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Synthesis, biological evaluation, and molecular modeling of berberine derivatives as potent acetylcholinesterase inhibitors

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ABSTRACT

By targeting the dual active sites of acetylcholinesterase (AChE), a new series of berberine derivatives was designed, synthesized, and evaluated as AChE inhibitors. Most of the derivatives inhibited AChE in the sub-micromolar range. Compound **8c**, berberine linked with phenol by a 4-carbon spacer, showed the most potent inhibition of AChE. A kinetic study of AChE and BuChE indicated that a mix-competitive binding mode existed for these berberine derivatives. Molecular modeling studies confirmed that these hybrids target both the catalytic active site (CAS) and the peripheral anionic site (PAS) of AChE. This is the first report where AChE inhibitory activity has been associated with berberine as a lead molecule.

1. Introduction

Alzheimer's disease (AD), the most common dementia in the elderly, is a progressive neurodegenerative disorder characterized by memory loss and other cognitive impairments. Although the etiology of AD is not completely known, several diverse hallmarks such as β-amyloid (Aβ) deposits, τ-protein aggregation, oxidative stress, or low levels of acetylcholine (ACh) play significant roles in the pathophysiology of the disease.[1] The cholinergic hypothesis of AD suggests that low levels of ACh in specific regions of the brain result in learning and memory dysfunction.[2] This hypothesis indicates the therapeutic potential of increasing the levels of ACh through inhibition of the acetylcholinesterase (AChE). Acetylcholinesterase inhibitors (AChEIs) represent a well-established class of drugs for the symptomatic treatment of AD, which include tacrine (Cognex®), **1a**, Fig. 1), donepezil (Aricept®, **1b**, Fig. 1), galanthamine (Reminyl®, **1c**, Fig. 1), and huperzine A (**1d**, Fig. 1). Among them, galanthamine and huperzine A are naturally-occurring alkaloids from the genus Galanthus (Amaryllidaceae) and the club moss *Huperzia serrata* (Lycopodiaceae), respectively.

In the past decade, a series of crystal structures of AChE/inhibitor complexes have been reported,[3–6] which indicate that AChE has two binding sites: a catalytic active site (CAS) and a peripheral anionic site (PAS). The CAS of AChE is located at the bottom of a narrow gorge, which consists of two sub sites, an anionic and esteratic site. The second 'anionic' site, known as the PAS, lies around 14 Å from the active site. The PAS is involved in the formation of amyloid-β fibrils that are associated with plaque deposition in AD patients. AChE inhibitors simultaneously blocking both the catalytic and peripheral sites might not only alleviate the cognitive defect of AD patients by elevating ACh levels, but also act as disease-modifying agents delaying amyloid plaque formation.[7,8] This discovery stimulated a great interest toward a bivalent ligand strategy to design hybrid compounds for simultaneously inhibiting acetylcholine hydrolysis and AChE-induced Aβ aggregation. Generally, bivalent hybrid ligands are obtained by connecting two identical or distinct moieties through a linker of suitable length to make contact with both the CAS and PAS, including tacrine-related homo- and heterobivalent hybrids,[9,10] bis-galanthamine hybrids,[11] and huperzine A dimeric inhibitors,[12] with IC_{50} values from subnanomolar to picomolar.

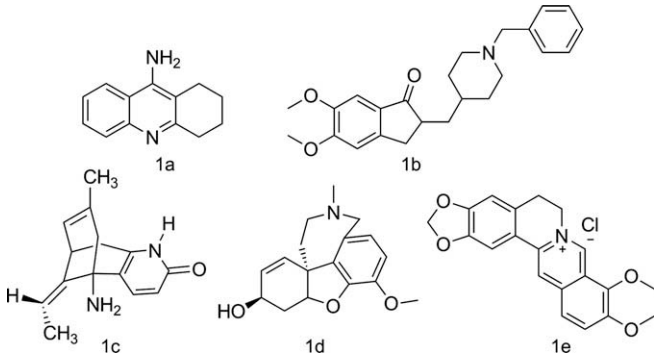

Figure 1. Stucture of tacrine (**1a**), donepezil (**1b**), galanthamine (**1c**), huperzine A (**1d**) and berberine (**1e**).

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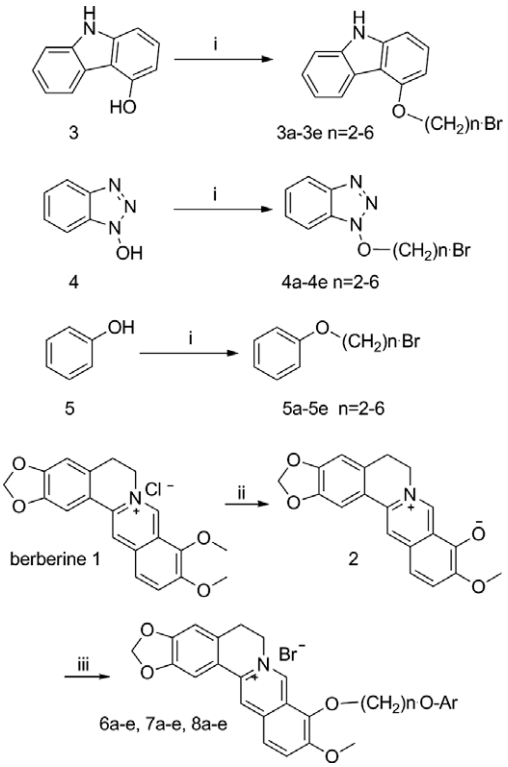
Berberine (**1e**, Fig. 1) could be isolated as the principal quaternary base from a traditional Chinese herb, *Coptis chinensis* Franch, the roots of which are widely used as traditional medicines for treating diarrhea[13] and gastrointestinal disorders.[14] It can inhibit acetylcholinesterase[15] and play an important role in metabolic syndrome.[16] Chemically, berberine possesses a quaternary nitrogen and three aromatic rings that might bind to CAS or PAS of AChE by π–π stacking and electronic interaction. Enlightened by the interesting structure of berberine, we designed several series of berberine derivatives by combining it with diverse aromatic rings that have two binding dual sites. The berberine moiety was expected to bind the PAS of AChE, and the aromatic rings of non-berberine moiety would interact with the catalytic center of AChE through a cation–π interaction. In this paper, we disclose the synthesis of berberine derivatives and their high AChE inhibitory activity, with IC_{50} values in the sub-micromolar range.

2. Results and discussion

2.1. Chemistry

The synthetic pathway of 9-substituted berberine derivatives **6a–e**, **7a–e**, **8a–e** are shown in Scheme 1. First, the reaction of 4-hydroxycarbazole, 1-hydroxybenzotriazole and phenol in butanone with α,ω-dibromoalkanes in the presence of K_2CO_3 was performed to provide responding ω-bromoalkyl ether derivatives (**3a–e**, **4a–e**, and **5a–e**). The selective demethylation of berberine **1** at 190 °C under vacuum gave berberrubine **2**[17] in 68% yield. Finally, the target compounds **6a–e**, **7a–e**, **8a–e** were obtained by reaction of berberrubine **2** with **3a–e**, **4a–e**, and **5a–e**, respectively, in CH_3CN for 12–24 h.



Scheme 1. 9-Substituted berberine derivatives **6a–e**, **7a–e**, **8a–e**. Reagents and conditions: (i) $Br(CH_2)nBr$, K_2CO_3, KI butanone, 1–4 h; (ii) 190 °C, 20–30 mmHg, 15 min; (iii) **3a–e**, **4a–e**, **5a–e**, K_2CO_3, CH_3CN, reflux.

2.2. In vitro inhibition studies of AChE and BuChE

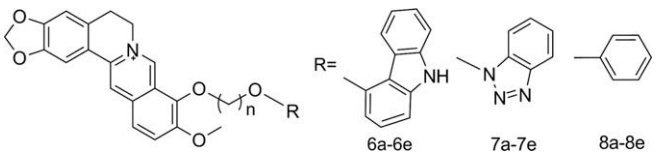
The AChE inhibitory effects of all target compounds were examined by the method of Ellman et al.[18] on AChE from electric eel using commercial galanthamine as the reference standard. The BuChE inhibitory on equine serum BuChE were also examined by the same method. The IC_{50} values for AChE and BuChE inhibition are summarized in Table 1.

All berberine derivatives were potent inhibitors of AChE, with IC_{50} values ranging from micromolar to sub-micromolar. A simple structure–activity relationship analysis showed that the AChE inhibitory potency closely related to the length of the alkylene chain. Compounds **6c, 7c**, and **8c**, with four methylene groups between the berberine and aromatic ring units, were the best inhibitors in their series. Especially, compound **8c** showed the best AChE inhibitory activity of all the berberine derivatives, with an IC_{50} value of 0.097 μM. However, the same trend was not shown for BuChE inhibition.

It is surprising that series **6**, which possesses berberine–carbazole hybrids, showed a remarkably low inhibitory activity compared to the bicyclic heterodimeric hybrids in series **7** and monocyclic heterodimeric hybrids in series **8**. Monocyclic derivatives (**8a–e**) showed most potent inhibitory activity of 9- to 20-fold more potent activity than 4-hydroxy carbazole derivatives. The relative rigidity of the 4-hydroxy carbazole scaffold could hinder penetration into the AChE gorge to reach the binding site.[19]

From the IC_{50} values of the compounds, it appeared that the 4-hydroxy carbazole derivatives (**6a–e**), showing a better activity toward BuChE than that of AChE. Compound **6a**, the most potent for BuChE inhibition, had an IC_{50} value of 0.029 μM, about 50-fold higher than for AChE. Recent studies indicated that inhibition of brain BuChE may represent an important therapeutic target for AD. It plays a key role and can partly compensate the function of

Table 1
In vitro inhibition and selectivity of compounds **1**, **6a–e**, **7a–e**, **8a–e**, and galanthamine on AChE and BChE activities



Compd	*n*	IC_{50} (μM) ± SEM		Selectivity for AChE[c]
		AChE[a]	BuChE[b]	
1		0.374 ± 0.024	18.2 ± 0.683	48.6
6a	2	1.23 ± 0.161	0.029 ± 0.004	0.024
6b	3	1.12 ± 0.08	0.143 ± 0.009	0.128
6c	4	0.856 ± 0.096	0.099 ± 0.006	0.116
6d	5	1.68 ± 0.059	0.107 ± 0.011	0.064
6e	6	2.10 ± 0.131	0.086 ± 0.007	0.04
7a	2	0.375 ± 0.022	2.48 ± 0.220	6.6
7b	3	0.359 ± 0.034	2.89 ± 0.430	8.0
7c	4	0.224 ± 0.019	4.17 ± 0.391	18.6
7d	5	0.280 ± 0.039	2.49 ± 0.139	8.9
7e	6	0.515 ± 0.016	2.83 ± 0.142	5.5
8a	2	0.224 ± 0.017	3.00 ± 0.080	13.4
8b	3	0.123 ± 0.007	2.22 ± 0.213	18.0
8c	4	0.097 ± 0.005	4.89 ± 0.035	50.4
8d	5	0.398 ± 0.024	2.69 ± 0.166	6.8
8e	6	0.520 ± 0.083	4.18 ± 0.147	8.0
Galanthamine		0.623 ± 0.099	15.7 ± 0.787	25.3

[a] 50% inhibitory concentration (means ± SEM of three experiments) of AChE from electric eel.
[b] 50% inhibitory concentration (means ± SEM of three experiments) of BuChE from equine serum.
[c] Selectivity for AChE = IC_{50} (BuChE)/IC_{50} (AChE).

AChE.[20,21] Consequently, the berberine derivatives could be interesting dual AChE/BChE inhibitors for Alzheimer's disease.

2.3. Kinetic study of AChE and BuChE

Determination of the inhibition type is important in understanding the mechanism of inhibition and the inhibitor binding sites. Graphical analysis of the steady-state inhibition data could give information about the binding mode of the selected compounds. Based on the in vitro inhibition experiments, berberine and its derivative **8c**, one of the most potent AChE inhibitors, were used to investigate the mechanisms of inhibition of AChE and BuChE (Fig. 2). The estimates of inhibition constants K_i (Table 2) were estimated from the plots of the slope versus the concentration of berberine and **8c**.

In the Lineweaver–Burk plots of Figure 2A and B, lines crossing the x axis in the same point revealed unchanged K_m and decreased v_{max} with increasing inhibitor concentrations. This is a typical trend for non-competitive inhibition, which was similar to that of propidium.[22] The inhibitory behavior of **8c** on AChE and BuChE, as illustrated in Figure 2C and D, is similar to that of bis-tetrahydroaminoacridine[23] inhibitors of AChE. Therefore, from the kinetic profile, we concluded that compound **8c** causes a mixed type of inhibition and could interact with both CAS and PAS.

Recent studies for non-catalytic functions of AChE suggested that the PAS, besides its role in allosteric regulation of AChE-catalyzed hydrolysis of released acetylcholine, also mediates heterologous protein associations, which contribute to cell recognition and adhesion processes during synaptogenesis, and the nucleation of amyloid peptides during Alzheimer's disease onset. AChEIs interact with both CAS and the PAS and inhibit AChE-induced Aβ fibrillogenesis.[24–26] For example, xanthostigmine derivatives,[27] a class of long-lasting AChE inhibitors, and bis-(−)-nor-meptazinols,[28] decreased Aβ aggregation and may be disease-modifying agents for AD. Because the berberine derivatives have a binding mode similar to xanthostigmine derivatives and bis-(−)-nor-meptazinols, berberine could be a lead compound for AD treatment.

Table 2
AChE and BuChE inhibition constants of some selected derivatives

Compound	Enzyme	$k_i \pm SE$ (μM)	Type of inhibition
Berberine	AChE	0.289 ± 0.075	Non-competitive
8c	AChE	0.045 ± 0.004	Mixed
Berberine	BuChE	9.20 ± 0.791	Non-competitive
8c	BuChE	1.02 ± 0.076	Mixed

Based on the kinetic analysis, we assumed that the rather wide shape of berberine binds to the PAS, and this binding might change the conformation of the enzyme and sterically block the gorge, making it harder for bulky berberine derivatives to enter the active site in the base of a deep, narrow gorge. Therefore, steric factors contribute to AChE inhibitory activity in the berberine hybrids, as the monocyclic benzene berberine hybrids have stronger activity than the tricyclic berberine carbazole hybrids.

2.4. Molecular modeling studies

To explore the interaction mode and the structure–activity relationships of 9-substituted berberine derivatives with AChE, molecular docking simulations were performed with the software AUTODOCK based on the structure of the complex of *T. californica* enzyme (*Tc*AChE) (PDB entry 2CMF). Based on the in vitro inhibition

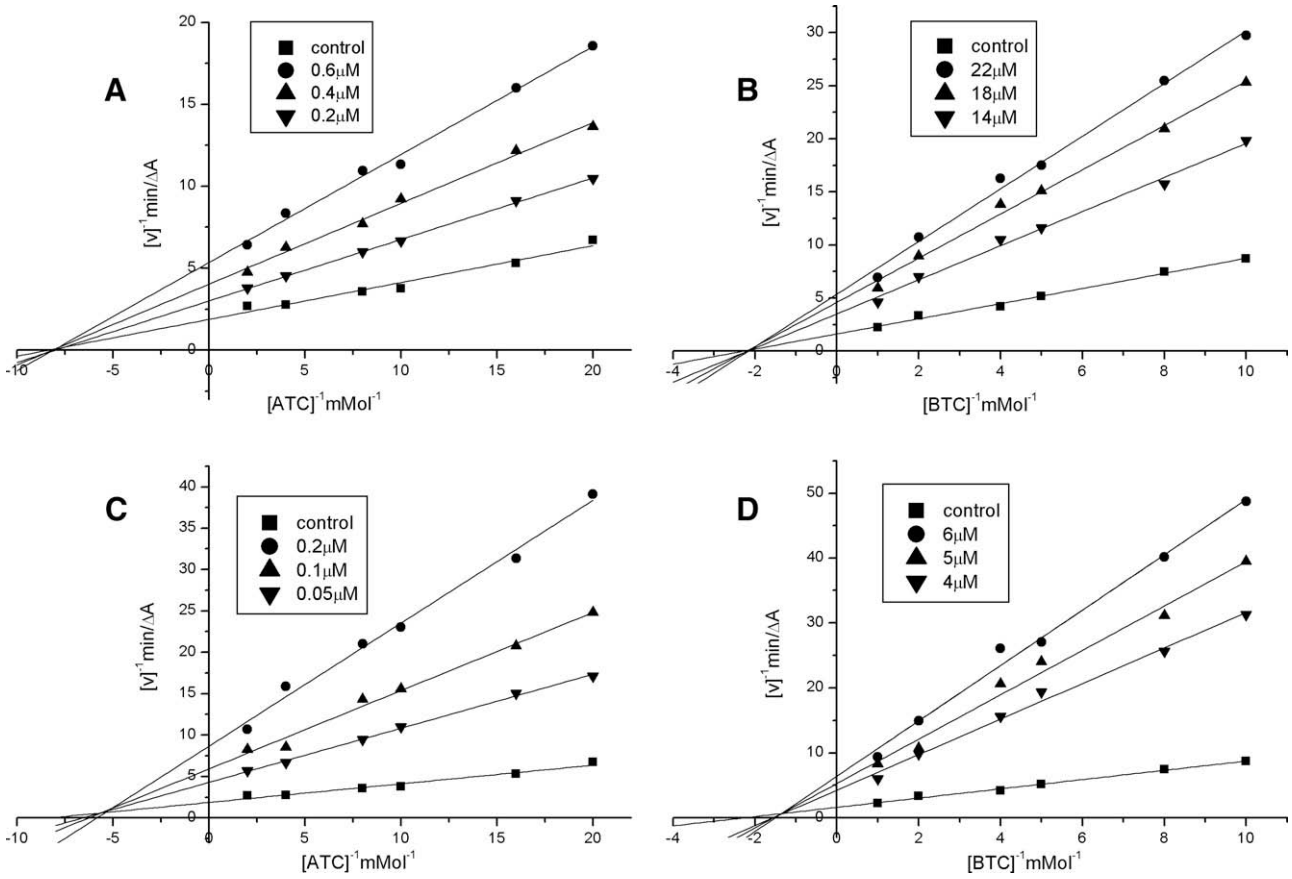


Figure 2. Steady state inhibition by berberine (A), **8c** (C) of AChE hydrolysis of ATCh and berberine (B), **8c** (D) of BuChE hydrolysis of BuTCh; the plots show non-competitive inhibition for berberine on AChE (A) and BuChE (B), and mixed-type inhibition for **8c** on AChE (C) and BuChE (D).

results, we selected compound **8c**, the highest AChE inhibitor, respectively, as ligand example.

As shown in Figure 3, the bivalent phenyl–berberine hybrid **8c** could favorably interact with the central catalytic pocket and the PAS along the active-site gorge of the enzyme with its three major functional groups: the berberine moiety, alkyl chain, and phenyl moiety. The phenyl ring of **8c** displays a classic parallel π–π stacking with the electron-rich indole ring of the Trp86 side chain near the bottom of the gorge. Its coplanar berberine moiety could stack above the PAS residue Trp279. Residue Phe330, which forms the 'bottle neck' of the active site gorge, adopts an 'open' conformation, allowing large groups to reach the base of the gorge. The phenyl moiety between Trp84 and Phe330 form face-to-face π–π stacking interactions in a 'sandwich' form. Indeed, the four-methylene aliphatic spacer (alkyl tether) links them, snaking along the gorge, and is long enough to allow a proper interaction between **8c** and both sites of the AChE. The conformation of the side chain fits the shape of the gorge. Compound **8c**, the highest AChE inhibitor, respectively, as a typical example to understand the ligand–protein interactions.

In addition, the berberine moiety, linked at the rim of the gorge, gives a face-to-edge π–π interaction with Phe278. The long tether could fold with a proper conformation in the gorge that might favorably interact with Tyr70, Asp72, Tyr121, Trp279, Ile287, Phe330, and Tyr334 by hydrophobic interactions. Therefore, the simultaneous interactions of phenyl–berberine hybrid **8c** in the central pocket, gorge, and peripheral pocket of *Tc*AChE produce inhibitory potency.

3. Conclusion

In summary, three series of berberine derivatives were designed, synthesized, and evaluated for their inhibitory activity against AChE. Most of them are potent inhibitors of AChE, with IC_{50} values in the micromolar and sub-micromolar range. The most potent inhibitor, **8c**, berberine linked with phenol by 4-carbon spacers, inhibited AChE with IC_{50} of 0.097 μM. The preliminary structure–activity relationship showed that the potency of AChE inhibition was mainly influenced by the function at the end of the chain, as well as the length of the connecting tether. A kinetic study indicated that the berberine derivatives cause a mixed type of inhibition and could interact with both CAS and PAS, consistent with the results of molecular molding. Thus, berberine and its derivatives bind and inhibit the peripheral anionic site of the AChE and may allow for rational design of novel AChE inhibitors.

4. Experimental section

4.1. Chemistry

The NMR spectra were recorded with TMS as the internal standard on a Varian 400 MHz spectrometer. Coupling constants were given in Hz. MS spectra were recorded on a Agilent LC–MS 6120 instrument with an ESI mass selective detector. Flash column chromatography was performed with silica gel (200–300 mesh) purchased from Qingdao Haiyang Chemical Co. Ltd or alumina from Sinopharm Chemical Reagent Co. Ltd. All the reactions were monitored by thin layer chromatography on silica gel.

Berberine chloride was isolated from Chinese herbal medicine *C. chinensis* Franch and recrystallized from hot water. Compound **2** was prepared according to the reported procedure.[17] Compounds **3a–e**, **4a–e**, **5a–e**, **6a–e**, **7a–e**, and **8a–e** were synthesized followed the reported procedures.

4.2. General procedures for the preparation of 3a–e, 4a–e, 5a–e

To a stirred suspension of selective compounds (**3**–**5**) (10 mmol) and K_2CO_3 (15 mmol) in butanone (50 mL), KI (1 mmol) and dib-

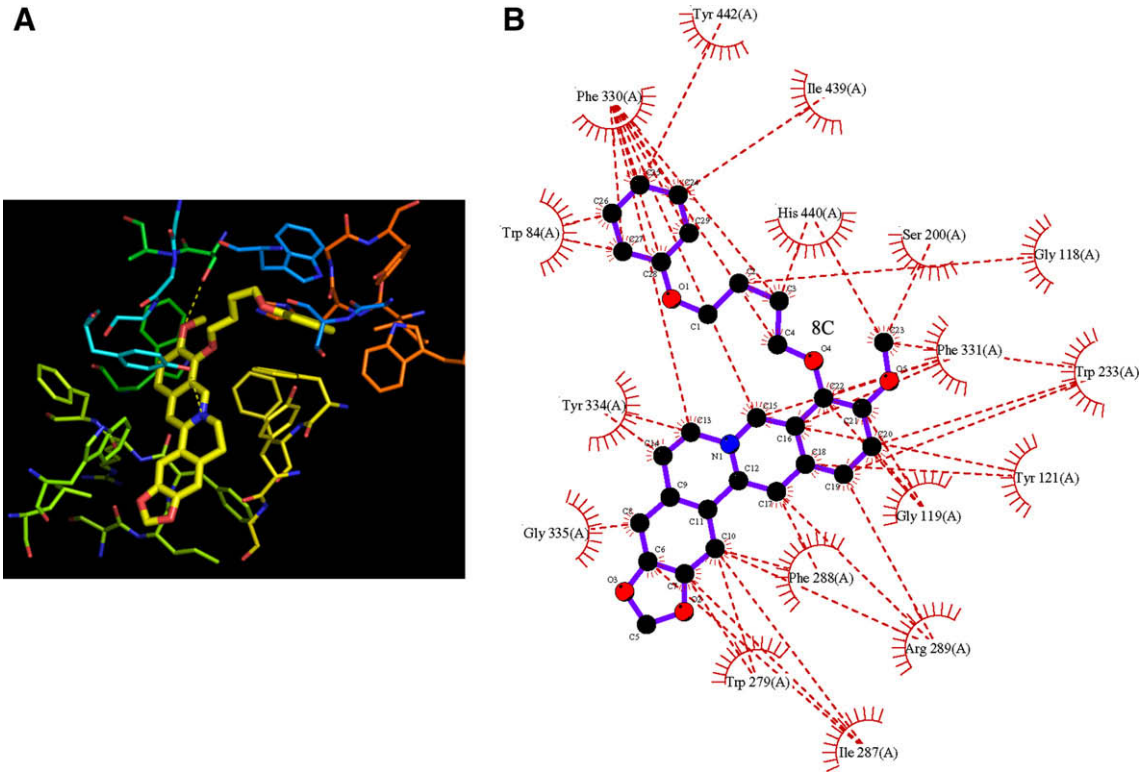


Figure 3. Predicted binding orientation of the **8C** on the binding domain of AChE. (A) Predicted positions of **8C** in the active site of AChE. (B) Schematic representation of the **8C** (purple) interactions with AChE (red). Hydrogen bonds are indicated with dashed lines. Residues involved in hydrophobic interactions with the ligand are shown with red rays.

romoalkanes (12 mmol) were added. The mixture was stirred at room temperature for 1–4 h, filtered, and then evaporated under vacuum. The crude product was chromatographed on a silica gel column, eluted with EtOAc/petroleum ether as eluent to afford the proposed compound.

4.2.1. 4-(2-Bromoethoxy)-9*H*-carbazole (3a)

4-Hydroxycarbazole (**3**) was treated with 1,2-dibromoethane according to general procedure to give the desired product **3a** as white solid, yield 71%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.37 (d, J = 7.6 Hz, 1H), 8.00 (br s, exchangeable with D_2O, 1H), 7.30–7.41 (m, 2H), 7.24–7.27 (m, 2H), 7.02 (d, J = 8.4 Hz, 1H), 6.60 (t, J = 8.0 Hz, 1H), 4.52 (t, J = 6.0 Hz, 2H), 3.82 (t, J = 6.0 Hz, 2H).

4.2.2. 4-(3-Bromopropoxy)-9*H*-carbazole (3b)

4-Hydroxycarbazole (**3**) was treated with 1,3-dibromopropane according to general procedure to give the desired product **3b** as a white solid, yield 76%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.22 (d, J = 7.6 Hz, 1H), 7.92 (br s, exchangeable with D_2O, 1H), 7.32–7.39 (m, 2H), 7.21–7.28 (m, 2H), 6.96 (d, J = 8.4 Hz, 1H), 6.64 (t, J = 8.0 Hz, 1H), 4.33 (t, J = 6.4 Hz, 2H), 3.72 (t, J = 6.4 Hz, 2H), 2.44–2.50 (m, 2H).

4.2.3. 4-(4-Bromobutoxy)-9*H*-carbazole (3c)

4-Hydroxycarbazole (**3**) was treated with 1,4-dibromobutane according to general procedure to give the desired product **3c** as a white solid, yield 70%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.27 (d, J = 7.6 Hz, 1H), 7.92 (br s, exchangeable with D_2O, 1H), 7.21–7.37 (m, 4H), 6.96 (d, J = 8.0 Hz, 1H), 6.61 (t, J = 8.0 Hz, 1H), 4.21 (t, J = 6.4 Hz, 2H), 3.52 (t, J = 6.4 Hz, 2H), 2.09–2.20 (m, 4H).

4.2.4. 4-(5-Bromopentyloxy)-9*H*-carbazole (3d)

4-Hydroxycarbazole (**3**) was treated with 1,5-dibromopentane according to general procedure to give the desired product **3d** as a white solid, yield 68%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.30 (d, J = 7.6 Hz, 1H), 7.99 (br s, exchangeable with D_2O, 1H), 7.23–7.38 (m, 4H), 7.00 (d, J = 8.0 Hz, 1H), 6.63 (t, J = 8.0 Hz, 1H), 4.22 (t, J = 6.4 Hz, 2H), 3.46 (t, J = 6.8 Hz, 2H), 1.97–2.04 (m, 4H), 1.54–1.80 (m, 2H).

4.2.5. 4-(6-Bromohexyloxy)-9*H*-carbazole (3e)

4-Hydroxycarbazole (**3**) was treated with 1,6-dibromohexane according to general procedure to give the desired product **3e** as a white solid, yield 67%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.20 (d, J = 7.6 Hz, 1H), 7.78 (br s, exchangeable with D_2O, 1H), 7.12–7.28 (m, 4H), 6.84 (d, J = 8.0 Hz, 1H), 6.53 (t, J = 8.0 Hz, 1H), 4.08 (t, J = 6.4 Hz, 2H), 3.31 (t, J = 6.8 Hz, 2H), 1.77–1.88 (m, 4H), 1.44–1.51 (m, 4H).

4.2.6. 1-(2-Bromoethoxy)-benzotriazole (4a)

1-Hydroxybenzotriazole (**4**) was treated with 1,2-dibromoethane according to general procedure to give the desired product **4a** as a white solid, yield 80%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.01 (d, 1H, J = 8.8 Hz), 7.70 (d, 1H, J = 8.4 Hz), 7.55 (t, 1H, J = 8.0 Hz), 7.42 (t, 1H, J = 7.6 Hz), 4.86 (t, J = 6.4 Hz, 2H), 3.69 (t, J = 6.4 Hz, 2H).

4.2.7. 1-(3-Bromopropoxy)-benzotriazole (4b)

1-Hydroxybenzotriazole (**4**) was treated with 1,3-dibromopropane according to general procedure to give the desired product **4b** as a white solid, yield 78%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.94 (d, J = 8.4 Hz, 1H), 7.54 (d, J = 8.4 Hz, 1H), 7.46 (t, J = 8.0 Hz, 1H), 7.33 (t, J = 8.0 Hz, 1H), 4.64 (t, J = 6.0 Hz, 2H), 3.66 (t, J = 6.4 Hz, 2H), 2.32–2.38 (m, 2H).

4.2.8. 1-(4-Bromobutoxy)-benzotriazole (4c)

1-Hydroxybenzotriazole (**4**) was treated with 1,4-dibromobutane according to general procedure to give the desired product **4c** as a white solid, yield 79%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.01 (d, J = 8.4 Hz, 1H), 7.51–7.59 (m, 2H), 7.40 (t, J = 7.6 Hz, 1H), 4.60 (t, J = 6.0 Hz, 2H), 3.53 (t, J = 6.4 Hz, 2H), 2.16–2.23 (m, 2H), 2.02–2.09 (m, 2H).

4.2.9. 1-(5-Bromopentyloxy)-benzotriazole (4d)

1-Hydroxybenzotriazole (**4**) was treated with 1,5-dibromopentane according to general procedure to give the desired product **4d** as a white solid, yield 77%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.00 (d, J = 8.4 Hz, 1H), 7.58 (d, J = 8.4 Hz, 1H), 7.52 (t, J = 8.4 Hz, 1H), 7.39 (t, J = 8.0 Hz, 1H), 4.57 (t, J = 6.4 Hz, 2H), 3.46 (t, J = 6.4 Hz, 2H), 1.87–2.00 (m, 4H), 1.71–1.78 (m, 2H).

4.2.10. 1-(6-Bromohexyloxy)-benzotriazole (4e)

1-Hydroxybenzotriazole (**4**) was treated with 1,6-dibromohexane according to general procedure to give the desired product **4e** as a white solid, yield 79%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.00 (d, J = 8.4 Hz, 1H), 7.57 (d, J = 8.4 Hz, 1H), 7.52 (t, J = 7.6 Hz, 1H), 7.39 (t, J = 7.6 Hz, 1H), 4.56 (t, J = 6.4 Hz, 2H), 3.44 (t, J = 6.4 Hz, 2H), 1.85–1.95 (m, 4H), 1.51–1.63 (m, 4H).

4.2.11. (2-Bromoethoxy)benzene (5a)

Phenol (**5**) was treated with 1,2-dibromoethane according to general procedure to give the desired product **5a** as oil, yield 87%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.17–7.23 (m, 2H), 6.83–6.90 (m, 3H), 4.39 (t, J = 6.0 Hz, 2H), 3.81 (t, J = 6.4 Hz, 2H).

4.2.12. (3-Bromopropoxy)benzene (3b)

Phenol (**5**) was treated with 1,3-dibromopropane according to general procedure to give the desired product **5b** as oil, yield 90%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.16–7.22 (m, 2H), 6.81–6.89 (m, 3H), 4.02 (t, J = 6.0 Hz, 2H), 3.52 (t, J = 6.4 Hz, 2H), 2.20–2.26 (m, 2H).

4.2.13. (4-Bromobutoxy)benzene (5c)

Phenol (**5**) was treated with 1,4-dibromobutane according to general procedure to give the desired product **5c** as oil, yield 86%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.25–7.30 (m, 2H), 6.87–6.96 (m, 3H), 3.99 (t, J = 6.0 Hz, 2H), 3.48 (t, J = 6.4 Hz, 2H), 2.03–2.10 (m, 2H), 1.90–1.97 (m, 2H).

4.2.14. (5-Bromopentyloxy)benzene (5d)

Phenol (**5**) was treated with 1,5-dibromopentane according to general procedure to give the desired product **5d** as oil, yield 81%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.26–7.31 (m, 2H), 6.89–6.98 (m, 3H), 3.87 (t, J = 6.4 Hz, 2H), 3.40 (t, J = 6.4 Hz, 2H), 1.76–1.90 (m, 4H), 1.58–1.67 (m, 2H).

4.2.15. (6-Bromohexyloxy)benzene (5e)

Phenol (**5**) was treated with 1,6-dibromohexane according to general procedure to give the desired product **5e** as oil, yield 79%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.28–7.34 (m, 2H), 6.90–6.99 (m, 3H), 3.73 (t, J = 6.0 Hz, 2H), 3.38 (t, J = 6.4 Hz, 2H), 1.73–1.82 (m, 4H), 1.52–1.69 (m, 4H).

4.3. General procedures for the preparation of 6a–e, 7a–e, 8a–e

Compounds **3a**–**e**, **4a**–**e**, **5a**–**e** (2.4 mmol) was added to a magnetically stirred suspension of **2** (2 mmol), K_2CO_3 (6 mmol) in CH_3CN (15 mL). The mixture was heated in reflux for 12–24 h, and monitored by TLC. When the mixture was cooled to room temperature, filtered, and then evaporated under vacuum. The crude product was chromatographed on an Al_2O_3 column, eluted with $CHCl_3$/MeOH (100:1–50:1) as eluent to afford the proposed compound.

4.3.1. 9-*O*-[2-(9*H*-Carbazole-4-yloxy)ethyl] berberine bromide (6a)

Berberrubine (**2**) was treated with 4-(2-bromoethoxy)-9*H*-carbazole (**3a**) according to general procedure to give the desired product **6a** as a yellow solid, yield 61%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.20 (br s, exchangeable with D_2O, 1H), 9.64 (s, 1H), 8.71 (s, 1H), 8.24 (d, J = 9.2, 1H), 7.94 (d, J = 9.0, 1H), 7.61 (s, 1H), 7.31–7.33 (m, 3H), 7.18 (d, J = 6.9, 1H), 7.08–7.03 (m, 1H), 6.95 (s, 1H), 6.80–6.71 (m, 2H), 6.16 (s, 2H), 4.88 (t, J = 6.7, 2H), 4.71 (t, J = 6.2, 2H), 4.55 (t, J = 6.5, 2H), 4.13 (s, 3H), 2.68 (t, J = 6.5, 2H); LC/MS (ESI) m/z: $[M-Br]^+$ 531.6. Anal. Calcd for $C_{33}H_{27}BrN_2O_5$: C, 64.82; H, 4.45; N, 4.58. Found: C, 64.77; H, 4.40; N, 4.45.

4.3.2. 9-*O*-[3-(9*H*-Carbazole-4-yloxy)propyl] berberine bromide (6b)

Berberrubine (**2**) was treated with 4-(3-bromopropoxy)-9*H*-carbazole (**3b**) according to general procedure to give the desired product **6b** as a yellow solid, yield 55%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.35 (br s, exchangeable with D_2O, 1H), 9.80 (s, 1H), 8.88 (s, 1H), 8.13 (d, J = 9.2, 1H), 8.07 (d, J = 7.7, 1H), 7.97–7.90 (m, 1H), 7.76 (s, 1H), 7.44 (d, J = 8.1, 1H), 7.31 (t, J = 7.9, 3H), 7.06 (dd, J = 9.1, 4.4, 2H), 6.77 (d, J = 7.9, 1H), 6.17 (s, 2H), 4.78 (t, J = 6.2, 2H), 4.67 (t, J = 6.2, 2H), 4.51 (t, J = 5.9, 2H), 3.92 (s, 3H), 3.11 (t, J = 6.2, 2H), 2.54 (t, J = 6.1, 2H); LC/MS (ESI) m/z: $[M-Br]^+$ 545.6. Anal. Calcd for $C_{34}H_{29}BrN_2O_5$: C, 65.29; H, 4.67; N, 4.48. Found: C, 65.21; H, 4.55; N, 4.51.

4.3.3. 9-*O*-[4-(9*H*-Carbazole-4-yloxy)butyl] berberine bromide (6c)

Berberrubine (**2**) was treated with 4-(2-bromobutoxy)-9*H*-carbazole (**3c**) according to general procedure to give the desired product **6c** as a yellow solid, yield 50%; ^{1}H NMR (400 MHz, MeOD) δ: 9.22 (s, 1H), 8.18 (s, 1H), 7.96 (d, J = 7.8, 1H), 7.67 (s, 1H), 7.57 (d, J = 9.1, 1H), 7.50–7.43 (m, 2H), 7.37 (d, J = 8.1, 1H), 7.29–7.24 (m, 1H), 7.15 (t, J = 8.0, 1H), 6.96 (t, J = 7.4, 1H), 6.82 (t, J = 4.0, 2H), 6.57 (d, J = 7.9, 1H), 6.12 (s, 2H), 4.68 (d, J = 5.9, 2H), 4.41 (dt, J = 10.6, 5.7, 4H), 3.94 (s, 3H), 2.99 (d, J = 6.2, 2H), 2.27–2.19 (m, 4H); LC/MS (ESI) m/z: $[M-Br]^+$ 559.6. Anal. Calcd for $C_{35}H_{31}BrN_2O_5$: C, 65.73; H, 4.89; N, 4.30. Found: C, 65.59; H, 4.81; N, 4.22.

4.3.4. 9-*O*-[5-(9*H*-Carbazole-4-yloxy)butyl] berberine bromide (6d)

Berberrubine (**2**) was treated with 4-(2-bromopentyloxy)-9*H*-carbazole (**3d**) according to general procedure to give the desired product **6d** as a yellow solid, yield 48%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.32 (br s, exchangeable with D_2O, 1H), 9.76 (s, 1H), 8.91 (s, 1H), 8.14 (dd, J = 13.4, 8.5, 2H), 7.96 (d, J = 9.1, 1H), 7.78 (s, 1H), 7.43 (d, J = 8.1, 1H), 7.29 (t, J = 7.9, 2H), 7.11–6.99 (m, 3H), 6.70 (d, J = 7.9, 1H), 6.18 (s, 2H), 4.88 (t, J = 6.0, 2H), 4.36 (t, J = 6.6, 2H), 4.27 (t, J = 6.1, 2H), 4.02 (s, 3H), 3.00 (t, J = 6.1, 2H), 2.10–2.00 (m, 4H), 1.82 (dt, J = 14.7, 7.4, 2H); LC/MS (ESI) m/z: $[M-Br]^+$ 571.7. Anal. Calcd for $C_{36}H_{33}BrN_2O_5$: C, 66.16; H, 5.09; N, 4.29. Found: C, 66.11; H, 5.15; N, 4.13.

4.3.5. 9-*O*-[6-(9*H*-Carbazole-4-yloxy)butyl] berberine bromide (6e)

Berberrubine (**2**) was treated with 4-(2-bromohexyloxy)-9*H*-carbazole (**3e**) according to general procedure to give the desired product **6e** as a yellow solid, yield 49%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.23 (br s, exchangeable with D_2O, 1H), 9.72 (s, 1H), 8.87 (d, J = 7.4, 1H), 8.32 (s, 1H), 8.13 (dd, J = 12.4, 8.3, 1H), 7.78 (d, J = 6.4, 1H), 7.79 (s, 1H),7.43 (d, J = 8.0, 1H), 7.27 (t, J = 7.9, 2H), 7.11–6.99 (m, 3H), 6.75 (d, J = 7.9, 1H), 6.18 (s, 2H), 4.88 (d, J = 6.2, 2H), 4.33 (t, J = 6.7, 2H), 4.23 (t, J = 5.9, 2H), 4.01 (s, 3H), 3.16 (t, J = 6.1, 2H), 1.97–2.05 (m, 4H), 1.68–1.74 (m, 4H); LC/MS (ESI) m/z: $[M-Br]^+$ 571.7. Anal. Calcd for $C_{37}H_{35}BrN_2O_5$: C, 66.57; H, 5.28; N, 4.20. Found: C, 66.49; H, 5.25; N, 4.27.

4.3.6. 9-*O*-[2-(Benzotriazole-1-yloxy)ethyl] berberine bromide (7a)

Berberrubine (**2**) was treated with 1-(2-bromoethoxy)-benzotriazole (**4a**) according to general procedure to give the desired product **7b** as a yellow solid, yield 71%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.03 (s, 1H), 9.03 (s, 1H), 8.29 (d, J = 9.2, 1H), 8.15–8.09 (m, 2H), 7.94 (d, J = 8.3, 1H), 7.88 (s, 1H), 7.73–7.68 (m, 1H), 7.56–7.52 (m, 1H), 7.17 (s, 1H), 6.24 (s, 2H), 5.14 (t, J = 6.4, 2H), 5.02 (t, J = 6.1, 2H), 4.79 (t, J = 6.0, 2H), 4.11.(s, 3H), 3.31 (t, J = 6.1, 2H); LC/MS (ESI) m/z: $[M-Br]^+$ 483.4. Anal. Calcd for $C_{27}H_{23}BrN_4O_5$: C, 57.56; H, 4.11; N, 9.94. Found: C, 57.39; H, 4.10; N, 9.88.

4.3.7. 9-*O*-[3-(Benzotriazole-1-yloxy)propyl] berberine bromide (7b)

Berberrubine (**2**) was treated with 1-(3-bromopropoxy)-benzotriazole (**4b**) according to general procedure to give the desired product **7b** as a yellow solid, yield 66%;^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.83 (s, 1H), 8.94 (s, 1H), 8.22 (d, J = 9.2, 1H), 8.09 (d, J = 8.4, 1H), 8.01 (d, J = 9.1, 1H), 7.87 (d, J = 8.4, 1H), 7.81 (s, 1H), 7.64 (t, J = 7.4, 1H), 7.51–7.45 (m, 1H), 7.10 (s, 1H), 6.18 (s, 2H), 4.91 (dd, J = 14.5, 6.3, 4H), 4.56 (t, J = 6.2, 2H), 4.07 (s, 3H), 3.21 (t, J = 6.4, 2H), 2.43 (m, 2H); LC/MS (ESI) m/z: $[M-Br]^+$ 497.4. Anal. Calcd for $C_{28}H_{25}BrN_4O_5$: C, 58.24; H, 4.36; Br, 13.84; N, 9.70. Found: C, 58.20; H, 4.25; Br, 13.84; N, 9.51.

4.3.8. 9-*O*-[4-(Benzotriazole-1-yloxy)butyl] berberine bromide (7c)

Berberrubine (**2**) was treated with 1-(4-bromobutoxy)-benzotriazole (**4c**) according to general procedure to give the desired product **7c** as a yellow solid, yield 61%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.79 (s, 1H), 8.94 (s, 1H), 8.21 (d, J = 9.2, 1H), 8.08 (d, J = 8.5, 1H), 8.00 (d, J = 9.1, 1H), 7.86 (d, J = 8.4, 1H), 7.81 (s, 1H), 7.68–7.62 (m, 1H), 7.52–7.46 (m, 1H), 7.10 (s, 1H), 6.18 (s, 2H), 4.95 (t, J = 6.1, 2H), 4.70 (t, J = 6.3, 2H), 4.38 (t, J = 6.2, 2H), 4.04 (s, 3H), 3.22 (t, J = 6.1, 2H), 2.20–2.11 (m, 2H), 2.11–2.02 (m, 2H); LC/MS (ESI) m/z: $[M-Br]^+$ 511.5. Anal. Calcd for $C_{29}H_{27}BrN_4O_5$: C, 58.89; H, 4.60; N, 9.47. Found: C, 58.77; H, 4.55; N, 9.50.

4.3.9. 9-*O*-[5-(Benzotriazole-1-yloxy)pentyl] berberine bromide (7d)

Berberrubine (**2**) was treated with 1-(5-bromopentyloxy)-benzotriazole (**4d**) according to general procedure to give the desired product **7d** as a yellow solid, yield 60%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.77 (s, 1H), 8.93 (s, 1H), 8.20 (d, J = 9.2, 1H), 8.07 (d, J = 8.4, 1H), 8.00 (d, J = 9.1, 1H), 7.84 (d, J = 8.3, 1H), 7.79 (s, 1H), 7.63 (t, J = 7.6, 1H), 7.51–7.44 (m, 1H), 7.09 (s, 1H), 6.18 (s, 2H), 4.94 (t, J = 6.2, 2H), 4.63 (t, J = 6.4, 2H), 4.34 (t, J = 6.5, 2H), 4.05 (s, 3H), 3.18 (t, J = 6.4, 2H), 1.97 (dd, J = 14.2, 6.8, 2H), 1.90 (dd, J = 14.4, 6.7, 2H), 1.80–1.72 (m, 2H); LC/MS (ESI) m/z: $[M-Br]^+$ 525.5. Anal. Calcd for $C_{30}H_{29}BrN_4O_5$: C, 59.51; H, 4.83; N, 9.25. Found: C, 59.44; H, 4.91; N, 9.43.

4.3.10. 9-*O*-[6-(Benzotriazole-1-yloxy)hexyl] berberine bromide (7e)

Berberrubine (**2**) was treated with 1-(6-bromohexyloxy)-benzotriazole (**4e**) according to general procedure to give the desired product **7e** as a yellow solid, yield 57%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.76 (s, 1H), 8.93 (s, 1H), 8.20 (d, J = 9.2, 1H), 8.07 (d, J = 8.5, 1H), 7.99 (d, J = 9.1, 1H), 7.83 (d, J = 8.3, 1H), 7.80 (s, 1H), 7.63 (t, J = 7.6, 1H), 7.51–7.45 (m, 1H), 7.10 (s, 1H), 6.18 (s, 2H), 4.94 (t, J = 6.2, 2H), 4.59 (t, J = 6.4, 2H), 4.32 (t, J = 6.7, 2H), 4.05 (s, 3H), 3.20 (t, J = 6.2, 2H), 1.97–1.89 (m, 2H), 1.88–1.80 (m,

2H), 1.67–1.56 (m, 4H); LC/MS (ESI) *m*/*z*: $[M-Br]^+$ 539.7. Anal. Calcd for $C_{31}H_{31}BrN_4O_5$: C, 60.10; H, 5.04; N, 9.04. Found: C, 60.15; H, 5.08; N, 9.11.

4.3.11. 9-*O*-[2-(Phenylol-1-yloxy)ethyl] berberine bromide (8a)

Berberrubine (**2**) was treated with (2-bromoethoxy)benzene (**5a**) according to general procedure to give the desired product **8a** as a yellow solid, yield 71%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.76 (s, 1H), 8.93 (s, 1H), 8.20 (d, J = 9.2 Hz, 1H), 8.02 (d, J = 9.2 Hz, 1H), 7.79 (s, 1H), 7.28 (t, J = 8.0 Hz, 2H), 7.07 (s, 1H), 6.93 (t, J = 7.6 Hz, 1H), 6.86 (d, J = 7.6 Hz, 2H), 6.17 (s, 2H), 4.78 (t, J = 6.0 Hz, 2H), 4.67 (t, J = 6.1 Hz, 2H), 4.43 (t, J = 6.5 Hz, 2H), 4.06 (s, 3H), 3.12 (t, J = 6.4 Hz, 2H); LC/MS (ESI) *m*/*z*: $[M-Br]^+$ 442.4. Anal. Calcd for $C_{27}H_{24}BrNO_5$: C, 62.08; H, 4.63; N, 2.68. Found: C, 62.01; H, 4.60; N, 2.78.

4.3.12. 9-*O*-[3-(Phenylol-1-yloxy)propyl] berberine bromide (8b)

Berberrubine (**2**) was treated with (3-bromopropoxy)benzene (**5b**) according to general procedure to give the desired product **8b** as a yellow solid, yield 75%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.78 (s, 1H), 8.94 (s, 1H), 8.18 (d, J = 9.2 Hz, 1H), 7.98 (d, J = 9.2 Hz, 1H), 7.80 (s, 1H), 7.31 (t, J = 8.0 Hz, 2H), 7.09 (s, 1H), 6.93–6.99 (m, 3H), 6.18 (s, 2H), 4.87 (t, J = 6.0 Hz, 2H), 4.48 (t, J = 6.0 Hz, 2H), 4.26 (t, J = 6.4 Hz, 2H), 4.00 (s, 3H), 3.19 (t, J = 6.0 Hz, 2H), 2.34 (t, J = 6.4 Hz, 2H); LC/MS (ESI) *m*/*z*: $[M-Br]^+$ 456.5. Anal. Calcd for $C_{28}H_{26}BrNO_5$: C, 62.69; H, 4.89; N, 2.61. Found: C, 62.60; H, 4.77; N, 2.78.

4.3.13. 9-*O*-[4-(Phenylol-1-yloxy)butyl] berberine bromide (8c)

Berberrubine (**2**) was treated with (4-bromobutoxy)benzene (**5c**) according to general procedure to give the desired product **8c** as a yellow solid, yield 76%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.81 (s, 1H), 8.97 (s, 1H), 8.23 (d, J = 9.2 Hz, 1H), 8.04 (d, J = 9.2 Hz, 1H), 7.84 (s, 1H), 7.34 (t, J = 7.6 Hz, 2H), 7.14 (s, 1H), 6.95–7.00 (m, 3H), 6.22 (s, 2H), 4.99 (t, J = 6.0 Hz, 2H), 4.42 (t, J = 6.0 Hz, 2H), 4.13 (t, J = 5.2 Hz, 2H), 4.09 (s, 3H), 3.25 (t, J = 6.0 Hz, 2H), 2.02–2.10 (m, 4H); LC/MS (ESI) *m*/*z*: $[M-Br]^+$ 470.5. Anal. Calcd for $C_{29}H_{28}BrNO_5$: C, 63.28; H, 5.13; N, 2.54. Found: C, 63.33; H, 5.14; N, 2.49.

4.3.14. 9-*O*-[5-(Phenylol-1-yloxy)pentyl] berberine bromide (8d)

Berberrubine (**2**) was treated with (5-bromopentyloxy)benzene (**5d**) according to general procedure to give the desired product **8d** as a yellow solid, yield 66%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.75 (s, 1H), 8.92 (s, 1H), 8.19 (d, J = 9.1, 1H), 8.00 (d, J = 9.2, 1H), 7.79 (s, 1H), 7.33–7.23 (m, 2H), 7.09 (s, 1H), 6.95–6.89 (m, 3H), 6.17 (s, 2H), 4.95 (t, J = 6.0, 2H), 4.31 (t, J = 6.0, 2H), 4.05 (s, 3H), 4.02 (t, J = 6.4, 2H), 3.21 (t, J = 6.4, 2H), 2.01–1.79 (m, 4H), 1.64 (tdd, J = 24.2, 11.8, 6.8, 2H); LC/MS (ESI) *m*/*z*: $[M-Br]^+$ 484.5. Anal. Calcd for $C_{30}H_{30}BrNO_5$: C, 63.83; H, 5.36; N, 2.48. Found: C, 63.81; H, 5.30; N, 2.56.

4.3.15. 9-*O*-[2-(Phenylol-1-yloxy)hexyl] berberine bromide (8e)

Berberrubine (**2**) was treated with (6-bromohexyloxy)benzene (**5e**) according to general procedure to give the desired product **8e** as a yellow solid, yield 61%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.74 (s, 1H), 8.92 (s, 1H), 8.19 (d, J = 9.1, 1H), 8.00 (d, J = 9.2, 1H), 7.79 (s, 1H), 7.32–7.24 (m, 2H), 7.09 (s, 1H), 6.91 (dd, J = 7.5, 4.6, 3H), 6.17 (s, 2H), 5.95 (t, J = 6.4, 2H), 4.30 (dd, J = 11.9, 6.6, 2H), 4.05 (s, 3H), 3.98 (dd, J = 13.0, 6.6, 2H), 3.21 (dd, J = 10.8, 5.4, 2H), 1.95–1.72 (m, 4H), 1.54 (dd, J = 8.6, 5.6, 4H). LC/MS (ESI) *m*/*z*: $[M-Br]^+$ 498.6. Anal. Calcd for $C_{31}H_{32}BrNO_5$: C, 64.36; H, 5.58; N, 2.42. Found: C, 64.32; H, 5.51; N, 2.53.

4.4. Biological activity

4.4.1. In vitro inhibition studies on AChE and BuChE

Acetylcholinesterase (AChE, E.C. 3.1.1.7, from electric eel), butylcholinesterase (BuChE, E.C. 3.1.1.8, from equine serum), 5,5′-dithiobis-(2-nitrobenzoic acid) (Ellman's reagent, DTNB), acetylthiocholine chloride (ATC), butylthiocholine chloride (BTC), and tarcine hydrochloride were purchased from Sigma–Aldrich. Berberine derivatives were dissolved in DMSO and diluted in 0.1 M KH_2PO_4/K_2HPO_4 buffer (pH 8.0) to provide a final concentration range. DMSO was diluted to a concentration in excess of 1 in 10,000, and no inhibitory action on either AChE or BuChE was detected in separate prior experiments.

4.4.2. In vitro AChE assay

All the assays were carried out under 0.1 M KH_2PO_4/K_2HPO_4 buffer, pH 8.0, using a Shimadzu UV-2450 Spectrophotometer. Enzyme solutions were prepared to give 2.0 units/mL in 2 mL aliquots. The assay medium (1 mL) consisted of phosphate buffer (pH 8.0), 50 μL of 0.01 M DTNB, 10 μL of enzyme, and 50 μL of 0.01 M substrate (ACh chloride solution). Test compounds were added to the assay solution and preincubated at 37 °C with the enzyme for 15 min followed by the addition of substrate. The activity was determined by measuring the increase in absorbance at 412 nm at 1 min intervals at 37 °C. Calculations were performed according to the method of the equation in Ellman et al.[18] Each concentration was assayed in triplicate.

In vitro BuChE assay was similar with the method described above.

4.5. Kinetic characterization of ChE inhibition

Kinetic characterization of AChE and BuChE was performed using a reported method.[29] Test compound was added into the assay solution and pre-incubated with the enzyme at 37 °C for 15 min, followed by the addition of substrate. Kinetic characterization of the hydrolysis of ATC catalyzed by AChE was done spectrometrically at 412 nm. A parallel control with no inhibitor in the mixture, allowed adjusting activities to be measured at various times. The plots were assessed by a weighted least square analysis that assumed the variance of V to be a constant percentage of V for the entire data set. Slopes of these reciprocal plots were then plotted against the concentration of the inhibitors in a weighted analysis and K_i was determined as the ratio of the replot intercept to the replot slope.

Kinetic characterization of BuChE assay use the similar method described above.

4.6. Molecular modeling

The initial model of AChE for docking studies was built based on the X-ray crystal structure of the bistacrine–AChE complex which was obtained from the Protein Data Bank (PDB entry 2CMF). The original ligand was removed while water molecules present in the PDB file were maintained in their position. 3D structures of the 9-substituted berberine derivatives were generated and optimized by DISCOVERY STUDIO 1.7 package (Accelrys Inc., San Diego, CA). AUTODOCK 3.0.5 package[30] was used to perform docking simulations, which adopts the hybrid Lamarckian Genetic Algorithm as searching algorithm and allows full flexibility of the ligand. Solvation parameters and Kollman charges for all atoms in AChE were assigned by using AUTODOCK Tools. The grid for energy evaluation was centered at residue Trp84 in AChE with grid points in the *x*-, *y*-, *z*-axes set to 50 × 50 × 50 and separated by 0.375 Å. The initial population size and maximum number of energy evaluations were set to 100 and 1.0 × 107 respectively. The docked results within an

RMSD of 1.5 Å were clustered, and the final results of each ligand were selected considering both the embedded empirical binding free energy evaluation and the clustering analysis.

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References and notes

1. Scarpini, E.; Scheltens, P.; Feldman, H. *Lancet Neurol.* **2003**, *2*, 539.
2. Talesa, V. N. *Mech. Ageing Dev.* **2001**, *122*, 1961.
3. Harel, M.; Schalk, I.; Ehret-Sabatier, L.; Bouet, F.; Goeldner, M.; Hirth, C.; Axelsen, P. H.; Silman, I.; Sussman, J. L. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 9031.
4. Bolognesi, M. L.; Andrisano, V.; Bartolini, M.; Cavalli, A.; Minarini, A.; Recanatini, M.; Rosini, M.; Tumiatti, V.; Melchiorre, C. *Farmaco* **2005**, *60*, 6.
5. Edwin, H. R.; Boris, B.; Harry, M. G.; Dawn, M. W.; David, S.; Larry, D. W.; Paul, R. C.; Yuan-Ping, P.; Israel, S.; Joel, L. S. *J. Med. Chem.* **2006**, *49*, 5491.
6. Aviv, P.; Qiong, X.; Harry, M. G.; Wei, F.; Yun, T.; Yun, T.; Israel, S.; Zhuibai, Q.; Joel, L. S. *J. Med. Chem.* **2009**, *52*, 2543.
7. Pang, Y. P.; Quiram, P.; Jelacic, T.; Hong, F.; Brimijoin, S. *J. Biol. Chem.* **1996**, *271*, 23646.
8. Munoz-Torrero, D.; Camps, P. *Curr. Med. Chem.* **2006**, *13*, 399.
9. Pelayo, C.; Rachid, E. A.; Jordi, M.; Diego, M.; Torrero; Albert, B.; Josep, E. B.; Nuria, M. V.; Xavier, B.; Modesto, O.; Francisco, J. L. *J. Med. Chem.* **2000**, *43*, 4657.
10. Rodriguez-Franco, M. I.; Fernandez-Bachiller, M. I.; Perez, C.; Hernandez-Ledesma, B.; Bartolome, B. *J. Med. Chem.* **2006**, *49*, 459.
11. Guillou, C.; Mary, A.; Renko, D. Z.; Gras, E.; Thal, C. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 637.
12. Jin, G.; Luo, X.; He, X.; Jiang, H.; Zhang, H.; Bai, D. *Arzneim.-Forsch.* **2003**, *53*, 753.
13. Zhang, M. F.; Shen, Y. Q. *Zhongguo Yao Li Xue Bao* **1989**, *10*, 174.
14. Zhou, H.; Mineshita, S. *J. Pharmacol. Exp. Ther.* **2000**, *294*, 822.
15. Cho, K. M.; Yoo, I. D.; Kim, W. G. *Biol. Pharm. Bull.* **2006**, *29*, 2317.
16. Li, Y. H.; Peng, Y.; Kong, W. J.; Wang, Y. X.; Hu, C. Q.; Zuo, Z. Y.; Wang, Y. M.; Gao, H.; Gao, L. M.; Feng, Y. C.; Du, N. N.; Liu, Y.; Song, D. Q.; Jiang, J. D. *J. Med. Chem.* **2009**, *52*, 492.
17. Iwasa, K.; Kamigauchi, M.; Ueki, M.; Taniguchi, M. *Eur. J. Med. Chem.* **1996**, *31*, 469.
18. Ellman, G. L.; Courtney, K. D.; Andres, B., Jr.; Featherstone, R. M. *Biochem. Pharmacol.* **1961**, *7*, 88.
19. Aldo, A.; Andrea, C.; Massimiliano, G.; Massimo, G.; Alberto, L.; Alessandra, L.; Rita, M.; Mirella, R.; Maurizio, R.; Aldo, R. *J. Med. Chem.* **2001**, *44*, 4011.
20. Mesulam, M. M.; Guillozet, A.; Shaw, P.; Levey, A.; Duysen, E. G.; Lockridge, O. *Neuroscience* **2002**, *110*, 627.
21. Lane, R. M.; Potkin, S. G.; Enz, A. *Int. J. Neuropsychopharmacol.* **2006**, *9*, 101.
22. Bolognesi, M. L.; Andrisano, V.; Bartolini, M.; Cavalli, A.; Minarini, A.; Recanatini, M.; Rosini, M.; Tumiatti, V.; Melchiorre, C. *Farmaco* **2005**, *60*, 465.
23. Carlier, P. R.; Chow, E. S. H.; Han, Y. F.; Liu, J.; El, Y. J.; Pang, Y. P. *J. Med. Chem.* **1999**, *42*, 4225.
24. Inestrosa, N. C.; Alvarez, A.; Perez, C. A.; Moreno, R. D.; Vicente, M.; Linker, C.; Casanueva, O. I.; Soto, C.; Garrido, J. *Neuron* **1996**, *16*, 881.
25. Johnson, G.; Moore, S. W. *Biochem. Biophys. Res. Commun.* **1999**, *258*, 758.
26. Soreq, H.; Seidman, S. *Nat. Rev. Neurosci.* **2001**, *2*, 294.
27. Federica, B.; Angela, R.; Lorna, P.; Alessandra, B.; Silvia, G.; Manuela, B.; Vincenza, A.; Andrea, C.; Maurizio, R.; Piero, V. *J. Med. Chem.* **2005**, *48*, 4444.
28. Xie, Q.; Wang, H.; Xia, Z.; Lu, M.; Zhang, W.; Wang, X.; Fu, W.; Tang, Y.; Sheng, W.; Li, W.; Zhou, W.; Zhu, X.; Qiu, Z.; Chen, H. *J. Med. Chem.* **2008**, *51*, 2027.
29. Maria, L. B.; Vincenza, A.; Manuela, B.; Rita, B.; Carlo, M. *J. Med. Chem.* **2005**, *48*, 24.
30. Morris, G. M.; Goodsell, D. S.; Halliday, R. S.; Huey, R.; Hart, W. E.; Belew, R. K.; Olson, A. J. *J. Comput. Chem.* **1998**, *19*, 1639.