European Journal of Medicinal Chemistry 46 (2011) 1572–1581

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Syntheses and characterization of novel oxoisoaporphine derivatives as dual inhibitors for cholinesterases and amyloid beta aggregation

Yan-Ping Li [1], Fang-Xian Ning [1], Meng-Bi Yang, Yong-Cheng Li, Min-Hua Nie, Tian-Miao Ou, Jia-Heng Tan, Shi-Liang Huang*, Ding Li, Lian-Quan Gu, Zhi-Shu Huang**

School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou 510006, People's Republic of China

ARTICLE INFO

Article history:
Received 18 November 2010
Received in revised form
27 January 2011
Accepted 3 February 2011
Available online 1 March 2011

Keywords:
Oxoisoaporphine derivatives
Inhibitors
Acetylcholinesterase
Butyrylcholinesterase
Amyloid beta

ABSTRACT

A series of 3-substituted (**5c**–**5f**, **6c**–**6f**) and 4-substituted (**10a**–**10g**) oxoisoaporphine derivatives were synthesized. It was found that all these synthetic compounds had IC_{50} values at micro or nano molar range for cholinesterase inhibition, and most of them could inhibit amyloid β (Aβ) self-induced aggregation with inhibition ratio from 31.8% to 57.6%. The structure–activity relationship studies revealed that the derivatives with higher selectivity on AChE also showed better inhibition on Aβ self-induced aggregation. The results from cell toxicity study indicated that most quaternary methiodide salts had higher IC_{50} values than the corresponding non-quaternary compounds. This study provided potentially important information for further development of oxoisoaporphine derivatives as lead compounds for the treatment of Alzheimer's disease.

1. Introduction

Alzheimer's disease (AD), a progressive and fetal neurodegenerative disorder, is affecting more and more elder people all around the world [1]. Data have revealed that until 2009 the patients of AD were 17 million and the number would reach 70 million by 2050 [2]. Current drugs available for AD therapy are AChE (Acetylcholinesterase) inhibitors (tacrine, doepenzil, rivastigmine and galantanmine) [3] and an NMDA (*N*-methyl D-aspartate) antagonist memantine [4]. However, these drugs could only improve symptoms and exert limited effects on most patients. Therefore, more effective drugs are urgently required for AD therapy.

The pathology of AD includes Tau protein hyperphosphorylation, aggregated amyloid beta (Aβ) protein deposits, cholinergic system dysfunction, neuro loss and so on [5]. Among the above pathologic factors, acetylcholine (ACh) and Aβ play important roles in this process. The cholinergic hypothesis implies that the cognitive and memory deterioration of AD is due to the low level of choline, especially ACh in the brain [6]. AChE catalyzes rapid hydrolysis of ACh in central and peripheral nervous systems. The enzyme has a deep and narrow active site gorge, which contains a catalytic active site (CAS) in the bottom and a peripheral cationic site (PAS) in the entry [7]. Generally inhibitors of AChE may bind to either one or both of the two sites and inhibit the activity of the enzyme, resulting in increasing the level of ACh and improved learning and memorizing ability. On the other hand, the amyloid cascade hypothesis implies that overproduction and aggregation of Aβ initiates the process of AD by small oligomers and extracellular fibril tangles [8]. Aβ is a small peptide of almost 4 KDa, and is a proteolytic derivative by β and γ secretases in amyloid precursor protein (APP). Compounds which could reduce the production of Aβ and inhibit its aggregation have proven to attenuate AD symptoms in AD mice models [9]. More importantly and interestingly, it has been widely accepted that compounds interfering with both AChE and Aβ would be more promising for drug development in AD, and many series of compounds have been synthesized and reported [10–13].

Oxoisoaporphine alkaloids are isolated from the rhizomes of *Menispermum dauricum* DC, and the use of rhizomes of the plant have a long history in traditional Chinese medicine. We have previously reported two series of synthetic derivatives, oxoisoaporphine and oxoaporphine alkaloids, as cholinesterase inhibitors. The 9-substituted oxoisoaporphine derivatives (Fig. 1) have exhibited good AChE inhibitory activity with IC_{50} values in the nano molar range and high selectivity for AChE over BuChE (45- to 1980-fold) [14]. The 4-substituted oxoaporphine derivatives have also shown AChE inhibitory activity, but their activity is lower than the activity

* Corresponding author. Tel.: +8620 39949052.
** Corresponding author. Tel.: +8620 39949056; fax: +8620 39943056.
E-mail addresses: lsshsl@mail.sysu.edu.cn (S.-L. Huang), ceshzs@mail.sysu.edu.cn (Z.-S. Huang).
[1] These two authors contributed equally to this paper.

0223-5234/$ – see front matter © 2011 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2011.02.005

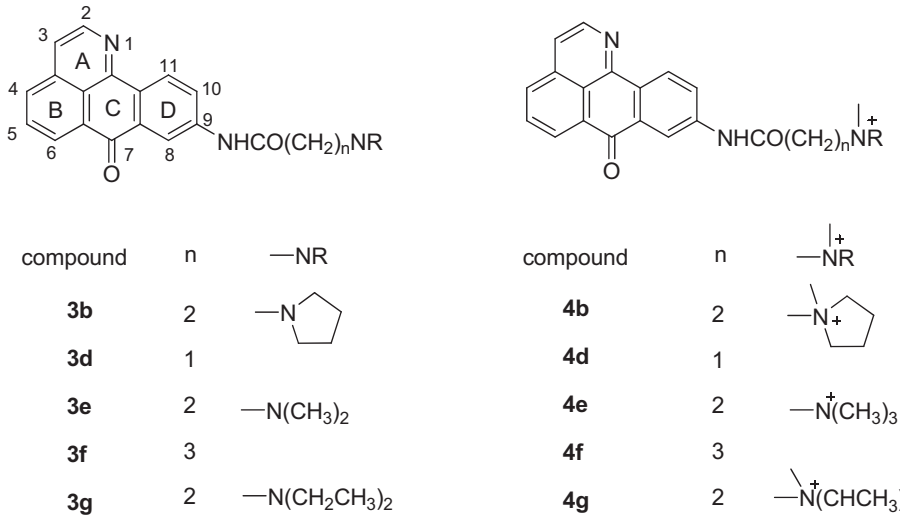


Fig. 1. Structures of 9-substituted oxoisoaporphine derivatives.

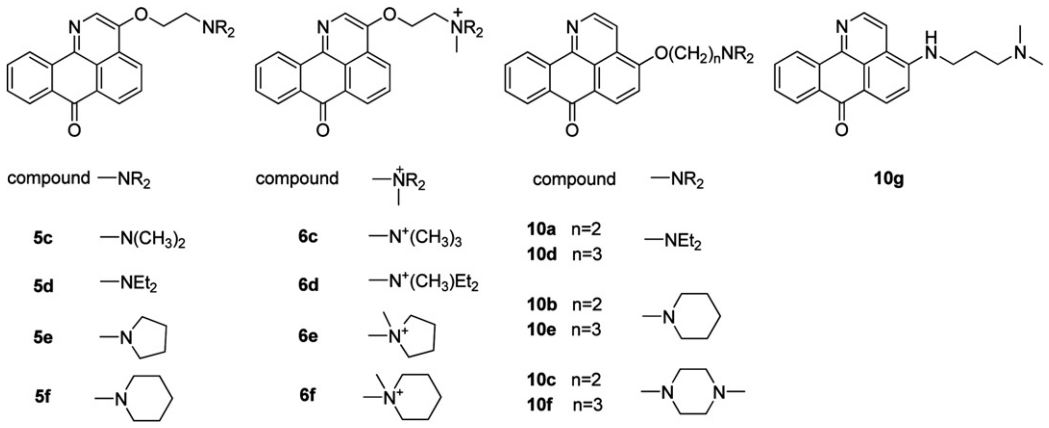
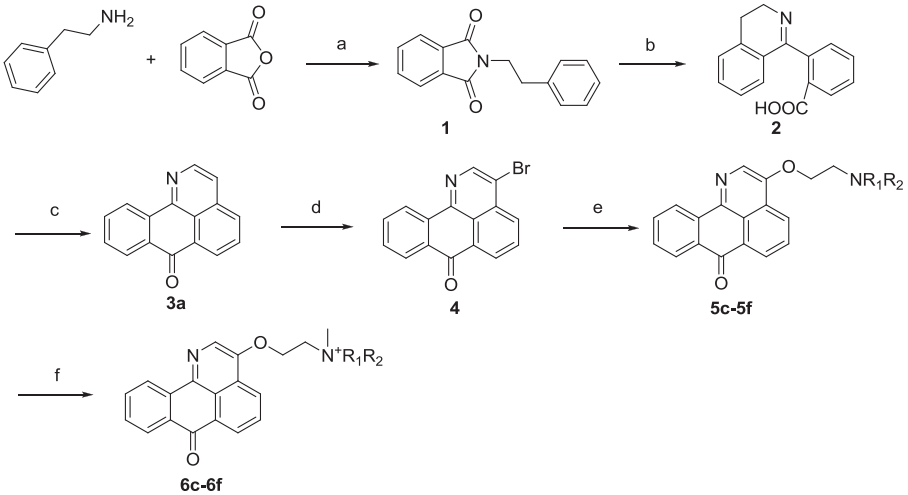
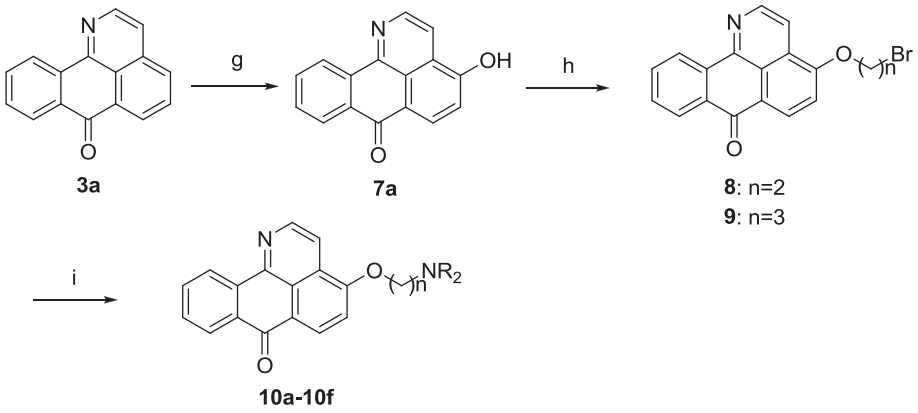


Fig. 2. Structures of 3-substituted (**5c**–**5f**, **6c**–**6f**) and 4-substituted (**10a**–**10g**) oxoisoaporphine derivatives.

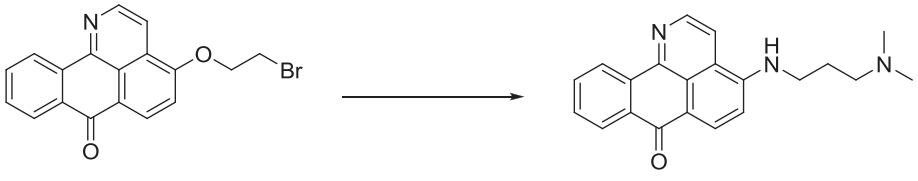
of the 9-substituted oxoisoaporphine derivatives for about 2–3 orders of magnitude [15]. Since the oxoisoaporphine derivatives were more effective cholinesterase inhibitors than oxoaporphine derivatives, in the present study, two new series of 3-substituted (**5c**–**5f**, **6c**–**6f**) and 4-substituted (**10a**–**10g**) oxoisoaporphine derivatives (Fig. 2) were synthesized to further study their structure–activity relationship. Their inhibition on AChE and BuChE, as well as Aβ (1–42) self-induced aggregation was studied using the *Ellman* methods [16] and Thioflavin-T fluorescence assay [17]. Molecular docking study with MOE software (Molecular Operating



Scheme 1. Synthesis of 3-substituted oxoisoaporphine derivatives (**5c**–**5f**, **6c**–**6f**). Reagents and conditions: (a) EtOH, reflux; 78%; (b) anhydrous $AlCl_3$, 220 °C; (c) concentrated H_2SO_4, 230 °C, two steps 32%; (d) Br_2, $PhNO_2$, I_2, 90%; (e) 18-crown-6, KOH, $R_1R_2NCH_2CH_2OH$, DMF, 60 °C, 17–36%; (f) $CH_3I/CHCl_3$, rt, 24 h, 93%.



Scheme 2. Synthesis of 4-substituted oxoisoaporphine derivatives (**10a**–**10f**). Reagents and conditions: (g) $NH_2OH{\cdot}HCl$ or $NH_2NH_2H_2O$, NaOH, DEG, 140 °C, 76–83%; (h) K_2CO_3, DMF, $Br(CH_2)_nBr$, 41–47%; (i) NHR_2, K_2CO_3, CH_3CN, KI, reflux, 22–85%.



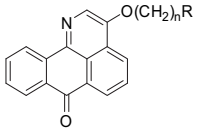
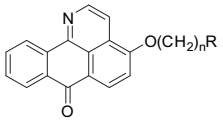
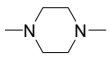
Scheme 3. Synthesis of compound **10g**. Reagents and conditions: $NH_2(CH_2)_3N(CH_3)_2$, ethanol, reflux, 80%.

Environment, Chemical Computing Group, Montreal, Canada) was also carried out to understand the binding mode and selectivity of these derivatives.

2. Chemistry

Two efficient methods were used for the synthesis of 3-substituted and 4-substituted oxoisoaporphine derivatives based on those previously reported [18,19]. The synthetic pathway for the preparation of 3-substituted oxoisoaporphine derivatives was shown in Scheme 1 [18]. The key step was the conversion of compound **4** to compounds **5c**–**5f** through reaction with the corresponding N-substituted alcohol ($R_1R_2NCH_2CH_2OH$) by using reagent KOH/DMF/18-crown-6. The use of 18-crown-6 made the reaction much easier to happen at relatively mild condition. This method was simple and efficient for the synthesis of aryl ether. The reaction yields of **5c**–**5f** were 26%, 27%, 36% and 17%,

Table 1
Inhibitory activity of 3-substituted and 4-substituted oxoisoaporphine derivatives to AChE and BuChE.

	Compound	R	*n*	IC_{50} (nM)		Selectivity for AChE[c]
				AChE[a]	BuChE[b]	
	5c	$-N(CH_3)_2$	2	1560 ± 40	1640 ± 48	1.0
	5d	$-N(CH_2CH_3)_2$	2	700 ± 50	1486 ± 136	2.1
	**5e**		2	471 ± 40	790 ± 11	1.6
	5f		2	375 ± 60	723 ± 12	1.9
	6c	$-\overset{+}{N}(CH_3)_3$	2	933 ± 5	972 ± 68	1.0
(**5c-6f**)	**6d**	$-\overset{+}{N}(CH_3)(CH_2CH_3)_2$	2	258 ± 8	708 ± 63	2.7
	6e		2	145 ± 6	302 ± 11	2.1
	6f		2	95 ± 8	255 ± 15	2.7
	**10a**	$-N(CH_2CH_3)_2$	2	201 ± 11	3068 ± 16	15.3
	10d		3	34.3 ± 1.7	2696 ± 303	78.6
	10b		2	88 ± 1.4	2208 ± 104	25.1
	10e		3	22 ± 1	905 ± 16	41.1
	10c		2	432 ± 13	2748 ± 247	6.4
(**10a-10f**)	**10f**		3	405 ± 26	5498 ± 40	13.6
	10g			398 ± 36	3350 ± 5	8.4
	Tacrine			105 ± 10	11 ± 5	0.1

[a] Inhibitor concentration (mean ± SEM of three experiments) required for 50% inactivation of AChE.
[b] Inhibitor concentration (mean ± SEM of three experiments) required for 50% inactivation of BuChE.
[c] Selectivity for AChE = IC_{50} (BuChE)/IC_{50} (AChE).

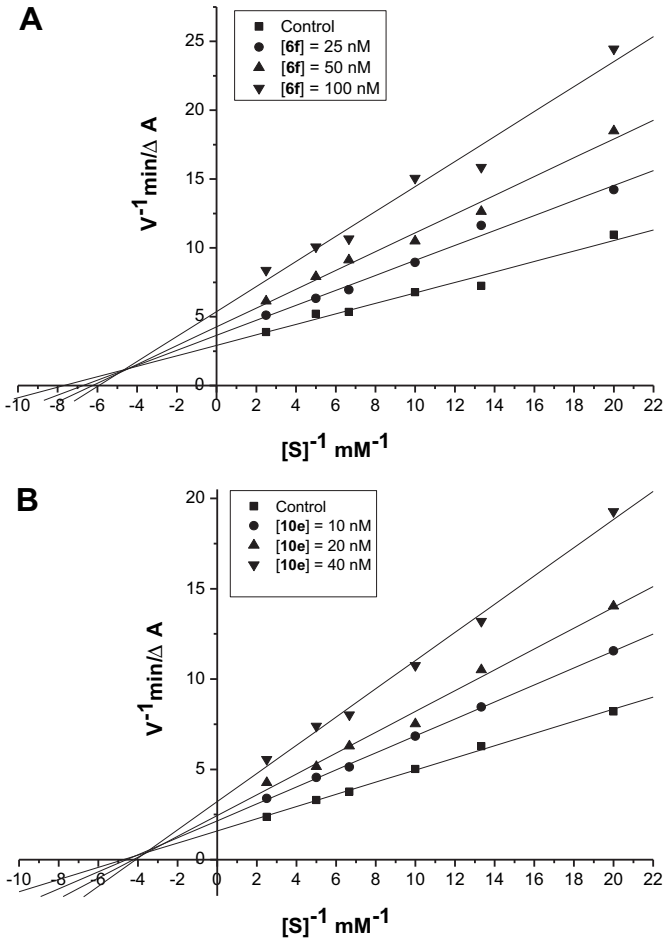


Fig. 3. Steady-state analysis for the inhibition of AChE hydrolysis of acetylthiocholine (substrate) by **6f** (A) and **10e** (B). The Lineweaver–Burk reciprocal plots of initial velocity and substrates concentrations were presented. Lines were derived from a weighted least-square analysis of the data points.

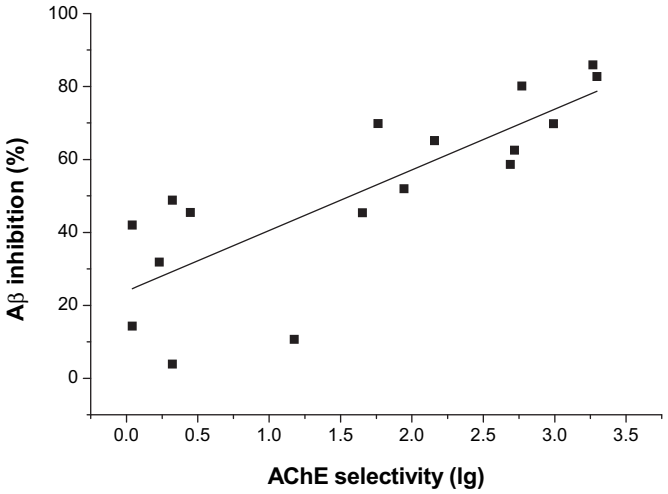


Fig. 4. Linear relationship between AChE selectivity (lg) and Aβ inhibition for oxoisoaporphine derivatives.

respectively. When using the alcohol with increasing carbon chain the reaction yields reduced and the products could not be separated and obtained by the column. Finally, compounds **5c**–**5f** reacted with iodomethane to give compounds **6c**–**6f** with quantitative yield.

The synthesis of 4-substituted oxoisoaporphine derivatives was shown in Scheme 2. The introduction of a hydroxyl group into aromatic ring of 1-azabenzanthrone to get key intermediate **7a** was carried out through the reaction of 1-azabenzanthrone with hydrazine hydrate or hydroxylamine hydrochloride in the presence of sodium hydroxide in diethylene glycol (DEG) at 140 °C [19]. This method permitted the introduction of a phenolic hydroxyl group in a one-step reaction, avoiding the disadvantages of traditional methods for the synthesis of phenolic derivatives (alkali fusion of arylsulfonates, hydrolysis of benzene halides, etc) which involve harsh reaction conditions and multistep procedures [20]. The desired products, compounds **10a**–**10f** were obtained from intermediate **7a** through initial reaction with dibromo-alkanes followed with corresponding secondary amines. When compound **8** reacted with 2-dimethylaminopropylamine, compound **10g** was obtained as shown in Scheme 3. The chemical structures of all above synthesized compounds were characterized using ^{1}H and ^{13}C NMR, and the key intermediate **7a** was further characterized with H–H cosy, HMBC, HMQC, and HRMS. The purity of all synthetic products was determined with HPLC to be over 95%.

3. Results and discussion

3.1. Cholinesterases inhibitory activity

The *in vitro* inhibition study of above synthetic compounds to AChE and BuChE were carried out following the method of *Ellman* et al [16], using tacrine as a reference. AChE from *electric eel* and BuChE from *equine serum* were used in this study because of their high sequence homology to the human enzymes. As shown in Table 1, all these compounds had good inhibitory activity to AChE with IC_{50} values in micro to nano molar range, and some of them also showed good inhibitory activity to BuChE.

It was found that the 3-substituted oxoisoaporphine derivatives with a cyclic amine group (pyrrolidinyl and piperidinyl) in their side chains were stronger inhibitors than those with alkyl amine groups. For instance, compounds **5e** and **5f** had lower IC_{50} values

Table 2
Inhibition of self-induced Aβ (1–42) aggregation by oxoisoaporphine derivatives with curcumin as a reference.

Compound	Inhibition ratio (%)[a]	IC_{50} (μM)[b]	Compound	Inhibition Ratio (%)[a]	IC_{50} (μM)[b]	Compound	Inhibition Ratio (%)[a]	IC_{50} (μM)[b]
5c	14.29	16.23	**10a**	10.68	>25	**3b**	69.80	4.57
5d	3.88	9.21	**10b**	14.00	>25	**3d**	45.37	5.37
5e	31.83	11.99	**10c**	38.92	>25	**3e**	69.85	4.14
5f	34.20	>25	**10d**	40.09	>25	**3f**	65.15	3.27
6c	42.01	>25	**10e**	42.97	>25	**3g**	62.54	3.94
6d	45.47	>25	**10f**	33.65	>25	**4b**	85.91	2.12
6e	48.83	>25	**10g**	57.60	>25	**4d**	51.95	>25
6f	50.86	>25	Curcumin	33.26	–	**4e**	80.09	>25
						4f	58.60	>25
						4g	82.74	>25

[a] Inhibition of self-induced Aβ (1–42) aggregation with the tested compounds at concentration of 10 μM.
[b] Results obtained with MTT for SH-SY5Y cell line.

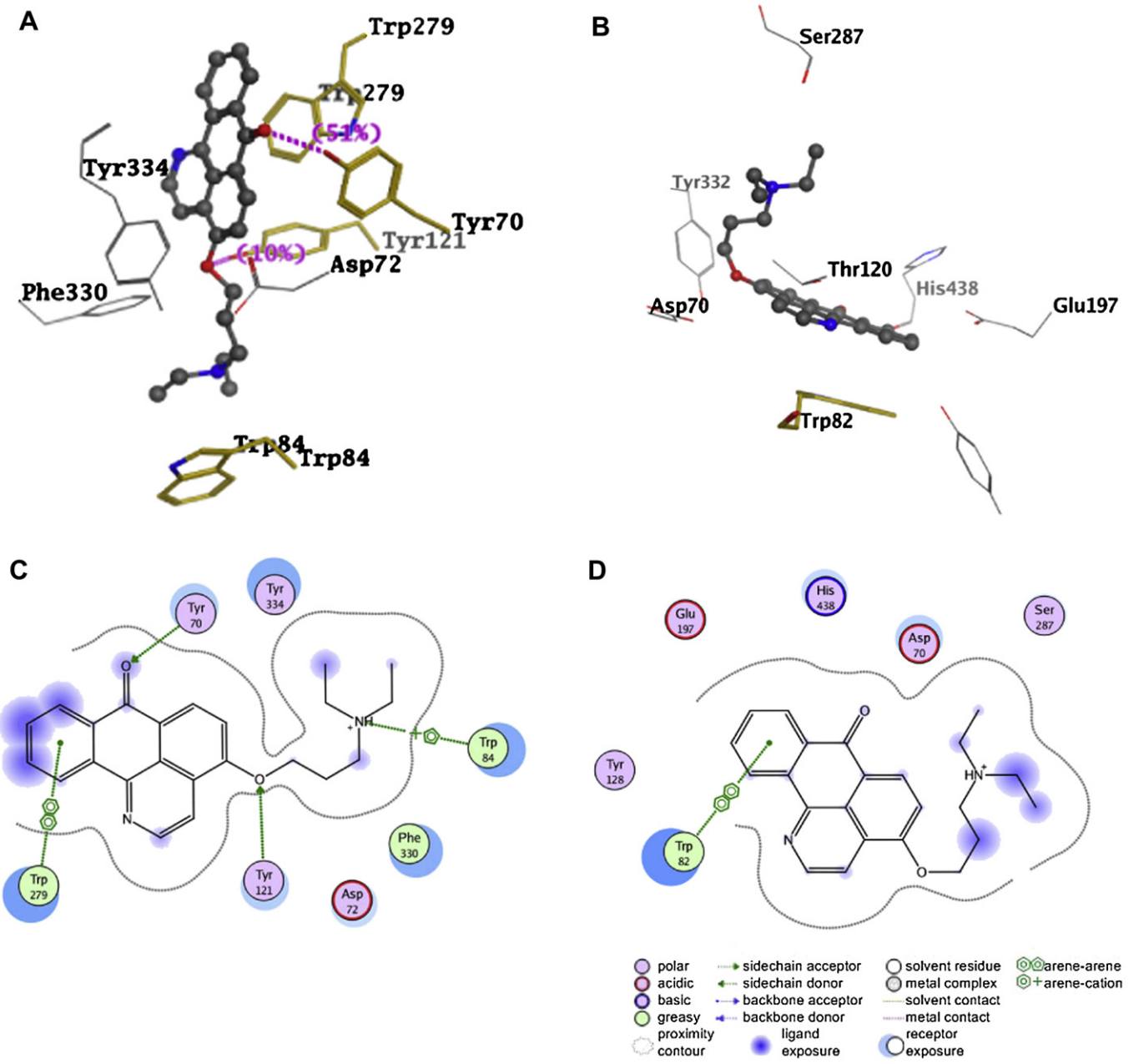


Fig. 5. Docking models of compound **10d** with *Tc*AChE (A, C) and *h*BuChE (B, D) generated with MOE.

than compounds **5c** and **5d** to both AChE and BuChE, and the compounds with piperidinyl groups in their side chains exhibited highest activity. In addition, compounds with quaternary amine were more efficient inhibitors to both enzymes than those with tertiary amine. For example, compound **6f** showed 4 times stronger inhibition for AChE (95 ± 8 nM) and BuChE (255 ± 15 nM) than compound **5f**, whose inhibition IC_{50} values were 375 ± 60 nM for AChE and 723 ± 12 nM for BuChE, respectively.

In comparison with 3-substituted compounds **5c**–**5f**, the 4-substituted oxoisoaporphine derivatives **10a**–**10g** were much better AChE inhibitors with lower IC_{50} values. The 4-substituted derivatives with piperidinyl group in their side chains also showed the highest inhibitory activity, just like the 3-substituted derivatives. For example, compounds **10b** and **10e** had strong AChE inhibitory activity with IC_{50} values of 88 ± 1.4 nM and 22 ± 1 nM, respectively, and compound **10e** was the best AChE inhibitor among all the newly synthesized compounds. Interestingly, the 4-substituted oxoisoaporphine derivatives were weaker BuChE inhibitors than the 3-substituted compounds, and their IC_{50} values for BuChE were 6–80 times higher than their corresponding IC_{50} values for AChE.

Our results also indicated that the selectivity of oxoisoaporphine derivatives for AChE was closely related with the substitutive position of the side chain. Compared with the high selectivity of the 9-substituted oxoisoaporphine derivatives reported previously [14], the 3-substituted oxoisoaporphine derivatives (**5c**–**5f**, **6c**–**6f**) had lower selectivity (1–2 fold) for AChE over BuChE, while the 4-substituted derivatives (**10a**–**10g**) had moderate selectivity (8–78 fold) for AChE over BuChE.

Because compounds **6f** and **10e** were the best AChE inhibitors in the two series of oxoisoaporphine derivatives, respectively, we further studied their inhibitory property using graphical analysis of steady-state inhibition data. As shown in Fig. 3, compounds **6f** and

10e could cause a mixed type of inhibition, which indicated that they could bind to both CAS and PAS of AChE simultaneously. It had been reported that some amino acids in PAS of AChE could promote Aβ fibrillation, and an inhibitor of PAS, propidium, was able to block this activity [21,22]. Therefore, the inhibitors which could bind to both CAS and PAS of AChE might have dual inhibition for both AChE activity and AChE induced Aβ aggregation.

3.2. Aβ (1–42) self-induced aggregation inhibitory activity and cell viability

Aβ was an important target in treating AD, and the inhibition of Aβ fibril formation was potentially important in AD therapy. Most of our synthetic compounds were found to inhibit Aβ (1–42) aggregation effectively as shown in Table 2 using Thioflavin-T fluorescence assay [17]. Compared with curcumin, which was a known active natural product for the inhibition of Aβ aggregation, compounds **5e**–**6f** and **10c**–**10g** showed similar or better inhibitory activity at 10 μM, with inhibition ratio from 31.83% to 57.60%. The compound **10g** showed the strongest inhibition among all these compounds. We also examined previously synthesized oxoisoaporphine derivatives for their anti-Aβ (1–42) aggregation activity (Table 2, compounds **3b**, **3d**,**e**, **3g**, **4b**, **4d**,**e** and **4g**), and it was found that most of these compounds also showed strong inhibition with ratio from 45.37% to 82.74%. The compounds with quaternary nitrogen in their side chains were found to have better inhibitory effects. The toxicity of the oxoisoaporphine derivatives was examined in human neuroblastoma SH-SY5Y cells, and the results were summarized as shown in Table 2. The cell viability was not affected by most compounds. Only compounds **5d**, **3b**, **3d**–**3g**, and **4b** had IC_{50} values lower than 10 μM, which might affect their applications in AD therapy.

The selectivity of cholinesterase inhibitors had been controversial in AD therapy. Although BuChE may be involved in AD pathology, the comparison of clinical appliance of tacrine, donepezil, rivastigmine and galantamine demonstrated that tacrine, a non-selective AChE inhibitor, had less favorable therapeutic index [23–25]. Meanwhile, evidences suggested that the activity of BuChE decreased in AD patients [26]. Consequently, we thought that selective AChE inhibitors were preferable for drug development of AD. A linear relationship was shown in Fig. 4 between AChE selectivity and Aβ aggregation inhibitory activity based on the data of compounds **3b**, **3d**–**3g**, **4d**, **4e**–**4f**, **5c**–**5e**, **6c**–**6e**, and **10a**. It was found that most of the synthetic oxoisoaporphine derivatives which had good selectivity on AChE also showed high anti-Aβ (1–42) aggregation activity. Therefore, these selective oxoisoaporphine derivatives were dual inhibitors, which were potential lead compounds for further development for AD therapy.

3.3. Molecular docking studies

To further study the interaction mode and selectivity of these derivatives for two cholinesterases, molecular docking study was performed using software package MOE 2008.10. The X-ray crystal structure of the *Tc*AChE complex with bis-taccrine (PDB code 1EVE) with a resolution of 2.50 Å was applied to build the starting model of AChE. Because the crystal structure of BuChE from *equine serum* was not available, human BuChE (PDB code 1P0I) with a resolution of 2.00 Å was used instead because of their high sequence homology.

The docking results are shown in Fig. 5, which indicated that compound **10d**, with the highest selectivity (AChE/BuChE = 78.60) and strong inhibitory activity ($IC_{50} = 34.3 \pm 1.7$ nM) to AChE, could interact with both CAS and PAS of AChE simultaneously (Fig. 5, A and C). The D ring of **10d** bound with Trp279 via π–π stacking interaction, and its side chain bound with Trp84 via cation–π interaction. Besides, two hydrogen bonds were also found. One formed between Tyr70 and the oxygen of C=O group in the mother ring of **10d** (O···O distance: 2.52 Å). The another formed between Tyr121 and the oxygen of the side chain (O···O distance: 3.23 Å). Thus, compound **10d** could fit into the gorge of AChE perfectly, which explained its high inhibitory effect. On the other hand, it was demonstrated that compound **10d** could also bind into the large catalytic cavity of BuChE (Fig. 5, B and D). This binding only involved π–π stacking interaction with Trp82, and hydrogen bond interaction was not observed. The most important difference between AChE and BuChE was the peripheral site. For AChE, the peripheral site was constituted with some large aromatic residues. But for BuChE, these residues were replaced with aliphatic amino acids. As a result, the binding modes of compound **10d** with these two enzymes were different, as shown in Fig. 5. Because compound **10d** could bind more tightly with AChE through more interactions, it had high selectivity for AChE over BuChE.

4. Conclusion

Two series of oxoisoaporphine derivatives were synthesized and found to be effective AChE and BuChE inhibitors, and most of them also had good inhibition for Aβ (1–42) aggregation. Their cholinesterase inhibitory activity was affected by the position of substitution and the type of side chains. The 4-substituted oxoisoaporphine derivatives were found to be more effective and selective AChE inhibitors than 3-substituted inhibitors. The compounds with pyrrolidinyl and piperidinyl groups in their side chains were found to have stronger AChE inhibition. The compounds with quaternary nitrogen in their side chains were found to have higher activity for both AChE inhibition and anti-Aβ (1–42) aggregation. It is possible that the positively charged side chains may interact with the CAS of AChE resulting in strong binding and inhibition of AChE. They may also interfere with the hydrogen bond formation during Aβ (1–42) aggregation resulting in higher anti-Aβ (1–42) aggregation activity. This study provided potentially important information for further development of oxoisoaporphine derivatives as lead compounds for the treatment of AD.

5. Experimental section

5.1. Chemistry

General Methods. Reagents were purchased from common commercial suppliers, and were used without further purification. Nuclear magnetic resonance spectra were recorded using a Varian Inova 500 NB or Bruker Avance III 400 MHz or Varian Mercury-Plus 300 instrument. Chemical shifts are reported in δ values (ppm) relative to internal TMS. HPLC analyses were performed on Shimadzu Prominence LC-20A equipment with an SPD-M20A PDA detector, using a Phenomenex Synergi Fusion-RP (C18, 4 μm, 250 mm) column. HRMS were recorded on Thermo MAT95XP using EI ionization. Mass spectra (MS) were obtained by electron spray ionization (ESI) in positive mode using an LCQ DECA XP LC-MS spectrometer.

5.1.1. Phenylethylphthalimide (*1*)

A mixture of phthalic anhydride (30 g, 0.2 mol) and β-phenylethylamine (26 g, 0.2 mol) in anhydrous ethanol was refluxed for 6 h. After cooling to 0–5 °C, crystals of phenylethylphthalimide **1** (43 g, 78%) were separated, which were collected through filtration and wash with ethanol. The product was pure enough for the next reaction. Mp 130–131 °C (lit [27,28]. 124–126 °C); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.78–7.82 (m, 2H), 7.67–7.71 (m, 2H), 7.17–7.29 (m, 5H), 3.92 (t, 2H, $J = 7.6$ Hz), 2.98 (t, 2H, $J = 7.6$ Hz); ESI-MS m/z: 252 $[M + H]^+$.

*5.1.2. 1-Azabenzanthrone (**3a**)*

To a mixture of anhydrous aluminum chloride (75 g, 0.56 mol) and sodium chloride (15 g, 0.26 mol) was slowly added phenylethylphthalimide **1** (53 g, 0.2 mol) at 180 °C for 30 min. The reaction was allowed to continue at 220–230 °C for 2 h. The product was cooled, finely ground and poured slowly into concentrated sulfuric acid (550 mL) at 90 °C. The mixture was stirred and heated at 230–240 °C for 2 h. After being cooled, the solution was poured into ice. Sodium hydroxide was added until pH = 3 was obtained, and the resultant precipitate was filtered off and washed in turn with dilute aqueous sodium hydroxide and water to give the crude product **18g**, which was extracted with acetic acid. The extract was condensed under reduced pressure, and the resultant precipitate was washed off, dried, and sublimed at 130–140 °C at a pressure of 1 mm mercury to give the product **3a** (15.3 g, 32%) as light-yellow solid, mp 182–183 °C (lit [27,28]. 181–183 °C); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.90 (d, 1H, J = 7.9 Hz), 8.77 (d, 1H, J = 5.6 Hz), 8.66 (d, 1H, J = 7.2 Hz), 8.41 (d, 1H, J = 7.8 Hz), 8.15 (d, 1H, J = 8.2 Hz), 7.90 (t, 1H, J = 7.3 Hz), 7.80 (t, 1H, J = 7.3 Hz), 7.74 (d, 1H, J = 5.8 Hz), 7.64 (t, 1H, J = 7.7 Hz); m/z calcd for $C_{16}H_9NO$ 231.07, ESI-MS m/z: 232.1 $[M + H]^+$.

*5.1.3. 3-Bromo-7H-dibenzo[de,h]quinolin-7-one (**4**)*

To a solution of bromine (1.8 mL) in nitrobenzene (33 mL) was added compound **3a** (1.1 g, 4.8 mmol) followed by one crystal of iodine. The mixture was heated under reflux for 5 h. After cooling and filtration, the pure compound **4** (1.35 g, 90%) was obtained [29]. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.76 (d, 1H, J = 7.6 Hz), 8.69 (d, 1H, J = 7.3 Hz), 8.57 (d, 1H, J = 8.3 Hz), 8.38 (d, 1H, J = 7.8 Hz), 8.30 (s, 1H), 7.90 (t, 1H, J = 7.8 Hz), 7.77 (t, 1H, J = 7.3 Hz), 7.57 (t, 1H, J = 7.8 Hz).

*5.2. General procedure for the synthesis of compounds **5c**–**5f***

The target products **5c**–**5f** were synthesized with the following general procedure. A solution of compound **4** (0.5 mmol) in 2 mL DMF, the corresponding alcohol ROH (1 mmol), 18-crown-6 (0.05 mmol), and finely ground potassium hydroxide (2 mmol) were mixed, and the resulting mixture was stirred at 80 °C for 6 h. Then the reaction mixture was cooled to room temperature, and poured into 5 mL of acidified water (pH 2). It was then extracted with chloroform, and the organic phase was dried over anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure. The residue was purified with flash column chromatography eluting with $CHCl_3/CH_3OH$ (100:3–100:5) to give a yellow solid.

*5.2.1. 3-(2-(Dimethylamino)ethoxy)-7H-dibenzo[de,h]quinolin-7-one (**5c**)*

Compound **5c** was obtained with a yield of 26% (40 mg). Purity: 99.0% (by HPLC). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.76 (d, 1H, J = 7.9 Hz,), 8.69 (d, 1H, J = 7.3 Hz), 8.55 (d, 1H, J = 8.3 Hz), 8.38 (d, 1H, J = 7.9 Hz), 8.33 (s, 1H), 7.89 (t, 1H, J = 7.9 Hz), 7.77 (t, 1H, J = 7.9 Hz), 7.57 (t, 1H, J = 7.8 Hz), 4.45 (t, 2H, J = 5.6 Hz), 2.96 (t, 2H, J = 5.6 Hz), 2.45 (s, 6H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 182.5, 150.1, 140.0, 136.4, 134.3, 130.7, 130.3, 130.0, 129.5, 128.2, 127.9, 127.0, 126.6, 126.1, 124.2, 122.4, 67.4, 57.6, 45.5 (2C). m/z calcd for $C_{20}H_{18}N_2O_2$ 318.14, ESI-MS m/z: 319.1 $[M + H]^+$.

*5.2.2. 3-(2-(Diethylamino)ethoxy)-7H-dibenzo[de,h]quinolin-7-one (**5d**)*

Compound **5d** was obtained with a yield of 27% (45 mg). Purity: 100% (by HPLC). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.76 (d, 1H, J = 7.9 Hz), 8.69 (d, 1H, J = 7.3 Hz), 8.54 (d, 1H, J = 8.3 Hz), 8.38 (d, 1H, J = 7.9 Hz), 8.33 (s, 1H), 7.90 (t, 1H, J = 7.7 Hz), 7.77 (t, 1H, J = 7.9 Hz), 7.57 (t, 1H, J = 8.1 Hz), 4.42 (t, 2H, J = 5.9 Hz), 3.09 (t, 2H, J = 5.9 Hz), 2.74 (q, 4H, J = 7.1), 1.15 (t, 6H, J = 7.1). ^{13}C NMR (MeOD, 100 MHz) δ 184.7, 151.8, 141.8, 137.9, 135.1, 132.3, 131.1, 130.8, 130.4, 129.5, 129.4, 128.3, 128.1, 126.2, 125.6, 124.0, 68.4, 62.0, 52.4 (2C), 11.7 (2C). m/z calcd for $C_{22}H_{22}N_2O_2$ 346.17, ESI-MS m/z: 347.2 $[M + H]^+$.

*5.2.3. 3-(2-(Pyrrolidin-1-yl)ethoxy)-7H-dibenzo[de,h]quinolin-7-one (**5e**)*

Compound **5e** was obtained with a yield of 36% (60 mg). Purity: 98.2% (by HPLC). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.75 (d, 1H, J = 7.7 Hz), 8.69 (d, 1H, J = 7.2 Hz), 8.55 (d, 1H, J = 7.9 Hz), 8.38 (d, 1H, J = 7.9 Hz), 8.32 (s, 1H), 7.89 (t, 1H, J = 7.8 Hz), 7.77 (t, 1H, J = 7.2 Hz), 7.57 (t, 1H, J = 7.1 Hz), 4.50 (t, 2H, J = 5.9 Hz), 3.13 (t, 2H, J = 5.9 Hz), 2.77 (m, 4H), 1.87 (m, 4H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 182.5, 150.1, 140.4, 136.4, 134.2, 130.7, 130.2, 129.9, 129.4, 128.1, 127.9, 126.9, 126.6, 126.0, 124.2, 122.4, 68.5, 54.1 (2C), 47.5, 23.3 (2C). m/z calcd for $C_{22}H_{20}N_2O_2$ 344.15, ESI-MS m/z: 345.2$[M + H]^+$.

*5.2.4. 3-(2-(Piperidin-1-yl)ethoxy)-7H-dibenzo[de,h]quinolin-7-one (**5f**)*

Compound **5f** was obtained with a yield of 17% (30 mg). Purity: 99.5% (by HPLC). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.76 (d, 1H, J = 7.3 Hz), 8.69 (d, 1H, J = 7.3 Hz), 8.53 (d, 1H, J = 8.3 Hz), 8.38 (d, 1H, J = 7.8 Hz), 8.33 (s, 1H), 7.90 (t, 1H, J = 7.8 Hz), 7.77 (t, 1H, J = 7.3 Hz), 7.57 (t, 1H, J = 7.2 Hz), 4.51 (br, 2H, J = 5.9 Hz), 3.03 (br, 2H, J = 5.9 Hz), 2.66 (m, 4H), 1.68 (m, 4H), 1.50 (m, 4H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 182.5, 149.2, 140.6, 136.2, 134.4, 130.8, 130.5, 130.1, 129.7, 128.1, 128.0, 127.0, 126.5, 126.3, 124.3, 122.4, 62.4, 60.9 (2C), 47.7, 20.6, 19.4 (2C). m/z calcd for $C_{23}H_{22}N_2O_2$ 358.17, ESI-MS m/z: 359.2 $[M + H]^+$.

*5.3. General procedure for preparation of quaternary methiodide salts **6c**–**6f***

A mixture containing compound **5c** (0.04 g), iodomethane (0.5 mL), and chloroform (3 mL) was stirred at 25 °C for 24 h. An orange-yellow solid was collected after filtration and wash with chloroform, and then dried in vacuum. The quaternary methiodide salt **6c** was obtained in a yield of 93% as an amorphous powder. Purity: 96% (by HPLC). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.76 (d, 1H, J = 7.9 Hz), 8.65 (d, 1H, J = 7.2 Hz), 8.63 (d, 1H, J = 8.3 Hz), 8.60 (s, 1H), 8.28 (d, 1H, J = 7.7 Hz), 8.12 (t, 1H, J = 7.8 Hz), 7.91 (t, 1H, J = 7.6 Hz), 7.70 (t, 1H, J = 7.9 Hz), 4.92 (br, 2H), 4.01 (br, 2H), 3.27 (s, 9H). ^{13}C NMR (DMSO-d_6, 125 MHz) δ 185.0, 151.0, 140.6, 138.1, 135.6, 131.7, 131.6, 131.4, 131.1, 130.1, 129.3, 128.6, 128.5, 126.8, 126.0, 124.5, 67.1, 65.0, 55.0 (3C). m/z calcd for $C_{21}H_{21}N_2O_2$ 333.16, ESI-MS m/z: 333.2 $[M]^+$.

Compound **6d**: Purity: 97.6% (by HPLC). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.75 (d, 1H, J = 7.4 Hz), 8.64 (d, 1H, J = 7.3 Hz), 8.60 (s, 1H), 8.58 (d, 1H, J = 7.8 Hz), 8.28 (d, 1H, J = 7.8 Hz), 8.13 (t, 1H, J = 7.8 Hz), 7.91 (t, 1H, J = 7.4 Hz), 7.70 (t, 1H, J = 7.9 Hz), 4.88 (t, 2H, J = 4.1 Hz), 3.96 (t, 2H, J = 4.1 Hz), 3.53 (q, 4H, J = 7.2 Hz), 3.15 (s, 3H), 1.34 (t, 6H, J = 7.2 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.4, 149.2, 140.6, 136.2, 134.4, 130.8, 130.5, 130.1, 129.7, 128.0, 127.9, 127.0, 126.4, 126.3, 124.3, 122.4, 62.6, 59.0, 56.5(2C), 47.3, 7.7(2C). m/z calcd for $C_{23}H_{25}N_2O_2$ 361.19, ESI-MS m/z: 361.2 $[M]^+$.

Compound **6e**: Purity: 97.7% (by HPLC). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.75 (d, 1H, J = 7.9 Hz), 8.66–8.60 (m, 3H), 8.28 (d, 1H, J = 7.8 Hz), 8.12 (t, 1H, J = 7.8 Hz), 7.91 (t, 1H, J = 7.6 Hz), 7.70 (t, 1H, J = 7.7 Hz), 4.92 (t, 2H, J = 4.1 Hz), 4.06 (t, 2H, J = 4.1 Hz), 3.72–3.65 (br, 4H), 3.21 (s, 3H), 2.12–2.21 (m, 4H). ^{13}C NMR (100 MHz, MeOD) δ 184.9, 150.8, 143.2, 137.9, 135.4, 132.6, 131.6, 131.4, 130.9, 129.8, 129.2, 128.3 (2C), 126.6, 125.8, 124.3, 66.7, 64.8, 64.3, 49.8 (2C), 22.6 (2C). m/z calcd for $C_{23}H_{23}N_2O_2$ 359.18, ESI-MS m/z: 359.2 $[M]^+$.

Compound **6f**: Purity: 96% (by HPLC). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.75 (d, 1H, J = 7.8 Hz), 8.66–8.60 (m, 3H), 8.28 (d, 1H, J = 7.8 Hz), 8.12 (t, 1H, J = 7.4 Hz), 7.91 (t, 1H, J = 7.6 Hz), 7.70 (t, 1H,

J = 7.5 Hz), 4.93 (t, 2H, J = 3.9 Hz), 4.06 (t, 2H, J = 3.9 Hz), 3.60–3.50 (m, 4H), 3.25 (s, 3H), 1.95–1.85 (m, 4H), 1.65–1.56 (m, 2H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 182.5, 150.2, 140.0, 136.4, 134.2, 130.7, 130.3, 130.0, 129.5, 128.2, 128.0, 126.9, 126.7, 126.2, 124.2, 122.4, 69.8, 67.4, 57.3, 54.4 (2C), 25.6 (2C), 23.9. m/z calcd for $C_{24}H_{25}N_2O_2$ 373.19, ESI-MS m/z: 373.2 $[M]^+$.

5.3.1. 4-Hydroxy-7H-dibenzo[de,h]quinolin-7-one (**7a**)

(a) A mixture of compound **3a** (0.50 g, 2.2 mmol) and hydrazine hydrate (1 mL) was stirred in diethylene glycol (15 mL) for 10 min. Then 2 mL of 40% NaOH solution was added dropwise, and the mixture was heated at 140 °C for 3 h. The reaction mixture was cooled to room temperature, and poured into water. The hydrochloric acid was added to adjust the solution to pH 3. The precipitate was filtered off and purified with silica gel chromatography using chloroform/methanol (100:5) as eluting solvent to afford compound **3a** as a red solid with a yield of 76% (0.40 g).

(b) A mixture of **3a** (0.50 g, 2.2 mmol) and hydroxylamine hydrochloride (2.7 g, 39 mmol) was stirred in diethylene glycol (15 mL) for 10 min. Then 5 mL of 40% NaOH solution was added dropwise, and the mixture was heated at 140 °C for 2 h. The reaction mixture was cooled to room temperature, and poured into water. The hydrochloric acid was added to adjust the solution to pH 3. The precipitate was filtered off to give pure compound **3a** with a yield of 83% (0.44 g).

^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.85 (d, 1H, J = 7.7 Hz), 8.78 (d, 1H, J = 5.3 Hz), 8.49 (d, 1H, J = 8.1 Hz), 8.29 (d, 1H, J = 7.6 Hz), 8.11 (d, 1H, J = 5.3 Hz), 7.87 (t, 1H, J = 7.3 Hz), 7.73 (t, 1H, J = 7.3 Hz), 7.33 (d, 1H, J = 8.1 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 180.6, 160.6, 146.9, 142.9, 136.1, 133.4, 133.0, 132.3, 130.4, 126.7, 126.3, 124.8, 123.5, 119.7, 116.2, 113.2; HRMS (EI) m/z calcd for $C_{16}H_9O_2N$ $[M^+]$: 247.0628, found 247.0629.

5.4. General procedure for the Syntheses of compounds **8**–**9**

To a solution of 4-hydroxy-7*H*-dibenzo[*de*,*h*]quinolin-7-one **7a** (1.5 g, 6.1 mmol) and powdered K_2CO_3 (1.6 g) in DMF (15 mL), an appropriate amount of dibromo-derivative(2 mL) was added. After stirring at 80 °C for 12 h, the solvent was removed under vacuum to give an oily residue that was purified using flash chromatography with PE/$CHCl_3$ = 1:1.

5.4.1. 4-(2-Bromoethoxy)-7H-dibenzo[de,h]quinolin-7-one (**8**)

It was synthesized from compound **7a** (1.5 g, 6.1 mmol) with powdered K_2CO_3 (1.6 g) in DMF (15 mL) and 1,2-dibromoethane (2 mL). It was purified using flash chromatography with $CHCl_3$/PE (1:1) to afford compound **8** as a yellow solid with a yield of 47% (1.0 g). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.93 (d, 1H J = 7.8 Hz), 8.81 (d, 1H, J = 5.7 Hz), 8.65 (d, 1H, J = 8.2 Hz), 8.44 (dd, 1H, J_1 = 7.8 Hz, J_2 = 1), 8.14 (d, 1H, J = 5.7 Hz), 7.81 (td, 1H, J_1 = 7.4 Hz, J_2 = 1.3 Hz), 7.66 (td, 1H, J_1 = 7.4 Hz, J_2 = 1.1 Hz), 7.16 (d, 1H, J = 8.2 Hz), 4.61 (t, 2H, J = 5.9 Hz), 3.84 (t, 2H, J = 5.9 Hz).

5.4.2. 4-(3-Bromopropoxy)-7H-dibenzo[de,h]quinolin-7-one (**9**)

It was synthesized from compound **7a** (1.5 g, 6.1 mmol) with powdered K_2CO_3 (1.6 g) in DMF (15 mL) and1,3-dibromopropane (2 mL). It was purified using flash chromatography with CH_2Cl_2/PE (1:1) to afford compound **9** as a yellow solid with a yield of 41% (0.91 g). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.92 (d, 1H, J = 7.8 Hz), 8.78 (d, 1H, J = 5.7 Hz), 8.65 (d, 1H, J = 8.3 Hz), 8.44 (dd, 1H, J_1 = 7.8 Hz, J_2 = 1.0 Hz), 8.05 (d, 1H, J = 5.7 Hz), 7.80 (td, 1H, J_1 = 7.3 Hz, J_2 = 1.3 Hz), 7.68 (td, 1H, J_1 = 7.3 Hz, J_2 = 1.1 Hz), 7.20 (d, 1H, J = 8.3 Hz), 4.44 (t, 2H, J = 6.0 Hz), 3.72 (t, 2H, J = 6.0 Hz), 2.54 (m, 2H).

5.5. General procedure for the syntheses of compounds **10a**–**g**

To a solution of amide (4 mL) and powered K_2CO_3 (1.2 g) in CH_3CN (100 mL), an appropriate amount of bromide was slowly added. After stirring at 50 °C for 4 h, the solvent was removed under vacuum to afford an oily residue that was purified using flash chromatography.

5.5.1. 4-(2-(Diethylamino)ethoxy)-7H-dibenzo[de,h]quinolin-7-one (**10a**)

It was synthesized from compound **8** (0.20 g , 0.57 mmol) with powered K_2CO_3 (1.2 g) in CH_3CN (100 mL) and diethyl amine (4 mL). It was purified using flash chromatography with $CHCl_3$/MeOH (100:2) to afford a light-yellow solid **10a** with a yield of 64% (120 mg). Purity: 97.7% (by HPLC). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.91 (d, 1H, J = 7.8 Hz), 8.78 (d, 1H, J = 5.6 Hz), 8.65 (d, 1H, J = 8.3 Hz), 8.44 (d, 1H, J = 7.8 Hz), 8.07 (d, 1H, J = 5.6 Hz), 7.80 (t, 1H, J = 7.3 Hz), 7.65 (t, 1H, J = 7.4 Hz), 7.18 (d, 1H, J = 8.3 Hz), 4.38 (t, 2H J = 5.7 Hz), 3.11 (t, 2H, J = 5.7 Hz), 2.76 (q, 4H, J = 7.1 Hz), 1.16 (t, 6H J = 7.1 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 182.3, 159.8, 148.1, 143.6, 136.9, 133.5, 132.8, 132.5, 130.3, 127.7, 127.5, 125.3, 123.8, 122.2, 115.8, 108.9, 68.1, 51.5, 48.1 (2C), 12.1 (2C). m/z calcd for $C_{22}H_{22}N_2O_2$ 346.17, ESI-MS m/z: 347.0 $[M + H]^+$.

5.5.2. 4-(2-(Piperidin-1-yl)ethoxy)-7H-dibenzo[de,h]quinolin-7-one (**10b**)

Compound **10b** was obtained as a light-yellow solid with a yield of 85% (170 mg). Purity: 98.6% (by HPLC). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.90 (d, 1H, J = 7.9 Hz), 8.77 (d, 1H, J = 5.6 Hz), 8.63 (d, 1H, J = 8.3 Hz), 8.43 (d, 1H, J = 7.8 Hz), 8.05 (d, 1H, J = 5.6 Hz), 7.79 (t, 1H, J = 7.3 Hz), 7.65 (t, 1H, J = 7.4 Hz), 7.16 (d, 1H, J = 8.3 Hz), 4.42 (t, 2H, J = 5.7 Hz), 2.99 (t, 2H, J = 5.6 Hz), 2.62 (br, 4H), 1.65 (t, 4H, J = 5.2 Hz), 1.49 (m, 2H, br). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 180.9, 159.5, 147.1, 143.7, 136.1, 133.8, 132.6, 132.1, 130.6, 126.9, 126.8, 124.9, 122.9, 121.0, 115.7, 110.3, 67.1, 56.7, 54.1 (2C), 25.3 (2C), 23.5. m/z calcd for $C_{23}H_{22}N_2O_2$ 358.17, ESI-MS m/z: 359.1 $[M + H]^+$.

5.5.3. 4-(2-(4-Methylpiperazin-1-yl)ethoxy)-7H-dibenzo[de,h]quinolin-7-one (**10c**)

Compound **10c** was obtained as a light-yellow solid with a yield of 65% (130 mg). Purity: 99.8% (by HPLC). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.91 (d, 1H, J = 7.9 Hz), 8.77 (d, 1H, J = 5.6 Hz), 8.64 (d, 1H, J = 8.3 Hz), 8.43 (d, 1H, J = 7.8 Hz), 8.05 (d, 1H, J = 5.6 Hz), 7.79 (t, 1H, J_1 = 7.3 Hz), 7.65 (t, 1H, J = 7.3 Hz), 7.16 (d, 1H, J = 8.3 Hz), 4.41 (t, 2H, J = 5.6 Hz), 3.03 (t, 2H, J = 5.6 Hz), 2.73 (br, 4H), 2.52 (br, 4H), 2.33 (s, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 180.9, 159.6, 147.1, 143.7, 136.1, 133.7, 132.6, 132.1, 130.6, 127.0, 126.8, 124.9, 122.9, 120.9, 115.7, 110.3, 67.4, 56.1, 54.6 (2C), 52.7 (2C), 45.4. m/z calcd for $C_{23}H_{23}N_3O_2$ 373.18, ESI-MS m/z: 374.1 $[M + H]^+$.

5.5.4. 4-(3-(Diethylamino)propoxy)-7H-dibenzo[de,h]quinolin-7-one (**10d**)

Compound **10d** was obtained as a light-yellow solid with a yield of 45% (70 mg). Purity: 97.4% (by HPLC). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.93 (d, 1H, J = 7.9 Hz), 8.79 (d, 1H, J = 5.6 Hz), 8.67 (d, 1H, J = 8.3 Hz), 8.44 (d, 1H, J_1 = 7.3 Hz), 8.09 (d, 1H, J = 5.6 Hz), 7.80 (t, 1H, J_1 = 7.7 Hz, J_2 = 0.9 Hz), 7.65 (t, 1H, J = 7.3 Hz), 7.20 (d, 1H, J = 8.3 Hz), 4.36 (t, 2H, J = 6.1 Hz), 2.75 (br, 2H), 2.62 (br, 4H), 2.16 (br, 2H), 1.08 (t, 6H, J = 6.7 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 182.5, 160.0, 148.2, 143.7, 137.0, 133.6, 132.9, 132.7, 130.3, 127.7, 127.5, 125.3, 123.9, 122.1, 115.7, 109.0, 67.4, 49.4, 47.2 (2C), 26.7, 11.5 (2C). m/z calcd for $C_{23}H_{24}N_2O_2$ 360.18, ESI-MS m/z: 361.2 $[M + H]^+$.

5.5.5. 4-(3-(Piperidin-1-yl)propoxy)-7H-dibenzo[de,h]quinolin-7-one (**10e**)

Compound **10e** was obtained as a light-yellow solid with a yield of 71% (120 mg). Purity: 97.8% (by HPLC). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.92 (d, 1H, J = 7.9 Hz), 8.78 (d, 1H, J = 5.6 Hz), 8.66 (d, 1H, J = 8.3 Hz), 8.44 (dd, 1H, J_1 = 7.3 Hz, J_2 = 0.9 Hz), 8.09 (d, 1H, J = 5.6 Hz), 7.80 (t, 1H, J_1 = 7.8 Hz, J_2 = 1.3 Hz), 7.65 (t, 1H, J_1 = 7.8 Hz, J_2 = 1.1 Hz), 7.20 (d, 1H, J = 8.3 Hz), 4.35 (t, 2H, J = 6.3 Hz), 2.60 (t, 2H, J = 5.8 Hz), 2.47 (br, 4H), 2.19 (t, 2H, J = 6.4Hz), 1.63 (br, 4H), 1.48 (br, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 182.3, 160.0, 148.1, 143.6, 136.9, 133.5, 132.8, 132.6, 130.2, 127.7, 127.5, 125.3, 123.8, 122.0, 115.8, 109.0, 67.8, 55.9, 54.9 (2C), 26.7, 26.1 (2C), 24.5. m/z calcd for $C_{24}H_{24}N_2O_2$ 372.18, ESI-MS m/z: 373.2 $[M + H]^+$.

5.5.6. 4-(3-(4-Methylpiperazin-1-yl)propoxy)-7H-dibenzo[de,h]quinolin-7-one (**10f**)

Compound **10f** was obtained as a light-yellow solid with a yield of 22% (40 mg). Purity: 97.6% (by HPLC). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.91(d, 1H, J = 7.9 Hz), 8.77 (d, 1H, J = 5.6 Hz), 8.64 (d, 1H, J = 8.3 Hz), 8.43 (dd, 1H, J_1 = 7.8 Hz), 8.07 (d, 1H, J = 5.6 Hz), 7.80 (t, 1H, J = 7.2 Hz), 7.65 (t, 1H, J = 7.2 Hz), 7.18 (d, 1H, J = 8.3 Hz), 4.34 (t, 2H, J = 6.2 Hz), 2.65 (t, 2H, J = 7.2 Hz), 2.64–2.40 (br, 8H), 2.32 (s, 3H), 2.17 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 182.4, 160.0, 148.2, 143.6, 136.9, 133.6, 132.8, 132.6, 130.3, 127.7, 127.5, 125.3, 123.9, 122.1, 115.8, 108.9, 67.5, 55.1 (2C), 55.0, 53.2 (2C), 46.0, 26.6. m/z calcd for $C_{24}H_{25}N_3O_2$ 387.19, ESI-MS m/z: 388.3 $[M + H]^+$.

5.5.7. 4-(3-(Dimethylamino)propylamino)-7H-dibenzo[de,h]quinolin-7-one (**10g**)

A solution of compound **8** (240 mg, 0.68 mmol) and 3-dimethylaminopropylamine (5 mL) in ethanol (50 mL) was heated under reflux for 2 h. Then, the solvent was evaporated, and the residue was purified using silica gel column chromatography with $CHCl_3$/MeOH (100:5) as eluting solvent to afford a red solid **10g** with a yield of 80% (180 mg). Purity: 96.3% (by HPLC). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.93 (d, 1H, J = 7.9 Hz), 8.71 (d, 1H, J = 5.7 Hz), 8.61 (d, 1H, J = 8.4 Hz), 8.47 (d, 1H, J = 7.8 Hz), 7.75 (t, 1H, J = 7.4 Hz), 7.64 (t, 1H, J = 7.4 Hz), 7.60 (d, 1H, J = 5.2 Hz), 6.71 (d, 1H, J = 8.5 Hz), 3.53 (q, 2H, J = 5.20 Hz), 2.70 (t, 2H, J = 5.20 Hz), 2.47 (s, 6H), 2.00 (quinter, 2H, J = 5.2 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 181.2, 150.7, 148.1, 142.3, 136.6, 134.5, 133.5, 132.5, 129.9, 127.1, 125.2, 124.7, 124.2, 117.0, 114.4, 106.0, 59.5, 45.5 (2C), 44.7, 24.2. m/z calcd for $C_{21}H_{21}N_3O$ 331.17, ESI-MS m/z: 332.1 $[M + H]^+$.

5.6. Biological assays

5.6.1. In vitro AChE and BuChE inhibition assay

All the assays were carried out in 0.1 M KH_2PO_4/K_2HPO_4 buffer, pH 8.0, using a Shimadzu 2450 Spectrophotometer. Enzyme solutions were prepared at the concentration of 2.0 unit/mL. The assay medium contained phosphate buffer (pH 8.0), 0.01 M DTNB, AChE, and 0.01 M substrate (acetylthiocholine chloride). The substrate was added to the medium with inhibitor after incubation for 15 min. The activity was determined by measuring the increase in absorbance at 412 nm in 1 min interval at 37 °C. The data were calculated based on the method of Ellman et al. *In vitro* BuChE assays were carried out using a similar method as described above.

5.6.2. Inhibition of Aβ (1–42) self-induced aggregation

The thioflavin-T fluorescence method was used, and Aβ (1–42) peptide (Anaspec Inc) was dissolved in phosphate buffer (pH 7.4, 0.01 M) to give a 40 μM solution. Compounds were firstly prepared in DMSO at a concentration of 10 mM. The final concentration of Aβ (1–42) and inhibitors were 20 μM and 10 μM, respectively. After incubating at 37 °C for 48 h, thioflavin-T (5 μM in 50 mM glycine–NaOH buffer, pH 8.0) was added. Fluorescence was measured at 450 nm (λ ex) and 485 nm (λ em). Each inhibitor was examined in triplicate. The fluorescence intensities were recorded, and the percentage of inhibition on aggregation was calculated with the following equation: (1 − IFi/IFc) × 100%. IFi and IFc were the fluorescence intensities obtained in the presence and absence of inhibitors, respectively, after subtracting the fluorescence of corresponding blanks.

5.6.3. Culture of SH-SY5Y cells and MTT assay of cell viability

SH-SY5Y cells, at passages between 3 and 16 after thawing, were maintained in Dulbecco's modified Eagle's medium (DMEM, GIBCO) containing 15 nonessential amino acids and supplemented with 10% fetal calf serum (GIBCO), 1 mM glutamine, 50 mg/μl penicillin, and 50 mg/μl streptomycin. Cultures were seeded into flasks containing supplemented medium, and were maintained at 37 °C in 5% CO_2 humidified air. For assays, SH-SY5Y cells were sub-cultured in 96-well plates at a seeding density of 10,000 cells per well. After 24 h, they were placed into medium with compounds. After 48 h, the survival of cells was determined with MTT assay. Briefly, after incubating with 20 μl of MTT (2.5 mg/mL) at 37 °C for 4 h, living cells containing MTT formazon crystals were solubilized in 100 μl of dimethyl sulfoxide (DMSO). The absorbance of each well was measured with a micro culture plate reader at the wavelength of 570 nm.

5.6.4. Molecular docking study

All calculations and analysis were carried out with Molecular Operating Environment (MOE) program (Chemical Computing Group, Montreal, Canada). The crystal structure of AChE (PDB code 1EVE) and BuChE (PDB code 1P0I) used in the docking study were obtained from the Protein Data Bank (www.rcsb.org). Heteroatoms and water molecules in the PDB files were removed at the beginning, and all hydrogen atoms were subsequently added to the proteins. Amber99 forcefield was assigned to the enzymes using MOE. Prior to the docking calculations, an energy minimization using the MMFF94 forcefield was performed on the enzymes using the minimization protocol.

Compound **10d** was drawn in MOE and all hydrogen atoms were added subsequently. Then the compounds were protonated using the protonate 3D protocol and energy minimized using the MMFF94 forcefield in MOE. When the enzymes and compound **10d** were ready for docking study, compound **10d** was modeled within the enzymes using the MOE docking protocol with the Alpha Triangle placement option and the London dG scoring function.

Acknowledgments

We thank the Natural Science Foundation of China (Grants U0832005, 90813011, 30701050), the International S&T Cooperation Program of China (2010DFA34630), and Science Foundation of Guangzhou (2009A1-E011-6) for financial support of this study.

References

[1] J.L. Cummings, N. Engl. J. Med. 351 (2004) 56–67.
[2] Alzheimer's Association. http://www.alz.org/national/documents/report_alzfactsfi-gures2009.pdf (2009).
[3] R.S. Doody, Alzheimer Dis. Assoc. Disord. 13 (Suppl. 2) (1999) S20–S26.
[4] S.H. Ferris, Expert Opin. Pharmacother. 4 (2003) 2305–2313.
[5] M. Citron, Nat. Rev. Neurosci. 5 (2004) 677–685.
[6] E. Scarpini, P. Scheltens, H. Feldman, Lancet Neurol. 2 (2003) 539–547.
[7] G. Kryger, I. Silman, J.L. Sussman, Structure 7 (1999) 297–307.
[8] J. Hardy, D. Allsop, Trends Pharmacol. Sci. 12 (1991) 383–388.
[9] K. Ono, M.M. Condron, L. Ho, J. Wang, W. Zhao, G.M. Pasinetti, D.B. Teplow, J. Biol. Chem. 283 (2008) 32176–32187.
[10] S. Rizzo, C. Riviere, L. Piazzi, A. Bisi, S. Gobbi, M. Bartolini, V. Andrisano, F. Morroni, A. Tarozzi, J.P. Monti, A. Rampa, J. Med. Chem. 51 (2008) 2883–2886.

[11] M. Rosini, V. Andrisano, M. Bartolini, M.L. Bolognesi, P. Hrelia, A. Minarini, A. Tarozzi, C. Melchiorre, J. Med. Chem. 48 (2005) 360–363.
[12] M. Rosini, E. Simoni, M. Bartolini, A. Cavalli, L. Ceccarini, N. Pascu, D.W. McClymont, A. Tarozzi, M.L. Bolognesi, A. Minarini, V. Tumiatti, V. Andrisano, I.R. Mellor, C. Melchiorre, J. Med. Chem. 51 (2008) 4381–4384.
[13] Q. Xie, H. Wang, Z. Xia, M. Lu, W. Zhang, X. Wang, W. Fu, Y. Tang, W. Sheng, W. Li, W. Zhou, X. Zhu, Z. Qiu, H. Chen, J. Med. Chem. 51 (2008) 2027–2036.
[14] H. Tang, F.X. Ning, Y.B. Wei, S.L. Huang, Z.S. Huang, A.S. Chan, L.Q. Gu, Bioorg. Med. Chem. Lett. 17 (2007) 3765–3768.
[15] H. Tang, Y.B. Wei, C. Zhang, F.X. Ning, W. Qiao, S.L. Huang, L. Ma, Z.S. Huang, L.Q. Gu, Eur. J. Med. Chem. 44 (2009) 2523–2532.
[16] G.L. Ellman, K.D. Courtney, V. Andres Jr., R.M. Feather-Stone, Biochem. Pharmacol. 7 (1961) 88–95.
[17] H. LeVine, Protein Sci. 2 (1993) 404–410.
[18] V.B. Bojinov, T.N. Konstantinova, Dyes Pigm. 32 (1996) 151–157.
[19] F.X. Ning, X. Weng, S.L. Huang, L.J. Gu, Z.S. Huang, L.Q. Gu, Chin. Chem. Lett. 22 (2011) 41–44.
[20] Q.Y. Xing, R.Q. Xu, Z. Zhou, W.W. Pei, Organic Chemistry, second ed. Higher Education Press, 1994.
[21] G.V. De Ferrari, M.A. Canales, I. Shin, L.M. Weiner, I. Silman, N.C. Inestrosa, Biochemistry 40 (2001) 10447–10457.
[22] M.L. Bolognesi, V. Andrisano, M. Bartolini, A. Cavalli, A. Minarini, M. Recanatini, M. Rosini, V. Tumiatti, C. Melchiorre, Farmaco 60 (2005) 465–473.
[23] M.M. Mesulam, M. Asuncion Moran, Ann. Neurol. 22 (1987) 223–228.
[24] D.R. Liston, J.A. Nielsen, A. Villalobos, D. Chapin, S.B. Jones, S.T. Hubbard, A. Shalaby, A. Ramirez, D. Nason, W.F. White, Eur. J. Pharmacol. 486 (2004) 9–17.
[25] D.E. Kuhl, R.A. Koeppe, S.E. Snyder, S. Minoshima, K.A. Frey, M.R. Kilbourn, Ann. Neurol. 59 (2006) 13–20.
[26] E.H. Rydberg, B. Brumshtein, H.M. Greenblatt, D.M. Wong, D. Shaya, L.D. Williams, P.R. Carlier, Y.P. Pang, I. Silman, J.L. Sussman, J. Med. Chem. 49 (2006) 5491–5500.
[27] S. Iwashima, T. Ueda, H. Honda, T. Tsujioka, M. Ohno, J. Aoki, T.J. Kan, Chem. Soc., Perkin Trans. 1 (1984) 2177–2187.
[28] H. Tang, X.D. Wang, Y.B. Wei, S.L. Huang, Z.S. Huang, J.H. Tan, L.K. An, J.Y. Wu, A.S. Chan, L.Q. Gu, Eur. J. Med. Chem. 43 (2008) 973–998.
[29] T. Ueda, Z. Abliz, M. Sato, M. Nishimura, S. Iwashima, J. Aoki, T. Kan, S. Matsunaga, R. Tanaka, J. Mol. Struct. 224 (1990) 313–322.