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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and structure–activity relationship of nuciferine derivatives as potential acetylcholinesterase inhibitors

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Abstract Acetylcholinesterase inhibitors (AChEIs) are currently the best available pharmacotherapy for Alzheimer patients, but because of bioavailability issues, there is still great interest in discovering better AChEIs. The aporphine alkaloid is an important class of natural products, which shows diverse biological activity, such as acetylcholinesterase inhibitory activity. To find new lead AChEIs compounds, eight aporphine alkaloids were synthesized by *O*-dealkylation, *N*-dealkylation, and ring aromatization reactions using nuciferine as raw material. The anti-acetylcholinesterase activity of synthesized compounds was measured using modified Ellman's method. The results showed that some synthesized compounds exhibited higher affinity to AChE than the parent compound nuciferine. Among these compounds, 1,2-dihydroxyaporphine (**2**) and dehydronuciferine (**5**) were the most active compounds ($IC_{50} = 28$ and 25 μg/mL, respectively). Preliminary analysis of structure–activity relationships suggested that aromatization of the C ring, the presence of the alkoxyl group at C1 and the hydroxy group at C2 position as well as the alkyl substituent at the N atom were favorable to the acetylcholinesterase inhibition. Molecular docking was also applied to predict the binding modes of compounds **1**, **2**, and **9** into the huperzine A binding site of AChE.

Keywords Aporphine alkaloids · Acetylcholinesterase inhibitors · Structure–activity relationships

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Introduction

Alzheimer's disease (AD), the most common form of dementia in the elderly, is a progressive neurodegenerative disorder characterized by cognitive impairments and memory loss. It has been reported that approximately 10 % of the population over the age of 65 years is affected by AD (Racchi *et al.*, 2004). To date, although the etiology of AD is not completely known, two diverse hypotheses, the amyloid hypothesis and the cholinergic hypothesis, play significant roles in the pathophysiology of the disease (Scarpini *et al.*, 2003). The former indicates that acetylcholinesterase (AChE) can accelerate formation of β-amyloid fibrils which can lead to AD (Hardy and Selkoe, 2002). The latter suggests that decreased levels of acetylcholine in the hippocampus and cortex is one of the most important causes of AD. Therefore, some acetylcholinesterase inhibitors (AChEIs) are effective agents for the treatment of AD symptoms (Lahiri *et al.*, 2002). Recent research demonstrated that AChEIs not only alleviate the cognitive defects of AD patients by elevating acetylcholine (ACh) levels, but also act as disease modifying agents by preventing the first step of AD, the assembly of β-amyloid peptides (Aβ) into amyloid plaques (Munoz and Camps,

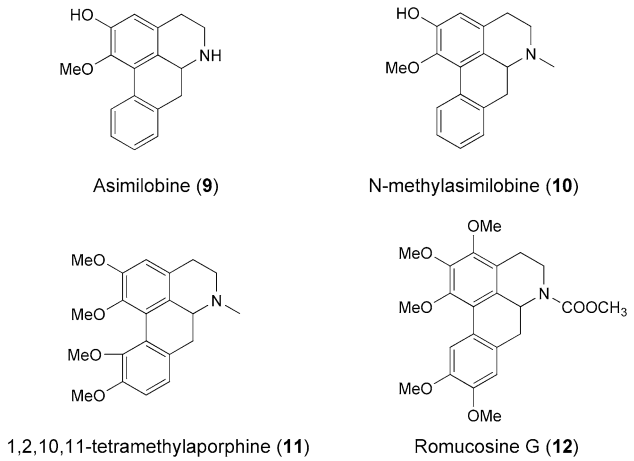


Fig. 1 The structures of compounds **9**–**12**

2006). This discovery stimulated great interest in the discovery of new AchEIs.

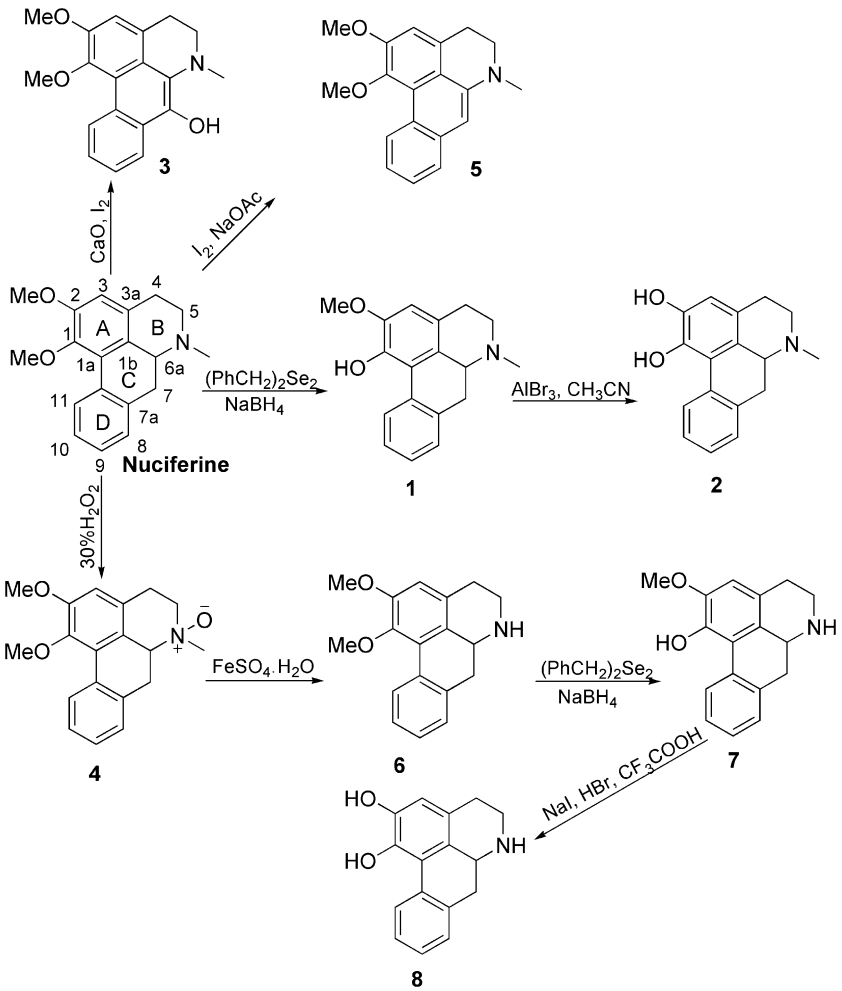
The aporphine alkaloid is an important and typical class of natural products. *N*-methylasimilobine (**10**) (Fig. 1), a nuciferine derivative, isolated from *Nelumbo nucifera*, as a potent AChEI, was reported in our previous paper (Yang *et al.*, 2012). To explore the structure–activity relationships of this type of compounds against AChE, we synthesized a series of nuciferine derivatives **1**–**8** (Scheme 1) by *O*-dealkylation, *N*-dealkylation, as well as aromatization and evaluated their activity against AChE.

Results and discussion

Chemistry

For preparing the target derivatives **1**–**8** from nuciferine, we followed the synthetic route outlined in Scheme 1. First, the regioselective *O*-demethylation of nuciferine at 140 °C with dibenzyl diselenide in the presence of sodium borohydride ($NaBH_4$) in dry *N*,*N*-dimethylformamide (DMF) gave 1-hydroxy-2-methoxyaporphine (**1**) in 76 % yield (Ahmad *et al.*, 1977). Next, *O*-demethylation of **1** with $AlBr_3$ in methyl cyanide at 60 °C gave

Scheme 1 Synthesis of derivatives **1**–**8**



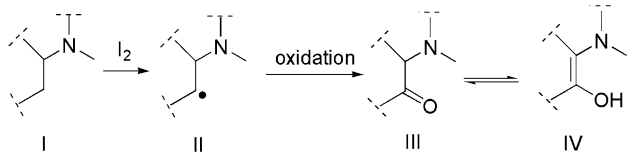


Fig. 2 The mechanism of forming 7-hydroxy-dehydronuciferine **3**

1,2-dihydroxyaporphine (**2**) in 63 % yield (Horie *et al.*, 1995). 7-Hydroxy-dehydronuciferine (**3**) was obtained in 61 % yield by reacting nuciferine with I_2 and CaO in the mixed tetrahydrofuran (THF) and methanol solution. It is worth mentioning that the obtainment of **3** was accidental. Originally, we planned to remove the methyl at the N-atom to obtain compound **6** using CaO and I_2 reported by Kirk (Acosta *et al.*, 1994). Unfortunately, **6** was not successfully obtained but resulted in a new product **3** identified by MS and NMR. The proposed mechanism (Fig. 2) was that the initial step was the formation of a benzyl radical (**II**) in the presence of I_2 and CaO, instead of a vinyl amine **5** through an iminium cation. Further oxidation would transform **II** into a ketone **III** which isomerizes to form the enol **IV.** Treatment of nuciferine with 30 % H_2O_2 in methanol at room temperature furnished nuciferine N-oxide (**4**) in 90 % yield. Reaction of nuciferine with I_2 and NaOAc in THF gave dehydronuciferine (**5**) in 50 % yield (Cava *et al.*, 1972). Reduction of **4** with hydrated ferrous sulfate ($FeSO_4{\cdot}7H_2O$) in methanol at room temperature afforded nornuciferine (**6**) in 40 % yield (Huang *et al.*, 2002) which was executed via *O*-demethylation using the same method of synthesizing **1** to yield 1-hydroxy-2-methoxynoraporphine (**7**) (73 % yield). However, synthesis of 1,2-dihydroxynoraporphine (**8**) was unsuccessful using many *O*-demethylation methods such as the use of dimethyl sulfide (Me_2S), methanesulfonic acid ($MeSO_3H$) (Couture *et al.*, 1996), HBr–HOAc (Zou *et al.*, 2008), $AlBr_3$ (Horie *et al.*, 1992), etc. as catalysts. Finally, compound **8** was obtained in 70 % yield using 40 % HBr as catalyst in the presence of sodium iodide in CF_3COOH solution at 140 °C high temperature.

Biological activities and structure–activity relationship

The AChE inhibitory activity of nuciferine derivatives was assayed according our previously described method (Yang *et al.*, 2012) using huperzine A as reference compound. Table 1 shows the activity of all compounds expressed either as inhibition ratio or IC_{50} values. As shown in Table 1, most of the synthesized compounds exhibited moderate activity against acetylcholinesterase. Compounds **2** and **5** had higher inhibition than others with IC_{50} values of 28 and 25 μg/mL, respectively. Although their activities were much less than Huperzine A and weaker than

Table 1 The inhibitory ratio and IC_{50} values of all compounds

Name	Inhibitory ratio % (final concentration, 0.103 mg/mL)	IC_{50} (μg/mL)
Nuciferine	25.0 ± 1.7	–
1	18.5 ± 1.2	–
2	72.9 ± 2.1	28 ± 0.4
3	61.7 ± 1.5	73 ± 0.4
4	37.3 ± 2.2	–
5	79.7 ± 2.0	25 ± 0.2
6	19.9 ± 1.6	–
7	2.0 ± 1.8	–
8	28.1 ± 2.3	–
9	59.0 ± 1.2	86 ± 0.3
10	94.0 ± 2.1	1.5 ± 0.2
Huperzine A	98.1 ± 0.5	0.017 ± 0.002

– The IC_{50} values were not measured for the corresponding compounds

N-methylasimilobine (**10**) reported in our group (Yang *et al.*, 2012), the activities obviously increased in comparison to the parent compound nuciferine. The preliminary structure–activity relationships could be drawn from the results as follows:

(1) The *O*-dealkylation of C1 decreased the AChE inhibition. For example, the inhibition ratio of *O*-demethylated product **1** (18.5 %) was lower than that of the parent compound nuciferine (25.0 %). This influence was also reflected between the compounds **2** and **10**, the IC_{50} values of them were 28 and 1.5 μg/mL, respectively. Moreover, this conclusion could also be summarized by comparing the inhibition ratio of **6** (19.9 %) and **7** (2.0 %). This indicated that the presence of the methoxy group at C1 is favorable to increasing the AChE inhibition.

(2) The hydroxyl group at the C2 position plays an important role for AChE inhibition. For example, compound **2**, sharing a hydroxyl at C2 position, was obtained from **1** by an *O*-dealkylation reaction. The activity of the former was approximately fourfold higher than the latter (the inhibition ratio was 72.9 and 18.5 %, respectively). This influence was also reflected between compounds **7** and **8**. This result was consistent with the previous molecular docking results reported by our group, which showed that the hydroxyl at C2 position of *N*-methylasimilobine (**10**) formed a H-bond with the carbonyl of Ser 293 in AchE (Yang *et al.*, 2012).

(3) The presence of the $N–CH_3$ is also important. Compound **8**, with an unsubstituted NH, failed to inhibit acetylcholinesterase, compared with compound **2**.

Compound **7**, a *N*-demethylated product of **1**, had relatively lower activity than **1**. This result was also consistent with our previous molecular docking results which demonstrated that the N-atom of compound **10** formed a H-bond with the hydroxyl of Tyr 124 in AChE. The fact that the inhibitory activity decreased after *N*-demethylation reaction could be explained by the reduction of electron-cloud density on the N-atom. The cleavage of the methyl group (a relatively stronger electron-donating group than the H-atom) could decrease the electron-cloud density on the N-atom causing reduction of H-bond forming ability and a decreased affinity between the enzyme and inhibitors.

(4) The aromatization of the C-ring significantly increases the inhibitory activity. For example, after the C-ring of nuciferine was aromatized to form compounds **3** and **5**, the inhibition ratio of the latter two were increased to 61.7 and 79.7 %, respectively. The aromatization of the C-ring could lead to an increase of π electron-cloud density and the aromaticity of the molecule, which reinforces the π–π interaction of inhibitor molecules with the aromatic amino acid residues of AChE.

The above analysis suggests that the aromatization of the C-ring and the alkoxyl of C1, the hydroxyl at the C2 position and the alkyl substituent on the N-atom were very important to maintain the AChE inhibition of nuciferine derivatives. In our future research, we will retain these pharmacophores to insure no loss of inhibitory activity.

The docking program Glide (Glide, version 5.5) was applied to predict the binding modes of the representative compounds (nuciferine, huperzine A, and compounds **1**, **2**, and **9**) into the huperzine A binding site of AChE. As shown in Figs. 3, 4, 5, 6, 7, and 8, huperzine A, nuciferine, compounds **1**, **2**, and **9** were docked into the active site of AChE around the residue Trp 84 (Trp 86 residue in the pdd code: 1C2B). In addition, the calculated binding free energy of compounds in the molecular docking study was found to correlate well with its inhibition activity, as shown in Table 2 and the RMSD for the re-dock study of huperzine A is 0.79 Å. These results could explain the observed differences in inhibitory activity. The aporphine alkaloids is an important class of natural products, which shows diverse biological activity, such as antiplatelet aggregation (Kuo *et al.*, 2001), anti-poliovirus (Boustie *et al.*, 1998), etc. However, except for a strong AChEI **10** reported by our group (Yang *et al.*, 2012) and two weak AChEIs **11**, **12** (Fig. 1) reported by Mankee et al. (2006), aporphine alkaloids with anti-AChE activity have been rarely obtained by isolation or synthesis. Moreover, this is the first report of the structure–activity relationships of this type of alkaloid against AChE. This paper describes the design and synthesis of a series of aporphine alkaloids, and measurement of their anti-AChE activity. The structure–activity relationship of these compounds was preliminarily analyzed and determined. Variations of activity between the different compounds tested suggest promising future structural modifications for activity improvement.

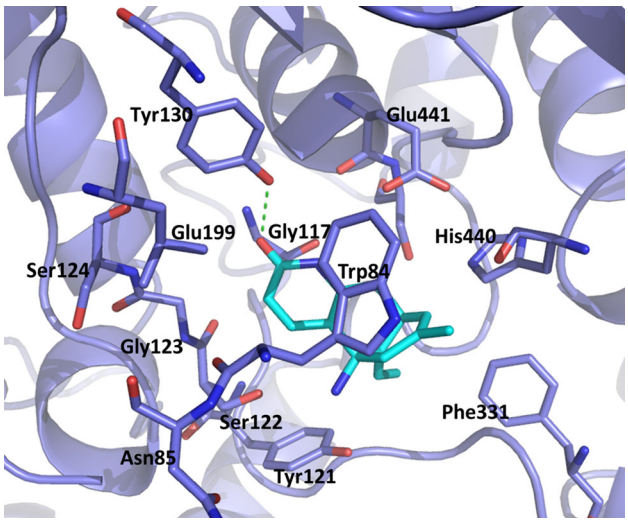


Fig. 3 The predicted binding model of compound **3** to AChE

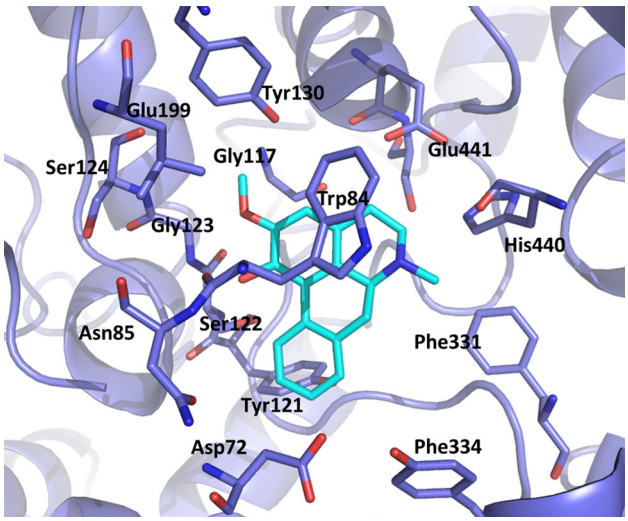


Fig. 4 The predicted binding model of compound **1** to AChE

Experimental

Materials and measurements

Acetylcholinesterase (EC 3.1.1.7, Sigma product NO. C2888), huperzine A were purchased from Sigma (St. Louis,

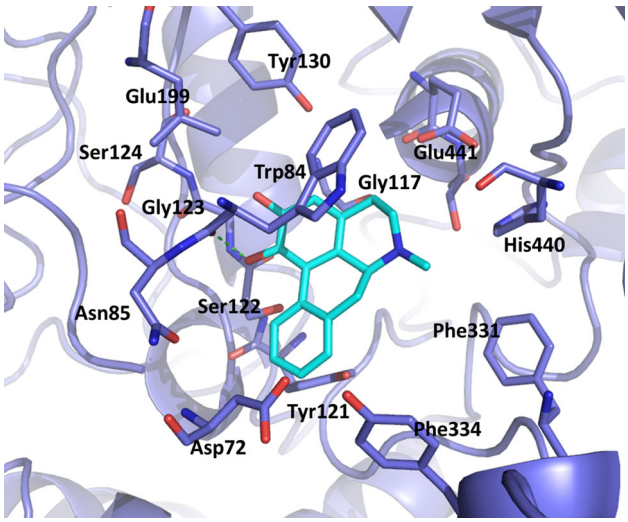


Fig. 5 The predicted binding model of compound **2** to AChE

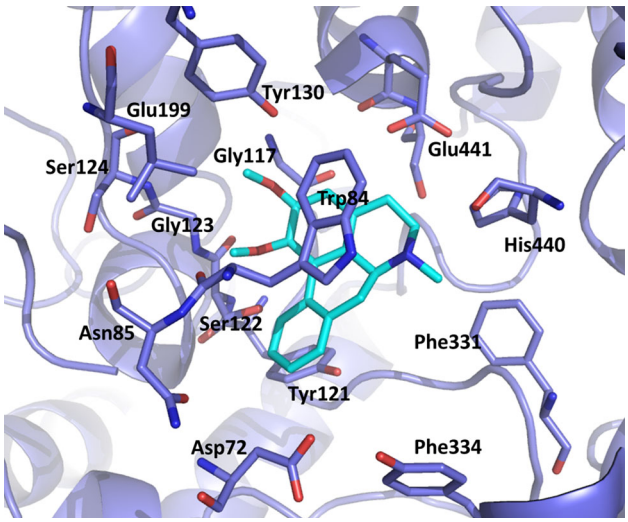


Fig. 7 The predicted binding model of nuciferine to AChE

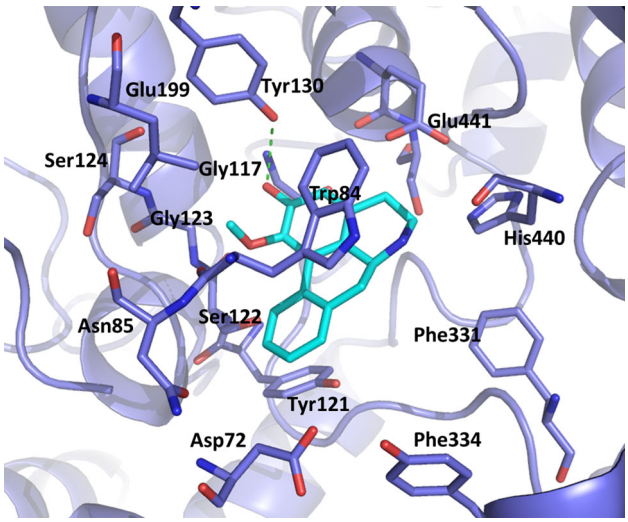


Fig. 6 The predicted binding model of compound **9** to AChE

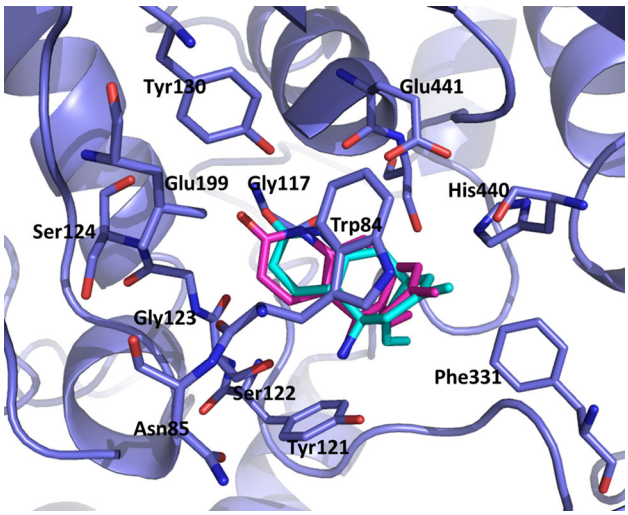


Fig. 8 Superposition of the crystal structure (*pink*) and the conformation (*cyan*) from docking result for huperzine A at the active site of AChE (Color figure online)

MO, USA). Sodium borohydride, *N*,*N*-dimethylformamide, dimethyl sulfide, methanesulfonic acid, aluminum bromide, and sodium iodide 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 leaves of *N. nucifera* were purchased from Huanghe Herb Market in Lanzhou. The herb 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. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Brucker Advance-AM-400 MHz spectrometer with TMS as the internal standard. Proton Chemical shifts are expressed in parts per million (ppm) and coupling constants in Hz. Mass spectra (ESI–MS, positive) was recorded on a Bruker Daltonics esquire 6000 spectrometer.

Table 2 The docking score of the studied compounds by Glide program

Inhibitors	Binding free energy (Kcal/mol)
Nuciferine	−7.58
1	−7.54
2	−8.39
9	−8.07
Huperzine A	−8.62

Extraction and isolation of (6aR)-1,2-dimethoxy-6-methyl-5,6,6a,7-tetrahydro-4H-dibenzo[de, g]quinoline (nuciferine)

The leaves of *N. nucifera* (10 kg) were crushed into a powder and extracted with 95 % ethanol (30 L × 2, 80 °C, 2 h). The supernatant was separated from the solid residue by paper filtration and evaporated to dryness under reduced pressure. The residue was suspended in water (1 L). The suspension solution was adjusted to pH = 2 by the addition of HCl (6 M), which would be filtered after one night. The filtrate solution was basified to pH = 11 with NaOH (1 M) and then extracted with chloroform (l L × 4) and evaporated to dryness to obtain total alkaloid (11.94 g). The total alkaloid extract was repeatedly isolated and purified using MCI–GEL and recrystallization to obtained nuciferine (5.153 g). M.p. 165–166 °C. ^{1}H NMR (400 MHz, $CDCl_3$), δ: 2.55 (s, 3H, N–CH_3), 2.50 (m, 1H), 2.59–2.71 (m, 2H), 3.02–3.20 (m, 4H), 3.65 (*s*, 3H, O–CH_3), 3.88 (s, 3H, O–CH_3), 6.63 (s, 1H), 7.23-7.33 (m, 3H), 8.36 (d, J = 8.0 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ:144.4 (C-1), 125.9 (C-1a), 129.0 (C-1b), 151.7 (C-2), 112.0 (C-3), 127.9 (C-3a), 28.4 (C-4), 52.3 (C-5), 71.0 (C-6a), 34.6 (C-7),136.0 (C-7a), 128.1 (C-8), 127.3 (C-9), 126.9 (C-10), 126.7 (C-11), 131.4 (C-11a), 43.9 (N–CH_3), 60.0 (C-1, OMe), 55.7 (C-2, OMe) (Guinaudeau *et al.*, 1979).

Synthesis

*(6aR)-2-methoxy-6-methyl-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinolin-1-ol (**1**, 1-hydroxy-2-methoxyaporphine)*

Compound **1** was prepared by the following method: To a stirred solution of dibenzyl diselenide (0.22 g, 0.7 mmol) in 10 mL of anhydrous *N*,*N*-dimethylformamide (DMF) was added excess $NaBH_4$ (0.3 g, 7.9 mmol) under N_2. After 20 min, nuciferine (0.28 g, 0.95 mmol) in 15 mL of DMF was introduced. The mixture was refluxed under nitrogen until all the alkaloid was consumed (monitored by TLC). The mixture was cooled and solvent was evaporated under reduced pressure. The residue was taken up in 5 % H_2SO_4 and the nonalkaloidal components were extracted with benzene. The acid solution was basified with 10 % NH_4OH (pH = 9) and exhaustively extracted with chloroform. The combined chloroform extracts were dried (anhydrous Na_2SO_4) and evaporated. The product was further purified by column chromatography (chloroform–methanol–triethylamine, 40:1:0.001, v/v/v) to give **1** (0.204 g, 76 %) as colorless needle crystals. M.p. 161–163 °C. ^{1}H NMR (400 MHz, $CDCl_3$), δ: 2.58 (s, 3H), 2.48–2.68 (m, 3H), 2.88–3.11 (m, 4H), 3.82 (s, 3H), 6.54 (s, 1H), 7.18–7.31 (m, 3H), 8.35 (d, J = 8.0 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 147.8 (C-1), 120.5 (C-1a), 129.9 (C-1b), 143.4 (C-2), 111.2 (C-3), 127.4 (C-3a), 28.8 (C-4), 54.6 (C-5), 64.3 (C-6a), 36.4 (C-7), 137.5 (C-7a), 129.2 (C-8), 128.9 (C-9), 127.5 (C-10), 125.0 (C-11), 133.7 (C-11a), 56.6 (C-2, OMe), 44.7 (N–CH_3). ESI MS: 282.3 $[M+H]^+$ (Barolo *et al.*, 2006).

*(6aR)-6-methyl-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinolin-1,2-diol (**2**, 1,2-dihydroxyaporphine)*

Compound **2** was prepared by the following method: To a solution of anhydrous $AlBr_3$ (1.0171 g, 3.8 mmol) in 10 mL dry MeCN was added compound **1** (0.182 g, 0.6 mmol) under N_2. The mixture was heated 70 °C for 48 h. The solvent was evaporated under reduced pressure. The residue was dissolved by acetone and collected the filtrate by filter. Purification by recrystallization from acrtone/chloroform gave the desired **2** (0.104 g, 65 %) as white amorphous powder. M.p. 162–164 °C. ^{1}H NMR (400 MHz, CD_3OD), δ: 2.83–2.96 (m, 3H), 3.18 (s, 3H, N–CH_3), 3.40–3.47 (m, 2H), 3.76 (dd, J = 12.4, 5.6 Hz 1H), 4.20 (br d, J = 13.6 Hz, 1H), 6.64 (s, 1H), 7.20–7.35 (m, 3H), 7.91 (s, 1H), 8.44 (d, J = 8.0 Hz 1H). ^{13}C NMR (125 MHz, CD_3OD), δ: 143.7 (C-1), 121.1 (C-1a), 122.8 (C-1b), 147.0 (C-2), 114.3 (C-3), 122.4 (C-3a), 27.1 (C-4), 54.1 (C-5), 63.4 (C-6a), 34.0 (C-7), 134.4 (C-7a), 129.8 (C-8), 128.9 (C-9), 128.1 (C-10), 128.1 (C-11),133.7 (C-11a), 41.9 (N–CH_3). ESI MS: 268.2 $[M+H]^+$ (Kelly *et al.*, 1976).

*1,2-dimethoxy-7-hydroxy-6-methyl-5,6-dihydro-4H-dibenzo[de,g]quinoline (**3**)*

Compound **3** was prepared by the following method: A mixture of nuciferine (0.095 g, 0.3 mmol) and calcium oxide (0.146 g, 2.6 mmol) in THF (2 mL) and methanol (2 mL) was chilled in an ice-bath. Iodine (0.164 g, 0.6 mmol) in THF (1 mL) was added. The mixture was stirred at 0 °C for 2.5 h. The mixture was filtered and the filtrate was evaporated under reduced pressure. Purification by flash chromatography on a silica–gel column (chloroform–methanol–triethylamine, 30:1:0.001, v/v/v) afforded **3** (0.057 g, 61 %) as colorless needle crystals. M.p. 258–260 °C. ^{1}H NMR (400 MHz, $CDCl_3$), δ: 2.26 (s, 3H), 2.90 (m, 1H), 3.13 (m, 1H), 3.40 (m, 2H), 3.99 (s, 3H), 4.07 (s, 3H), 7.15 (s, 1H), 7.23 (t, J = 8.0 Hz, 1H), 7.34 (d, J = 8 Hz, 1H), 7.42 (t, J = 8 Hz, 1H), 9.70 (d, J = 8.0 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 145.7 (C-1), 126.2 (C-1a), 121.5 (C-1b), 151.0 (C-2), 112.3 (C-3), 131.1 (C-3a), 27.0 (C-4), 50.2 (C-5), 143.4 (C-6a), 126.1 (C-7), 126.9 (C-7a), 127.9 (C-8), 123.7 (C-9), 126.2 (C-10), 126.2 (C-11),134.8 (C-11a), 41.6 (N–CH_3), 60.0

(C-1, OMe), 58.5 (C-2, OMe). ESI MS: 310.2 $[M+H]^+$ (Wafo *et al.*, 1999).

*(6S,6aR)-1,2-dimethoxy-6-methyl-6-oxo-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinoline (**4**, nuciferine N-oxide)*

Compound **4** was prepared by the following method: To a solution of nuciferine (0.295 g, 1 mmol) in 15 mL MeOH was added 7.5 mL 30 % H_2O_2 at room temperature. After the starting material had been consumed (ca 24 h), as monitored by TLC, the reaction mixture was extracted with $CHCl_3$ (200 mL × 3). The $CHCl_3$ layer after being washed with water (50 mL × 3) and dried over Na_2SO_4 was condensed under reduced pressure to give the residue, which was purified on a silica–gel column (chloroform–methanol–triethylamine, 30:1:0.001, v/v/v) to give **4** (0.279 g, 90 %) as green oil. ^{1}H NMR (400 MHz, $CDCl_3$), δ: 2.82–2.86 (m, 1H), 3.15–3.18 (m, 1H), 3.37–3.44 (m, 1H), 3.59–3.89 (m, 2H), 4.31–4.41 (m, 2H), 3.64 (s, 3H), 3.74 (s, 3H), 3.84 (s, 3H), 6.69 (s, 1H), 7.26–7.33 (m, 3H), 8.35 (d, $J = 8.0$ Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 146.1 (C-1), 120.2 (C-1a), 125.6 (C-1b), 153.4 (C-2), 110.9 (C-3), 127.9 (C-3a), 24.5 (C-4), 63.6 (C-5), 71.9 (C-6a), 56.3 (N–CH_3), 30.0 (C-7), 132.8 (C-7a), 127.9 (C-8), 128.2 (C-9), 128.4 (C-10), 128.3 (C-11), 128.2 (C-11a), 60.3 (C-1, OCH_3), 55.9 (C-2, OCH_3). ESI MS: 312.2 $[M+H]^+$ (Lu *et al.*, 1987).

*1,2-dimethoxy-6-methyl-5,6-dihydro-4H-dibenzo[de,g]quinoline (**5**, dehydronuciferine)*

Compound **5** was prepared by the following method: A solution of iodine (0.25 g, 1 mmol) in 20 mL THF was added dropwise during 30 min to a refluxing solution of nuciferine (0.3 g, 1 mmol) in 10 mL THF containing suspended anhydrous NaOAc (0.3 g, 4 mmol). The stirred mixture was refluxed gently for an addition 2 h and then evaporated to dryness. Dissolution of the residue with $CHCl_3$ (100 mL × 3) and evaporation of the extract gave an oil which crystallized from absolute EtOH to give **5** (0.146, 50 %) as colorless needle crystals. M.p. 130–131 °C. ^{1}H NMR (400 MHz, $CDCl_3$), δ: 3.09 (s, 3H), 3.28 (t, $J = 6.0$ Hz, 2H,), 3.38 (t, $J = 6.0$ Hz, 2H), 3.90 (s, 3H), 4.02 (s, 3H), 6.63 (s,1H), 7.02 (s, 1H), 7.34 (t, $J = 8.0$ Hz, 1H), 7.44 (t, $J = 8.0$ Hz, 1H), 7.65 (d, $J = 8.0$ Hz, 1H), 9.46 (d, $J = 8.0$ Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ:145.3 (C-1), 118.4 (C-1a), 125.3 (C-1b), 151.4 (C-2), 111.2 (C-3), 127.1 (C-3a), 30.5 (C-4), 59.3 (C-5), 123.5 (C-6a), 40.4 (N–CH_3), 126.3 (C-7), 134.2 (C-7a), 126.1 (C-8), 124.5 (C-9), 124.4 (C-10), 126.1 (C-11), 128.5 (C-11a), 56.1 (C-1, OCH_3), 50.0 (C-2, OCH_3). ESI MS: 294.1 $[M+H]^+$ (Wang *et al.*, 2009).

*(6aR)-1,2-dimethoxy-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinoline (**6**, nornuciferine)*

Compound **6** was prepared by the following method: To a stirred solution of **4** (0.31 g, 1 mmol) in 2 mL methanol was added $FeSO_4{\cdot}7H_2O$ (0.304 g, 2 mmol) at 0 °C. The reaction mixture was allowed to warm in the room temperature and stir for 12 h. A solution of saturated aqueous $NaHCO_3$ (10 mL) was added to the reaction mixture and the suspension was stirred at room temperature for 20 min and then extracted with chloroform (100 mL × 3). The combined chloroform layer was washed with water (50 mL × 3), brine and dried over Na_2SO_4. After removal of the organic solvent under reduced pressure, the residue was purified with flash chromatograph (eluting with chloroform–methanol–triethylamine, 10:1:0.001, v/v/v) to gave **6** (0.112 g, 40 %) as colorless needle crystals. M.p. 128–129 °C. ^{1}H NMR (400 MHz, $CDCl_3$), δ: 2.69–2.78 (m, 3H), 2.83–3.02 (m, 2H), 3.37–3.39 (m, 1H), 3.81–3.88 (m, 1H), 3.67 (s, 3H), 3.89 (s, 3H), 6.65 (s, 1H), 7.20–7.32 (m, 3H), 8.39 (d, $J = 8.0$ Hz, 1H). ^{13}C NMR (400 MHz, $CDCl_3$), δ: 145.5 (C-1), 126.7 (C-1a), 129.3 (C-1b), 152.6 (C-2), 111.7 (C-3), 129.3 (C-3a),29.6 (C-4), 42.6 (C-5), 53.3 (C-6a), 36.4 (C-7), 135.2 (C-7a), 128.4 (C-8), 127.9 (C-9), 127.6 (C-10), 127.2 (C-11), 132.0 (C-11a), 55.9 60.2 (C-1, OMe), (C-2, OMe). ESI MS: 282.2 $[M+H]^+$ (Guinaudeau *et al.*, 1983).

*(6aR)-2-methoxy-5,6,6a,7-tetrahydro-4H-Dibenzo[de,g]quinolin-1-ol (**7**, 1-hydroxy-2-methoxynoraporphine)*

Compound **7** was prepared from 0.4 mmol **6** and 0.35 mmol dibenzyl diselenide by the same way of synthesis of **1**. Compound **7** as colorless needle crystals, 0.068 g was obtained, yield 73 %. M.p. 208–210 °C. ^{1}H NMR (400 MHz, CD_3OD), δ: 2.61–2.68 (m, 2H), 2.79–2.95 (m, 3H), 3.25–3.30 (m, 1H), 3.69–3.74 (m, 1H), 3.86 (s, 3H), 6.66 (s, 1H), 7.12–7.24 (m, 3H), 8.41 (d, $J = 8.0$ Hz, 1H). ^{13}C NMR (400 MHz, CD_3OD), δ: 141.8 (C-1), 119.9 (C-1a), 123.3 (C-1b), 146.5 (C-2), 110.7 (C-3), 127.6 (C-3a), 28.4 (C-4), 42.8 (C-5), 53.1 (C-6a), 36.6 (C-7), 135.8 (C-7a), 128.4 (C-8), 128.4 (C-9), 126.2 (C-10), 126.0 (C-11), 132.4 (C-11a), 55.9 (C-2, OMe). ESI MS: 268.1 $[M+H]^+$(Han *et al.*, 1989; Guinaudeau *et al.*, 1979).

*(6aR)-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinolin-1,2-diol (**8**, 1,2-dihydroxynoraporphine)*

Compound **8** was prepared by the following method: The NaI (0.392 g, 2 mmol) was added to 8 mL 40 % HBr. After 30 min, then, a solution of **7** (0.134 g, 0.5 mmol) in

5 mL CF_3COOH was added to the reaction mixture. The reaction mixture was heated to 140 °C and refluxed for 5 h. The reaction mixture was cooled down to 100 °C and evaporated under reduced pressure. The flash chromatography (chloroform–methanol–triethylamine, 10:1:0.001, v/v/v) was performed to give **8** (0.088 g, 70 %) as colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$), δ: 2.72–2.76 (m, 2H), 2.88–2.91 (m, 1H), 3.05–3.08 (m, 2H), 3.48–3.52 (m, 1H), 4.05–4.08 (m, 1H), 6.50 (s, 1H), 7.08–7.14 (m, 3H), 8.33 (d, $J = 8.0$ Hz, 1H). ^{13}C NMR (400 MHz, $CDCl_3$), δ: 141.5 (C-1), 122.9 (C-1a), 126.5 (C-1b), 146.6 (C-2), 112.7 (C-3), 127.6 (C-3a), 30.4 (C-4), 42.6 (C-5), 55.1 (C-6a), 39.2 (C-7), 136.8 (C-7a), 128.2 (C-8), 127.6 (C-9), 126.5 (C-10), 127.8 (C-11), 132.3 (C-11a). ESI MS: $[M+H]^+$ (Wang *et al.*, 2009).

*(6aR)-1-methoxy-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinolin-2-ol (**9**, asimilobine)*

Compound **9** was purchased from Southwest Jiaotong University and identified by NMR spectroscopy. M.p. 177–179 °C, ^{1}H NMR (400 MHz, $CDCl_3$), δ: 3.58 (s, 3H), 6.66 (s, 1H), 7.22–7.30 (m, 3H), 8.29 (d, $J = 7.5$ Hz, 1H). ^{13}C NMR (400 MHz, $CDCl_3$), δ: 143.0 (C-1), 135.0 (C-1a), 125.5 (C-1b), 148.6 (C-2), 114.7 (C-3), 128.8 (C-3a), 27.2 (C-4), 42.5 (C-5), 53.5 (C-6a), 36.0 (C-7), 134.8 (C-7a), 128.4 (C-8), 127.9 (C-9), 126.3 (C-10), 127.1 (C-11), 131.3 (C-11a). (Rollinger *et al.*, 2006).

Bioassay procedures for AChE inhibition

The procedures of testing AChE inhibiting activity were same with those described in our previous paper (Yang *et al.*, 2012).

Molecular modeling

In order to further study the structure activity relationship, we docked five compounds (compounds **1**, **2**, **9**, nuciferine, and huperzine A) into the active site of AChE. We selected these five compounds due to their difference in inhibition activity. The structure of huperzine A was extracted from the co-crystal structure of AChE/huperzine A (PDB code: 1GPK). The structures of nuciferine and compound **1**, **2**, and **9** were constructed using Maestro (Maestro, version 9.0, Schrödinger, LLC, New York, NY, 2009) build panel. Then, all ligands were prepared using Ligprep (LigPrep, version 2.3, Schrödinger, LLC, New York, NY, 2009) which used MMFFs force field and gave the corresponding low energy 3D conformers of the ligands. In addition, the protonation states of ligands were assigned using the Ionizer method at a target pH value of 7.0 ± 2.0. The three-dimensional-structure of AChE/huperzine A complex was obtained from the Protein Data Bank (PDB code: 1GPK). Before docking the ligands into the protein active site, the protein structures were prepared including adding hydrogen atoms, assigning partial charges using the OPLS-2005 force field and assigning protonation states. The grid box was defined by centering on the huperzine A in the AChE active site. The docking of huperzine A, nuciferine and compound **1**, **2**, and **9** into the prepared grid were carried out using Glide program (Glide, version 5.5, Schrödinger, LLC, New York, NY, 2009) with the default parameter, for which standard precision (SP) mode was selected. The best scoring poses from the largest cluster were considered for further structural and interaction analysis.

Conclusion

Nuciferine was modified by *O*-dealkylation, *N*-dealkylation, and ring aromatization reactions and eight derivatives were obtained. The anti-acetylcholinesterase ability of these compounds was detected at the final concentration of 0.1 mg/mL. The results showed that 1,2-dihydroxy aporphine (**2**) and dehydronuciferine (**5**) had moderate anticholinesterase activity with IC_{50} values of 28 and 25 μg/mL. Analysis of the structure–activity relationship suggested that the alkoxyl of the C1, hydroxyl at the C2 position and the alkyl substituent on the N-atom are the basic pharmacophore for maintaining the AChE inhibition.

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