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Synthesis, Biological Activity Evaluation and Molecular Modeling Study on the New Isoconessimine Derivatives as Acetylcholinesterase Inhibitors

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New isoconessimine derivatives were synthesized from conessine (**1**) and evaluated as acetylcholinesterase (AChE) inhibitors. The derivatives were prepared via two reaction steps, *N*-demethylation and nucleophilic substitution. All of the synthesized derivatives exhibited more potential anti-acetylcholinesterase activities than conessine (**1**) (IC_{50}=16 μmol•L^{-1}) and isoconessimine (**2**) (IC_{50}>300 μmol•L^{-1}). Compound **7b** (3*β*-[methyl-[2-(4-nitrophenoxy)ethyl]amino]con-5-enine) showed the most potent inhibitory activity with an IC_{50} of 110 nmol/L which is close to that of reference compound huperzine A (IC_{50}=70 nmol/L). The mode of AChE inhibition by **7b** was reversible and non-competitive. In addition, molecular modeling was performed to explore the binding mode of inhibitor **7b** at the active site of AChE and the results showed that **7b** could be docked into the acetylcholinesterase active site and compound **7b** had hydrophobic interactions with Trp279 and Leu282.

Keywords isoconessimine derivatives, acetylcholinesterase inhibitors, molecular docking

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder which is characterized by memory loss and other cognitive impairments. There is not a clear theory on disease due to its complex etiology.[1] AD includes several diverse hallmarks such as *β*-amyloid (A*β*) deposits, *τ*-protein aggregation, oxidative stress, or low levels of acetylcholine (ACh).[2,3] The cholinergic hypothesis of AD suggests that low levels of ACh in specific regions of the brain result in learning and memory dysfunction. This hypothesis indicates that increase of ACh levels by inhibition of acetylcholinesterase is beneficial for the treatment of AD. Recently, evidences have been presented that AChE also played a key role in accelerating A*β* plaques deposition.[4] For this reason, acetylcholinesterase inhibitors (AChEIs) such as donezepil, rivastigmine, and galanthamine, have been considered currently mainly drugs for treatment of Alzheimer's disease.[5] Therefore, searching for new AChEIs is still drawn much attention.

Early findings indicated that the alkaloids isolated from *Holarrhena antidysenterica*, with conessine as a major alkaloid, appeared to be responsible for much of the antibacterial activity.[6] Recently, we found isoconessimine (**2**) and its derivatives conessine (**1**), conessimin (**3**), conarrhimin (**4**) and conimin (**5**) (Figure 1) showed moderate AChE inhibition activity with IC_{50} at μmol/L range.[7] To obtain more potent AChE inhibitors, a series of 3-N substitutional isoconessimine derivatives (**7a**—**7k**) were synthesized from conessine (**1**) and their anti-AChE activities were also tested.

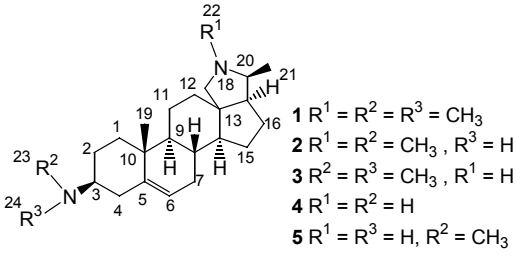

Figure 1 The structures of compounds **1**—**5**.

Up to now, there are many papers reported the design and synthesis of new AChEIs based on various chemical skeletons such as coumarin analogues,[8] berberine derivatives,[1] 2-phenoxy-indan-1-one deriva-

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tives,[9] oxoisoaporphine alkaloids,[10] rutaecarpine derivatives,[11] α-carboline derivatives,[12] *etc*. For these research work, the common strategies of increasing activity were introducing 2—4 atom side chains which enabled the inhibitors to snake along the gorge of AChE and introducing aromatic rings which would form a classic parallel π-π stacking with the electron-rich amino-acid residue of enzyme. For this, we introduced aryloxy-substitutional ethyl group which included both two atom side chain and aromatic ring to conessine skeleton and expected to obtain strong AChEIs.

Experimental

Materials and measurements

Acetylcholinesterase (EC 3.1.1.7, Sigma product NO. C2888, from *Electrophorus electricus*), huperzine A were purchased from Sigma (St. Louis, MO, USA). Phenols, ethylene dibromide, were purchased from Aladdin-Reagent Co, Ltd (Shanghai, China). Silica gel G plates and silica gel (200—300 mesh) were obtained from Qingdao Haiyang Chemical Co, Ltd (Qingdao, China). The seed of *Holarrhena antidysenterica* was purchased from BaYi Herb Market in Xining. It was identified by Associate Professor Lin Yang who majored in plant classification, School of Life Science and Engineering, Lanzhou University of Technology, Lanzhou, China. Other reagents were of analytical grade. Melting points were recorded on a Mel-Temp II melting-point apparatus and uncorrected. NMR spectra were recorded on a Brucker Advance-AM-400 MHz spectrometer with TMS as the internal standard. Mass spectra (ESI-MS, positive) were recorded on a Bruker Daltonics esquire 6000 spectrometer.

Extraction and isolation of conessine (1)

Conessine (**1**) was obtained according to our previous procedure.[7]

Synthesis of Isoconessimine (2)

A solution of dry conessine (**1**) 2 g (6 mmol) and 5 mL 30% hydrogen peroxide in MeOH (20 mL) was stirred at room temperature for 12 h. The reaction mixture was poured into water and extracted with chloroform (30 mL×3). The organic layer was washed with brine twice and concentrated in vacuum. The residue was purified by column chromatography (siliga gel, EtOAc/Methonal/Ammonia = 10/1/0.1) to give compound 3-*N*-oxide of the **1**. After treatment of 3-*N*-oxide with hydrated ferrous sulfate in methanol (20 mL) at room temperature for 12 h, saturated sodium bicarbonate (10 mL) was added into reaction mixture. After 0.5 h, the reaction mixture was poured into water, filtered and extracted with chloroform (30 mL×3). The organic layer was washed with brine twice and concentrated in vacuum. The crude product was chromatographed on a silica gel column, eluted with EtOAc/methonal/ammonia (20/1/0.2, *V*/*V*/*V*) to afford isoconessimine (**2**) (1.06 g, yield 55%). **2** was obtained as a white solid. m.p. 79—80 ℃; purity, 98% (HPLC); ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 0.94 (s, 3H), 1.04 (d, *J*=5.2 Hz, 3H), 2.21 (s, 3H), 2.33 (s, 3H), 2.98 (d, *J*=10.2 Hz, 1H), 5.36 (br s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) *δ*: 38.7, 28.9, 59.8, 37.9, 141.4, 120.7, 32.0, 33.4, 49.9, 37.1, 22.0, 39.5, 50.3, 55.8, 24.5, 27.6, 53.5, 64.6, 19.4, 63.1, 14.8, 41.2, 33.4; ESIMS *m/z*: 343.3 $[M+H]^+$. The NMR data is identical with those of literature.[13]

General procedure for the preparation of 6a—6k

The suspension of phenols (0.01 mol), sodium hydrate (0.012 mol), KI (0.05 g) and TBAB (0.05 g) in water (20 mL) was stirred. When the temperature reached 90 ℃, 0.015 mol ethylene dibromides were dropwise added into reaction mixtures and reflux for 12 h. After cooling, the reaction mixtures were poured into water and extracted with EtOAc. The organic layer was washed with brine twice and concentrated in vacuum. The residue was chromatographed on a silica gel column, eluted with EtOA/petroleum ether to afford **6a—6k** which were not very stable and must be used for next reaction immediately.

General procedure for the preparation of 7a—7k

A mixture of isoconessimine (**2**) (50 mg, 0.15 mmol), K_2CO_3 (62.5 mg, 0.75 mmol), KI (5 mg) and **6a—6k** in anhydrous acetonitrile (10 mL) was reflux for 16 h at atmosphere. After cooling, the reaction mixture was poured into water and extracted with chloroform. The organic layer was washed with brine twice and concentrated in vacuum. The residue was purified by column chromatography (siliga gel, EtOAc/methonal/ammonia) to give the desired product **7a—7k**.

3*β*-[Methyl-[2-(phenoxy)ethyl]amino]con-5-enine (7a) Yield 82%, white solid, m.p. 73—75 ℃; purity, 97.8 % (HPLC); ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 0.93 (s, 3H), 1.06 (d, *J*=6.4 Hz, 3H), 2.20 (s, N-CH_3, 3H), 2.40 (s, N-CH_3, 3H), 3.02 (d, *J*=10.4 Hz, 1H), 2.89 (t, *J*=6.4 Hz, 2H), 4.04 (t, *J*=6.4 Hz, 2H), 5.35 (br s, 1H), 6.89—6.99 (m, 3H), 7.29 (t, *J*=8.4 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) *δ*: 38.5, 27.4, 55.7, 34.6, 141.8, 120.7, 31.9, 33.4, 41.1, 37.0, 19.5, 38.5, 50.5, 53.4, 24.4, 24.7, 49.9, 66.5, 19.5, 64.2, 14.6, 38.9, 64.2, 66.5, 52.3, 114.5 (C×2), 129.3 (C×2), 141.8, 158.8; ESIMS *m/z*: 463.4 $[M+H]^+$.

3*β*-Methyl-[2-(4-nitrophenoxy)ethyl]-amino]con-5-enine (7b) Yield 91%, yellow solid, m.p. 141—143 ℃; purity, 98.5% (HPLC); ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 0.93 (s, 3H), 1.15 (d, *J*=6.4 Hz, 3H), 2.46 (s, N-CH_3, 6H), 3.18 (d, *J*=10.4 Hz, 1H), 2.93 (t, *J*=6.4 Hz, 2H), 4.14 (t, *J*=6.4 Hz, 2H), 5.35 (br s, 1H), 6.96 (d, *J*=8.0 Hz, 2H), 8.19 (d, *J*=8.0 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) *δ*: 38.4, 29.0, 55.6, 34.6, 141.4, 120.8, 31.9, 33.5, 41.2, 37.0, 22.0, 38.4, 50.8, 53.4, 24.8, 24.9, 49.8, 67.7, 19.6, 64.3, 14.5, 39.0, 64.3, 67.7 , 52.0, 114.4 (C×2), 125.9 (C×2), 141.6, 163.9; ESIMS *m/z*: 508.2 $[M+H]^+$.

3β-[Methyl-[2-(4-methylphenoxy)ethyl]-amino]-con-5-enine (7c) Yield 88%, white solid, m.p. 77—79 ℃; purity, 98.8% (HPLC); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.93 (s, 3H), 1.05 (d, J=6.0 Hz, 3H), 2.44 (s, N-CH_3, 3H), 2.28 (s, N-CH_3, 3H), 2.22 (s, CH_3, 3H), 3.00 (d, J=10.4 Hz, 1H), 2.87 (t, J=6.0 Hz, 2H), 4.01 (t, J=6.0 Hz, 2H), 5.35 (br s, 1H), 6.80 (d, J=8.0 Hz, 2H), 7.06 (d, J=8.0 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 38.7, 27.6, 55.8, 34.6, 141.9, 120.7, 32.0, 33.5, 41.2, 37.0, 22.0, 38.4, 50.4, 53.5, 24.5, 24.7, 50.0, 64.6, 19.5, 64.2, 14.8, 38.4, 63.2, 66.7, 52.3, 114.3 (C×2), 129.8 (C×2), 141.9, 156.7; ESIMS *m/z*: 477.3 $[M+H]^+$.

3β-[[2-(4-Methoxyphenoxy)ethyl]methylamino]-con-5-enine (7d) Yield 92%, white solid, m.p. 106—108 ℃; purity, 97.5% (HPLC); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.93 (s, 3H), 1.04 (d, J=6.0 Hz, 3H), 2.43 (s, N-CH_3, 3H), 2.21 (s, N-CH_3, 3H), 3.76 (s, OCH_3, 3H), 2.99 (d, J=10.4 Hz, 1H), 2.92 (t, J=6.0 Hz, 2H), 3.99 (t, J=6.0 Hz, 2H), 5.35 (br s, 1H), 6.81—6.85 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 38.6, 27.5, 55.7, 34.6, 141.9, 120.7, 32.0, 33.5, 41.1, 37.0, 22.0, 38.4, 50.5, 53.5, 24.5, 24.7, 50.0, 64.4, 19.5, 64.2, 14.7, 38.9, 63.3, 55.8, 67.3, 52.4, 114.5 (C×2), 120.7 (C×2), 153.7, 153.0; ESIMS *m/z*: 493.4 $[M+H]^+$.

3β-[[2-(2,4-Dichlorophenoxy)ethyl]methyamino]-con-5-enine (7e) Yield 79%, white solid, m.p. 73—75 ℃; purity, 97.8% (HPLC); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.94 (s, 3H), 1.04 (d, J=6.4 Hz, 3H), 2.43 (s, N-CH_3, 3H), 2.21 (s, N-CH_3, 3H), 2.99 (d, J=10.4 Hz, 1H), 2.93 (t, J=6.0 Hz, 2H), 4.06 (t, J=6.0 Hz, 2H), 5.35 (br s, 1H), 6.84 (d, J=8.0 Hz, 1H), 7.16 (d, J=8.0 Hz, 1H), 7.34 (d, J=2.0 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 38.7, 27.6, 55.8, 34.8, 141.8, 120.8, 32.0, 33.5, 41.1, 37.0, 22.0, 38.4, 50.4, 53.5, 24.5, 24.8, 50.0, 64.6, 19.5, 64.3, 14.8, 39.2, 63.2, 68.7, 52.1, 113.9, 123.6, 125.6, 127.5, 129.9, 153.3; ESIMS *m/z*: 531.2 $[M+H]^+$.

3β-[[2-(4-Chlorophenoxy)ethyl]methylamino]-con-5-enine (7f) Yield 86%, white solid, m.p. 91—93 ℃; purity, 98.0 % (HPLC); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.93 (s, 3H), 1.04 (d, J=6.0 Hz, 3H), 2.42 (s, N-CH_3, 3H), 2.22 (s, N-CH_3, 3H), 2.99 (d, J=10.4 Hz, 1H), 2.88 (t, J=6.0 Hz, 2H), 4.00 (t, J=6.0 Hz, 2H), 5.35 (br s, 1H), 6.82 (d, J=8.8 Hz, 2H), 7.21 (d, J=8.8 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 38.7, 27.6, 55.8, 34.6, 141.8, 120.8, 32.0, 33.5, 41.1, 37.0, 22.0, 38.4, 50.4, 53.5, 24.5, 24.7, 50.0, 64.5, 19.5, 64.2, 14.8, 38.9, 63.2, 67.1, 52.2, 115.7 (C×2), 129.2 (C×2), 125.4, 157.4; ESIMS *m/z*: 497.3 $[M+H]^+$.

3β-[[2-(2,4-Dinitrophenoxy)ethyl]methylamino]-con-5-enine (7g) Yield 90%, white solid. m.p. 145—147 ℃; purity, 97.8 % (HPLC); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.93 (s, 3H), 1.05 (d, J=6.0 Hz, 3H), 2.37 (s, N-CH_3, 3H), 2.25 (s, N-CH_3, 3H), 3.12 (d, J=10.4 Hz, 1H), 2.97 (t, J=6.0 Hz, 2H), 4.27 (t, J=6.0 Hz, 2H), 5.35 (br s, 1H), 7.23 (d, J=8.8 Hz, 1H), 8.42 (d, J=8.8 Hz, 1H), 8.76 (d, J=2.0 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 38.3, 27.3, 55.6, 34.7, 141.5, 120.9, 32.0, 33.5, 41.1, 37.0, 22.0, 38.3, 50.7, 53.4, 24.1, 24.8, 49.9, 64.4, 19.5, 63.8, 14.2, 39.0, 63.8, 70.3, 51.9, 114.5, 121.9, 129.0, 134.2, 140.0, 157.2; ESIMS *m/z*: 553.4 $[M+H]^+$.

3β-[Methyl-[2-(2-naphthoxy)ethyl]amino]con-5-enine (7h) Yield 85%, yellow oil; purity, 97.1% (HPLC); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.94 (s, 3H), 1.19 (d, J=6.4 Hz, 3H), 2.44 (s, N-CH_3, 3H), 2.28 (s, N-CH_3, 3H), 3.21 (d, J=10.4 Hz, 1H), 2.97 (t, J=6.0 Hz, 2H), 4.18 (t, J=6.0 Hz, 2H), 5.35 (br s, 1H), 7.14 (s, 1H), 7.15 (d, J=8.0 Hz, 1H), 7.32 (t, J=8.0 Hz, 1H), 7.43 (t, J=8.0 Hz, 1H), 7.70—7.76 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 38.4, 27.1, 55.5, 34.6, 141.7, 120.6, 31.9, 33.5, 41.1, 37.0, 22.0, 38.1, 50.9, 53.3, 23.9, 24.8, 49.8, 64.1, 19.5, 63.3, 13.9, 38.9, 63.3, 66.6, 52.3, 106.6, 119.0, 123.5, 126.3, 126.7, 127.6, 128.9, 129.3, 134.5, 156.8; ESIMS *m/z*: 513.2 $[M+H]^+$.

3β-[Methyl-[2-(1-naphthoxy)ethyl]amino]con-5-enine (7i) Yield 90%, white solid, m.p. 133—135 ℃; purity, 97.6% (HPLC); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.95 (s, 3H), 1.04 (d, J=6.0 Hz, 3H), 2.45 (s, N-CH_3, 3H), 2.22 (s, N-CH_3, 3H), 3.00 (d, J=10.4 Hz, 1H), 3.06 (t, J=6.0 Hz, 2H), 4.21 (t, J=6.0 Hz, 2H), 5.37 (br s, 1H), 6.80 (d, J=8.0 Hz, 1H), 7.34—7.49 (m, 4H), 7.78 (d, J=8.0 Hz, 1H), 8.26 (d, J=8.0 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 38.6, 27.5, 55.7, 34.8, 141.8, 120.8, 32.0, 33.4, 41.1, 37.0, 22.0, 38.4, 50.5, 53.5, 24.4, 24.9, 49.9, 64.4, 19.5, 64.1, 14.6, 39.1, 63.3, 67.2, 52.4, 104.5, 120.1, 122.1, 125.0, 125.6, 125.8, 126.3, 127.3, 134.4, 154.6; ESIMS *m/z*: 513.2 $[M+H]^+$.

3β-[[2-(4-Chloro-1-naphthoxy)ethyl]methyl-amino]con-5-enine (7j) Yield 85%, yellow oil, purity, 97.3% (HPLC); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.95 (s, 3H), 1.05 (d, J=6.0 Hz, 3H), 2.47 (s, N-CH_3, 3H), 2.23 (s, N-CH_3, 3H), 3.00 (d, J=10.4 Hz, 1H), 3.05 (t, J=6.0 Hz, 2H), 4.19 (t, J=6.0 Hz, 2H), 5.36 (br s, 1H), 6.71 (d, J=8.0 Hz, 1H), 7.43 (d, J=8.0 Hz, 1H), 7.51 (t, J=8.0 Hz, 1H), 7.60 (t, J=8.0 Hz, 1H), 8.18 (d, J=8.0 Hz, 1H), 8.29 (d, J=8.0 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 38.5, 27.4, 55.7, 34.8, 141.8, 120.8, 32.0, 33.5, 41.1, 37.0, 22.0, 38.4, 50.5, 53.5, 24.3, 24.9, 49.9, 64.2, 19.5, 64.2, 14.5, 39.0, 63.5, 67.5, 52.3, 104.6, 122.5, 123.1, 124.2, 125.7, 125.7, 126.6, 127.4, 131.3, 153.8; ESIMS *m/z*: 547.4 $[M+H]^+$.

3β-[[2-(2-Methoxyphenoxy)ethyl]methylamino]-con-5-enine (7k) Yield 82%, yellow oil. purity, 97.4% (HPLC); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.93 (s, 3H), 1.05 (d, J=6.0 Hz, 3H), 2.41 (s, N-CH_3, 3H), 2.22 (s, N-CH_3, 3H), 3.00 (d, J=10.4 Hz, 1H), 2.96 (t, J=6.0 Hz, 2H), 4.10 (t, J=6.0 Hz, 2H), 5.34 (br s, 1H), 6.87—6.92 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 38.6, 27.5, 55.8, 34.6, 141.8, 120.7, 32.0, 33.4, 41.1, 37.0, 22.0, 38.4, 50.4, 53.5, 24.4, 24.6, 49.9, 64.4, 19.5, 64.2, 14.7, 38.9, 63.3, 67.3, 52.1, 55.8, 111.7, 112.9, 120.8, 121.0, 148.3, 149.3; ESI-MS *m/z*: 493.4 $[M+H]^+$.

Bioassay procedures for AChE inhibition

The procedure of testing AChE inhibiting activity was same with those reported in our previous paper.[7]

Molecular modeling

The structure of **7b** was constructed using Maestro build panel (Maestro, version 9.0, Schrödinger, LLC, New York, NY, 2009). Then, the inhibitor was preprocessed by the LigPrep (LigPrep, version 2.3, Schrödinger, LLC, New York, NY, 2009) which used MMFFs force field[14] and gave the corresponding low energy 3D conformers of the ligand. The ionized state was assigned by using Epik (Epik, version 2.0, Schrödinger, LLC, New York, NY, 2009) at a target pH value of 7.0±2.0. The 3D crystal structure of the acetylcholinesterase for molecular docking was retrieved from the Protein Data Bank (PDB ID code 1VOT[15] and 2CKM[16]). The Protein Preparation Wizard was used to remove crystallographic water molecules, add hydrogen atoms, assign partial charges using the OPLS-2005 force field,[17] assign protonation states, and minimize the structure. The minimization was terminated when the root-mean-square deviation (RMSD) reached a maximum value of 0.30 Å. **7b** was docked into the active site of the acetylcholinesterase using the Glide (Glide, version 5.5, Schrödinger, LLC, New York, NY, 2009) with the standard precision (SP) scoring mode. The docking grid boxes for huperzine A and 7**b** were defined by centering on the inhibitors huperzine A and AA7 in the 1VOT and 2CKM, respectively. In molecular docking, 5000 poses were generated during the initial phase of the docking calculation, out of which best 500 poses were chosen for energy minimization by 500 steps of conjugate gradient minimizations. The best binding pose for **7b** was considered for the further analysis.

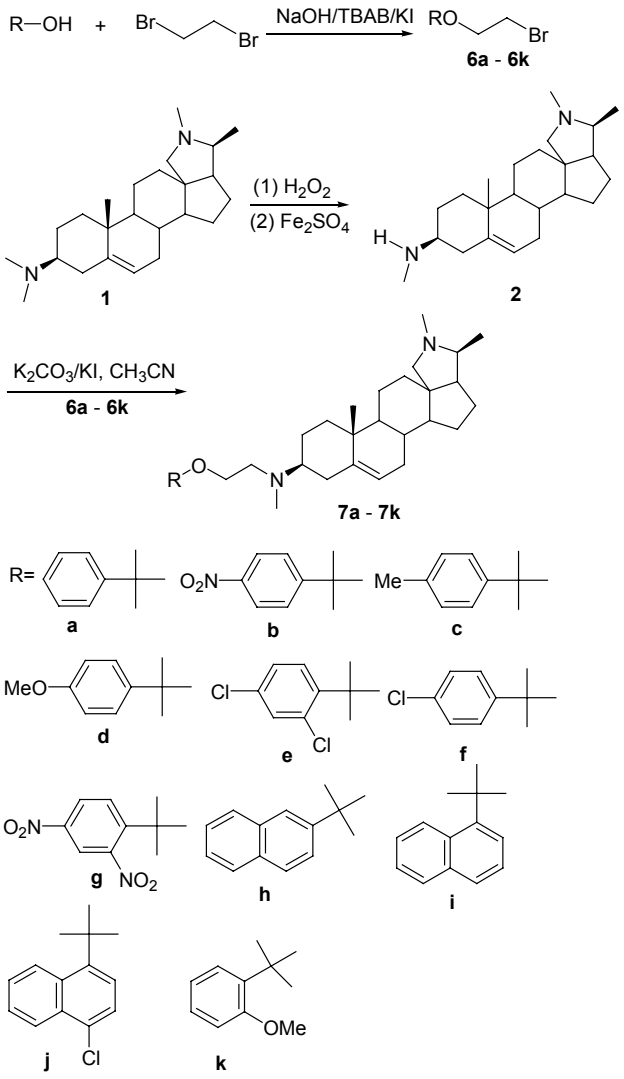
Results and Discussion

Conessine (**1**), the major alkaloid in seeds of the *Holarrhena antidysenterica*, can be readily obtained, and then was chosen as the starting material. Isoconessimine derivatives (**7a**—**7k**) were prepared according to the synthetic route as shown in Scheme 1.

Firstly, isoconessimine (**2**) was synthesized by utilizing the modified Polonovski reaction.[18,19] In brief, **1** was oxidized with 30% H_2O_2, and then reduced with Fe_2SO_4•$7H_2O$ at room temperature to yield **2**. Secondly, aryloxy-substitutional ethyl bromides (**6a**—**6k**) were prepared by reaction of phenols and ethylene dibromide in the presence of NaOH, KI and TBAB. Finally, nucleophilic substitution of **2** with **6a**—**6k** at basic condition easily afforded desired compounds **7a**—**7k** in 80%—90% yield.

The AChE inhibition activites of isoconessimine derivatives (**7a**—**7k**) were evaluated by colorimetric method in 96-well microplates described in our previous paper.[7] In fact, there are many methods used for detecting AChE inhibitory activity such as Colorimetric method, Nile Red-adsorbed Gold Nanoparticles method,[19] Mass Spectrometry assay,[21] *etc.* Among these methods, Colorimetric method is rapid, simple

Scheme 1 Synthesis of isoconessimine derivatives **7a**—**7k**



and convenient. So, in this paper we chose this method for detecting AChE inhibition. The results were summarized in Table 1. As shown in Table 1, all synthetized compounds (**7a**—**7k**) exhibited good inhibition against AChE with IC_{50} values ranging from micromolar to sub-micromolar and were more potent inhibitors than starting materials conessine (**1**) and isoconessimine (**2**) which indicated that the introduction of aryloxy-substitutional ethyl moiety was favorable for inhibitory activity. Compound **7b** with a *p*-nitro at aromatic ring, was the best AChE inhibitor with an IC_{50} value of 110 nmol/L. From the data, it seemed that: (1) Substitution at aromatic ring was beneficial to AChE inhibition which could be reflected in comparison of the activities of **7b**—**7k** (except for **7f** and **7J**) with **7a**; (2) The electron effect on aromatic ring was important for activity, but there was no regular pattern. It seemed that too high or too low electron-withdrawing effect was all disadvantageous. For example, compound **7b** with one nitro group at benzene ring showed the best AChE inhibition, while **7g** with two nitro group at benzene ring had approximately 13 times lower activity than **7b**. The cir-

cumstances for chlorine substitution at benzene ring were just contrary. Compound **7e** (with two chlorine atom) showed about 3 times more potent than **7f** (with only one chlorine atom). This phenomena could be explained by the much stronger electron-withdrawing ability of nitro group than that of chlorine. Electron-withdrawing effect of two nitro group might be too high, while that of one chlorine atom might be too low. However, in some case, the presence of electron-donor group also increased the activity, such as compound **7c**. The influence of electron effect on inhibitory activity maybe need to further explore. Determination of the inhibition type is important in understanding the mechanism of inhibition and the inhibitor binding sites. Compound **7b**, the most potent AChE inhibitor, was chosen for the kinetic study of AChE inhibition. The kinetic analysis of AChE inhibition by compound **7b** was shown in Figure 2. The K_m and V_{max} values were estimated from a Lineweaver-Burk plot. The V_{max} value of AChE against ACh was decreased from 0.278 to 0.175 (arbitrary unit) by the addition of compound **7b**.

Table 1 The acetylcholinesterase inhibitory activity of compunds **7a**—**7k** (expressed as IC_{50} values)

Compound	IC_{50}/(μmol•L^{-1})	Compound	IC_{50}/(μmol•L^{-1})
7a	2.10±0.20	**7g**	1.45±0.30
7b	0.11±0.03	**7h**	1.15±0.20
7c	0.70±0.08	**7i**	0.62±0.04
7d	1.40±0.20	**7j**	3.90±0.50
7e	0.88±0.05	**7k**	0.89±0.05
7f	2.30±0.40	**2**	>300
Huperzine A (positive control)	0.07±0.01		

However, the K_m values were both 0.20 mmol•L^{-1}, which were consistent with the values of 0.11—0.22 mmol•L^{-1} reported by Mahmood and Carmichael.[22] These results indicated that AChE inhibition of compound **7b** was reversible and non-competitive.

To explore the possible interaction mode between isoconessimine derivatives and the active site of AChE, molecular docking was applied. Compound **7b**, the most potent inhibitor, was chosen for this study. In this study, the performance of molecular docking was first evaluated by comparing the docked binding poses with the experimental structure for the inhibitor in the X-ray co-crystallized complex. Huperzine A in the X-ray cocrystallized complexes was re-docked into the binding sites and the conformation with the lowest GlideScore was adopted as the binding pose. The re-docked pose closely reproduced X-ray cocrystallized pose, and the RMSD for re-docked study of huperzine A was 0.09 Å. The calculated binding free energies (calculated from GlideScore) of huperzine A and **7b** in molecular docking study were −8.39 and −5.85 kcal•mol^{-1} which suggested that huperzine A is more efficacy against AChE. Figure 3 illustrated the structures of huperzine A and **7b** docked into the acetylcholinesterase. As shown in Figure 3, the binding pocket for huperzine A was formed by residues Trp84, Tyr116, Gly117, Gly118, Gly119, Tyr121, Ser122, Gly123, Leu127, Tyr130, Gly199, Ser200, Phe290, Phe330, Phe331, Ile439, His440, Gly441 and Tyr442. **7b** binding with the pocket was characterized by residues Tyr70, Asp72, Gln74, Ser81, Trp84, Gly118, Gly119, Tyr121, Ser122, Gly199, Ser200, Trp279, Phe290, Phe330, Trp334, Gly335 and His440. We found that **7b** can interact with the peripheral site of the enzyme, however, this might be primarily explained by the larger size of **7b**. Docking results indicate that huperzine A forms hydrogen bond with Tyr130, whereas **7b** interacts with Gly118. Beside, as can be seen from Figure 3, the lipophilic groups of huperzine A and **7b** interact with the non-polor amino acid side chains in the binding pocket through hydrophobic interactions.

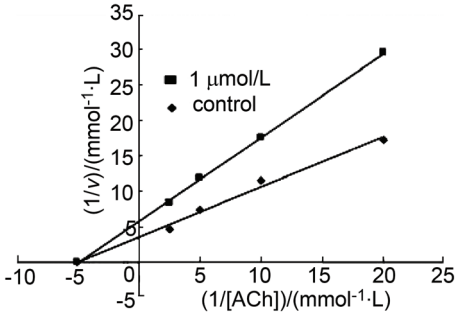


Figure 2 Lineweaver-Burk plot of 1/v vs. 1/[Ach]. In the absence of acetylcholinesterase (AChE) inhibitors, the K_m and V_{max} values were estimated as 0.20 mmol • L^{-1} and 0.278 (arbitrary unit) from the Lineweaver-Burk plot. In the presence of compound **7b**, the V_{max} value was decreased to 0.175 but the K_m value was not changed. These results indicated that AChE inhibitory activity of compound **7b** was reversible and non-competitive.

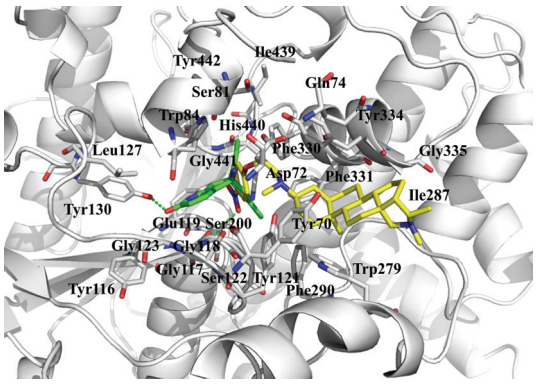


Figure 3 Stereo views illustrating the calculated binding modes of huperzine A and **7b** in the acetylcholinesterase. The acetylcholinesterase was represented as carton. Gray stick representations were shown for acetylcholinesterase residues in the protein binding pocket. Huperzine A and **7b** were shown as a green and yellow stick representation, respectively. Green dashed lines represented the protein-ligand hydrogen bonding.

Conclusions

11 new AChE inhibitors were obtained from conessine by *N*-demethylation and nucleophilic substitution reaction. The most potential inhibitor was **7b** with a 4-nitrophenoxyethyl at 3-N position of isoconessimine, which showed an IC_{50} value of 110 nmol/L, being close to those of huperzine A (IC_{50}=70 nmol/L). The mode of AChE inhibition by **7b** was proved to be reversible and non-competitive.

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