L 5, 5'-Dithiobis-(2-nitrobenzoic acid)(DTNB) as developer to generate the yellow anion 5-thio-2-nitro-benzoic acid. Then the absorbance of assay solution was measured at 412 nm by spectrophotometer. The IC_{50} values were calculated by Bliss method and expressed as Mean±SD. Rivastigmine was utilized as a positive control.

Kinetic Assay

Kinetic characterization of AChE was conducted by a reported method(13). Compound **3l**(or **3e**) was added into the assay solution and incubated with the enzyme at 30℃ for 25 min, followed by the addition of 100 μL acetylthiocholine iodide including five concentrations. The assay solution contained 100 μL compound **3l**(or **3e**), 100 μL DTNB, 2.76 mL 0.1 M Na_2HPO_4/NaH_2PO_4 buffer (pH 8.0). The hydrolysis of acetylthiocholine iodide catalyzed by AChE was determined spectrometrically at 412 nm. Additionally, the parallel control experiment was made without compound **3l**(or **3e**) in the mixture.

Molecular Docking

Molecular modeling was performed by Molecular Operating Environment (MOE) software package. 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 **3l** and compound **3e** 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

The synthetic routes to get the target compounds are outlined in **Scheme 1**. Compounds **2a**-**2c** were obtained by Claisen-Schmidit condensation with sodium hydroxide as the catalyst. The result showed that 10% sodium hydroxide was the suitable catalyst to gain the highest-yield and purity products. Compounds **3a**-**3l** were synthesized using compounds **2a**-**2c** and commercially minoalkyl halogenates in acetone in the presence of K_2CO_3 and NaI with moderate yields (75%-88%). The advantages of this step were mild reaction condition, simple operation, less by-product and easy purification. The structures of final compounds (**3a**-**3l**) were confirmed by IR, ^{1}H NMR and MS spectral methods. The purity of all final compounds was higher than 98% by HPLC assay.

Log P measurement

As showed in **Table 1**, the log P values of **3a~3l** were 1.67, 1.78, 1.80, 1.75, 1.67, 1.78, 1.80, 1.75, 1.65, 1.66, 1.81 and 1.73, respectively. The log P value of Rivastigmine is 1.68. According to the report, the suitable log P for optimum central nervous system(CNS) penetration was around 2.0±0.7(14). The results above indicated that **3a**-**3l** were sufficiently lipophilic to pass blood brain barriers *in vivo*.

Biological evaluation

Inhibition studies of AChE and BuChE

The obtained IC_{50} values of compounds **3a-3l** against AChE and BuChE *in vitro* are summarized in **Table 1**. The results showed that the alteration of fluorine atom position and amino alkyl groups markedly influenced the activity and the selectivity of chalcone derivates. Among these compounds, AChE inhibitory activity of 3-fluoro substituted chalcones was lower than that of 2-fluoro or 4-fluoro substituted chalcones. For dimethylamine or diethylamine containing chalcones, 2-fluoro substituted compounds had the most potent AChE inhibitory activities. However, for piperidine or pyrrolidine containing chalcones, 4-fluoro substituted compounds had the most potent AChE inhibitory activities.

Interestingly, the selectivity of compounds mentioned above in inhibiting AChE and BuChE was out of our expectation. It seemed that the position of fluorine atom was important for the selectivity. For all compounds but not dimethylamine substituted compounds(compound **3a, 3b, 3c**), 4-fluorine containing chalcones had higher selectivity than 2-fluorine or 3-fluorine containing chalcones in inhibiting AChE.

Kinetic studies

Compound **3l** and **3e** were selected for kinetic study with Michaelis-Menten to evaluate the inhibition profile(15). The graphical analysis of the steady-state inhibition data were shown in **Supplementary File**. Compound **3l** and **3e** both revealed the mixed-type inhibition against AChE with different bind equilibrium constants for the **c**ompetitive and uncompetitive inhibition.

The competitive inhibit constant(K_i) and uncompetitive inhibit constant(K_i') of compound **3l** were 0.38 and 2.95 μmol/L, however, that of compound **3e** were 15.01

and 8.23 μmol/L, respectively. It seemed that the primary inhibition type of compound **3l** was the competitive inhibition, but that of compound **3e** was the uncompetitive inhibition.

Molecular Docking

Among the synthesized compounds, compound **3l** with the most potent activity and high selectivity (IC_{50} for AChE=0.21±0.03, the selectivity for AChE over BuChE=65.0) and compound **3e** with the most poor activity and moderate selectivity **(**IC_{50} for AChE= 9.62±0.82, the selectivity for AChE over BuChE=8.21**)** were selected to conduct the molecular dock assay. The binding points of compound **3l** with AChE were Trp84, Trp279, Phe330 and Tyr334 (**Figure 2, A**), while that of compound **3e** were Trp84, Trp279 and Tyr334 (**Figure 2, C**). For both of them, the conformation of the side chain conformed to the shape of the mid-gorge, and the pyrrolidine or diethylamine substituent bound to Trp84 in the bottom of the gorge. However, compound **3l** but not **3e** could bind with Phe330 which was an important amino acid for hydrophobic interaction. The binding point of compound **3l** and compound **3e** with BuChE was the same amino acid---Leu241, but the partial structure of compound **3e** was out of the bind package of BuChE(**Figure 2, B,D**). These results might provided partially explanation for that why compound **3l** had a more potent AChE inhibitory activity and a higher selectivity for AChE over BuChE compared with compound **3e**.

Conclusion

In conclusion, for amino alkyl substituted fluoro-chalcones derivatives **3a-3l** which were designed and synthesized in present study, the alteration of fluorine atom position and amino alkyl groups markedly influenced the activity and the selectivity of chalcone derivates in inhibiting AChE and BuChE. Among them, compound **3l** posses the most potent inhibitory against AChE and the highest selectivity for AChE over BuChE. The kinetic study suggested that compound **3l** had a mixed-type inhibition against AChE. The molecular modeling study further indicated that this compound can bind with both the catalytic active site and the peripheral cationic site of AChE. Overall, compound **3l** could be considered as a candidate for further pharmacological

investigation for the treatment of Alzheimer's disease.

Conflict of interest

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

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Figure 1. The chemistry structure of some fluorinated drugs

Scheme 1. Reagents and conditions: (I) 10%NaOH, EtOH, 25□, stirred for 36h;（II） K_2CO_3, acetone, NaI, 56℃, reflux, overnight.

Figure 2. Molecular modeling of compound **3l** with AChE (**A**) and BuChE (**B**) , compound **3e** with AChE (**C**) and BuChE (**D**) , generated with MOE.

Table 1. Cholinesterase inhibitory activity and log P values of fluoro-chalcones derivatives

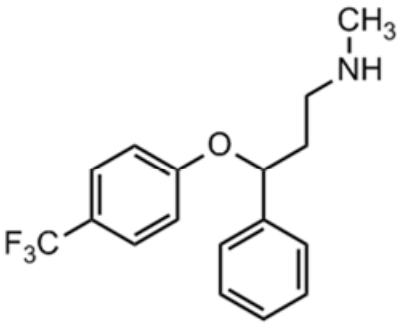
Compound	R^1	R^2	IC50[a] (μmol/L) ± SD		Selectivity for AChE[b]	LogP[c]
			AChE	BuChE		
3a	2-F		1.26±0.08	30.37±1.13	24.1	1.67
3b	3-F		3.60±0.26	73.46±1.84	20.4	1.67
3c	4-F		1.40±0.16	26.18±0.34	18.7	1.65
3d	2-F		2.67±0.20	22.48±1.17	8.42	1.78
3e	3-F		9.62±0.82	78.98±0.16	8.21	1.78
3f	4-F		4.85±0.10	65.48±0.72	13.5	1.66
3g	2-F		0.83±0.08	6.14±0.25	7.40	1.80
3h	3-F		2.20±0.47	29.21±4.76	13.3	1.80
3i	4-F		0.63±0.03	29.66±3.94	46.5	1.81
3j	2-F		0.23±0.03	6.81±0.36	29.6	1.75
3k	3-F		0.34±0.02	15.81±0.34	46.5	1.75
3l	4-F		0.21±0.03	13.65±0.18	65.0	1.73
Rivastigmine*	—	—	10.54±0.86	0.26±0.08	0.02	1.68

a. IC_{50} values of compounds represent the concentration that caused 50% enzyme activity loss.

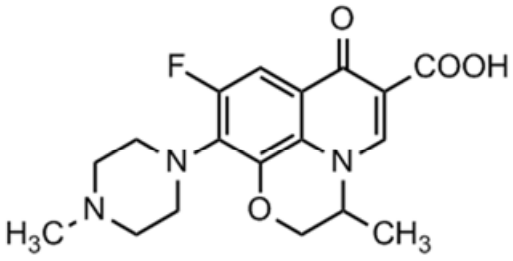
b. Selectivity for AChE is defined as IC_{50} (BuChE)/IC_{50} (AChE).

c. The partition coefficients of in the octanol/buffer solution at pH 7.4 were determined by the classical shake-flask method.

*Used for positive control



Fluoxetine



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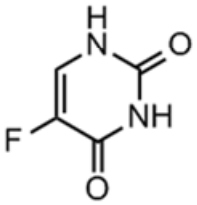
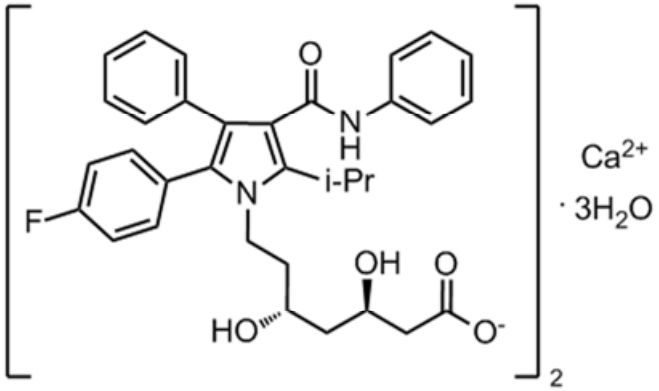
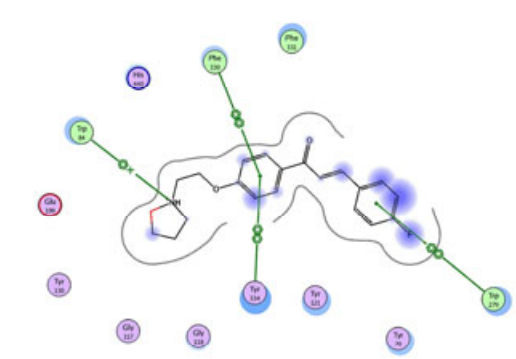


5-Fluoroucacil



Atorvastatin calcium



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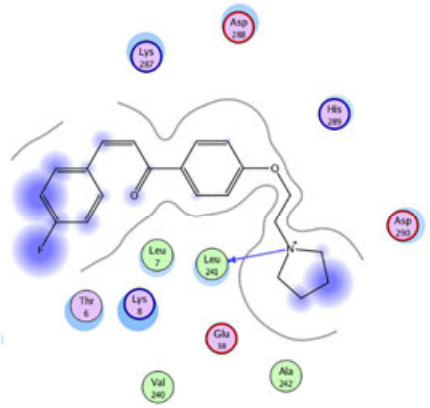

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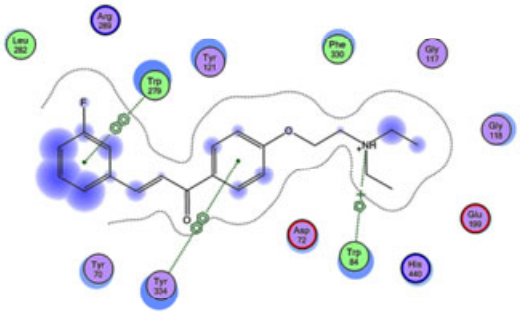

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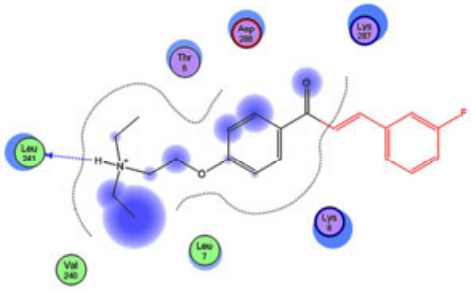

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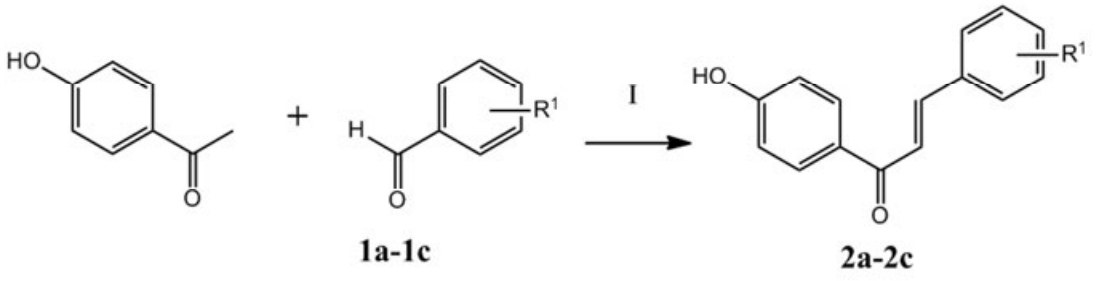
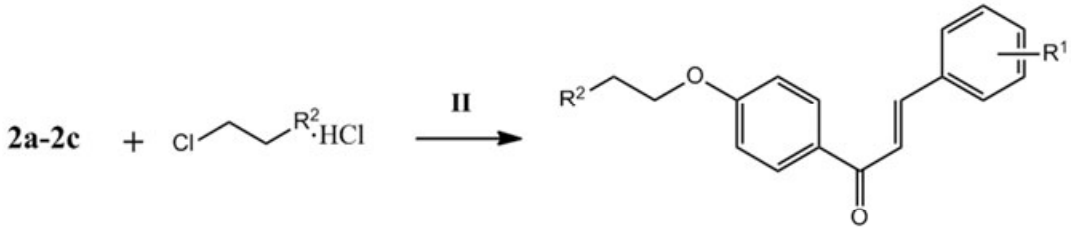


1a-1c

2a-2c



3a-3l