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Design, synthesis and pharmacological evaluation of chalcone derivatives as acetylcholinesterase inhibitors

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ABSTRACT

A novel series of chalcone derivatives (**4a**–**8d**) were designed, synthesized, and evaluated for the inhibition activity against acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). The log*P* values of the compounds were shown to range from 1.49 to 2.19, which suggested that they were possible to pass blood brain barriers in vivo. The most promising compound **4a** (IC_{50}: 4.68 μmol/L) was 2-fold more potent than Rivastigmine against AChE (IC_{50}: 10.54 μmol/L) and showed a high selectivity for AChE over BuChE (ratio: 4.35). Enzyme kinetic study suggested that the inhibition mechanism of compound 4a was a mixed-type inhibition. Meanwhile, the result of molecular docking showed its potent inhibition of AChE and high selectivity for AChE over BuChE.

1. Introduction

Alzheimer's disease (AD) is a progressive, chronic, neurodegenerative disorder, characterized by declining in memory and cognitive abilities. It is estimated that about 6% of the population worldwide aged over 65 currently suffer from AD.[1,2] The etiology of AD is still not full known, but many investigations have suggested that reduced level of the neurotransmitter acetylcholine (Ach), formation of β-amyloid peptide (Aβ) plaques, increased oxidative stress, inflammation and Tau-protein aggregation are thought to play significant roles in the process of this disease.[3–5]

Current treatment of AD mainly focuses on the inhibition of AChE activity aimed at rectifying the deficiency of cerebral acetylcholine.[6] Based on the cholinergic hypothesis, the deterioration of memory and cognition in AD patients is mainly caused by the extensive decline of ACh, which is released into presynaptic neuron to transport nervous signal and rapidly hydrolyzed by AChE.[7] Moreover, recent studies have identified that AChE could also play a key role in accelerating the assembly of β-amyloid into amyloid fibrils.[8] Up to now, several AChE inhibitors (such as donepezil, Rivastigmine, galantamine and tacrine) have been approved by European and US regulatory authorities for the clinical treatment of AD in the early to moderate stages.[9] However, these AChE inhibitors are known to have side effects or demerits such as hepatotoxicity, short half life, and gastrointestinal tract excitement.[10] Therefore, the investigation on searching for new and better AChE inhibitors is still of great interest.

Recently, AChE inhibitors from natural products had attracted significant attention because of their fewer side effects. Huperzine A and galantamine are successful examples of AChE inhibitors from natural products.[11] However, it is not so easy to find the potent compounds which could be potential drugs without any structure modifications for many natural products had weak bioactivities yet. In addition, the total chemical synthesis of some complicated natural products is usual difficult and costly. So, it is highly desirable to synthesize some derivates with natural compounds backbone according to the idea of rational molecular design to gain potential drugs for the therapy of AD. For the research and development of AChE inhibitors, many investigations revealed that nitrogen atom was important to the inhibition of AChE.[12] Several flavonoid and coumarin derivatives with terminal amine groups (Fig. 1) have been successfully designed and synthesized and some of them exhibited potent inhibitory activity against AChE.[13–15] Among them, ensaculin, a coumarin derivative, is under clinical investigation for potential AD management.[16]

Chalcones, natural compounds widely existed in fruits and vegetables, belong to the flavonoid family and consist of two aromatic rings connected by an α,β-unsaturated carbonyl group. Possibly due to the flexible structure, chalcones can bind effectively to many kinds of enzymes or receptors and exhibit diverse biological

Abbreviations: AD, Alzheimer's disease; AChE, acetylcholinesterases; ACh, acetylcholine; BuChE, butyrylcholinesterase; PAS, peripheral anionic sites; Log*P*, octanol/water partition; CNS, central nervous system; BBB, blood brain barrier.

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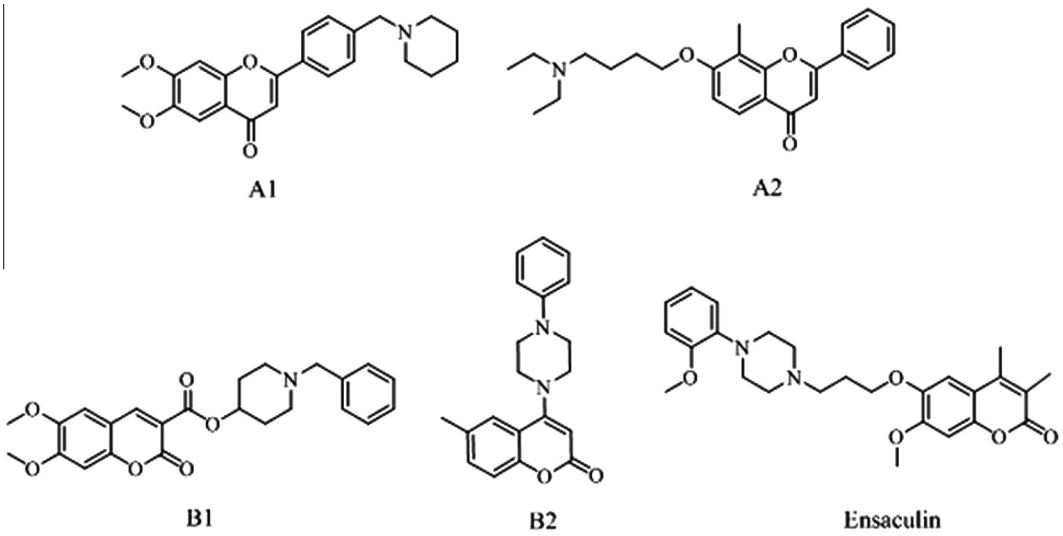


Figure 1. The chemical structure of flavonoid derivatives (A1, A2) and coumarin derivatives (B1, B2, Ensaculin) as the AChE inhibitors.

activities, such as anti-cancer, anti-infective, anti-inflammatory, anti-oxidant and anti-angiogenic effects.[17–20] But few investigations were conducted by pharmaceutical researchers about their biological activities of inhibiting AChE. Thus, based on the design experiences from existing AChE inhibitors, we designed and synthesized a series of chalcones with different basic side chains (n = 2–6) and evaluated their biological activity of inhibiting AchE. Moreover, we explored the binding mode of the compounds to AchE by kinetic experiments and measured the logarithm 1-octanol/water partition coefficient (log$P$ values). The log$P$ value was used to assess the ability of compounds to penetrate blood brain barrier (BBB). Furthermore, molecular docking studies were carried out to study the binding mode and selectivity of these compounds against AChE.

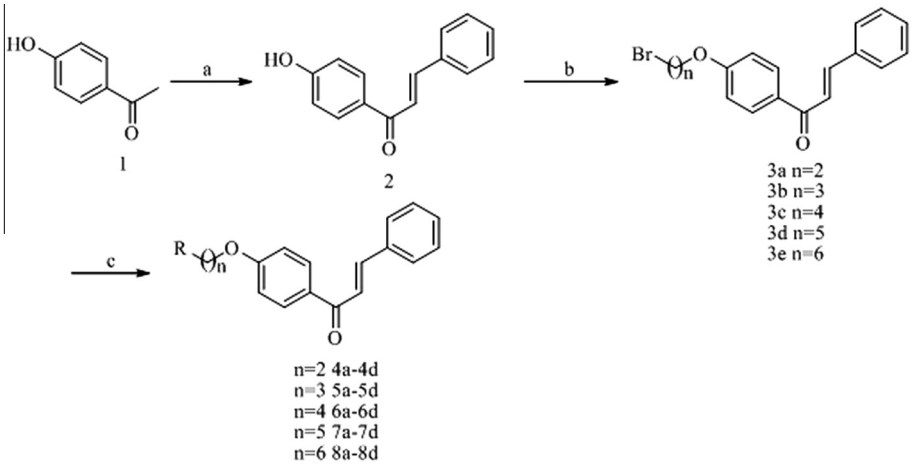
2. Results and discussion

2.1. Chemistry

The synthetic routes to target compounds 4a-8d are outlined in Scheme 1. Reaction of 4-hydroxyacetophenone 1 with benzaldehyde under the catalysis of NaOH/EtOH provided compound **2**.[21] Then, compound **2** was treated with dibromoalkanes bearing two to six carbons and K_2CO_3 in *N*,*N*-dimethylformamide (DMF) at 80 °C to generate compounds **3a**–**3e**. Finally, the target compounds **4a**–**8d** were obtained by refluxing **3a**–**3e** with commercially available secondary amines (dimethylamine, diethylamine, dipropylamine and dibutylamine) in acetone in the presence of K_2CO_3 and NaI. The structures of the designed compounds were characterized by proton nuclear magnetic resonance spectroscopy (^{1}H NMR), infrared spectrum (IR) and mass spectrometry (MS). Besides, the purities of all synthesized compounds were confirmed to be higher than 95% by HPLC.

2.2. Log*P* values

For a drug to treat AD, the ability to penetrate the BBB is vital. Although the factors that affect the penetration of a drug from the systemic circulation into the central nervous system (CNS) were complicated, log$P$ was thought as an important physical chemistry parameter to evaluate or predict the ability to cross BBB, which widely used in medicinal chemistry investigation.[22] Hansch[23] presumed that the log$P$ with optimum CNS penetration was around 2 ± 0.7. Log$P$ values of the synthesized compounds were measured by the classical shake-flask method[24] using RP-HPLC. As shown in Table 1, the log$P$ values of the tested



Scheme 1. Reagents and conditions: (a) benzaldehyde, NaOH, EtOH, rt; (b) Br(CH$_2$)$_n$Br, K_2CO_3, DMF, 80 °C; (c) second amine, K_2CO_3, NaI, acetone, reflux.

compounds ranged from 1.49 to 2.19, which indicated that all of the compounds were possible sufficiently lipophilic to pass BBB in vivo.

2.3. In vitro inhibition of AChE and BuChE

It has been reported that butyrylcholinesterase (BuChE) can non-specifically hydrolyzes Ach and its level is unchanged or even rises in advanced AD.[25,26] Thus, the inhibition of BuChE may also be beneficial to improve AD symptoms.[27]

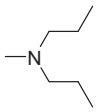
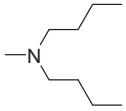
The inhibitory effects of the synthesized compounds on AChE and BuChE were evaluated using *Ellman* method, with Rivastigmine as the positive control. The half maximal inhibitory concentration (IC_{50} values) for AChE and BuChE inhibition and the selectivity for AChE were summarized in Table 1. According to the data, all of the synthesized compounds exhibited higher inhibitory activities against AChE than the precursor compound **2** (IC_{50} >500 μmol/L). Compounds **4a**, **4b**, **6a** and **6c** with IC_{50} values of 4.68, 7.63, 8.95, 5.91 μmol/L, respectively, showed potent activities compared to the reference compound Rivastigmine (IC_{50} = 10.54 μmol/L). Specifically, the most promising compound **4a** was 2-fold more active than Rivastigmine and also showed a high selectivity for AChE over BuChE (ratio: 4.35).

Based on the screening data (Fig. 2), it appeared that variation of the length of the chain linking chalcone backbone with terminal amine groups influenced their AChE and BuChE inhibitory activity significantly. In general, for compounds containing diethylamine or dimethylamine group, the inhibition activity against AChE was decreased as the chain length increased (**4a**, n = 2, inhibitory potency (=1/IC_{50}*100): 21.37; **5a**, n = 3, 9.55; **6a**, n = 4, 11.17; **7a**, n = 5, 7.65; **8a**, n = 6, 7.02; **4b**, n = 2, 13.11; **5b**, n = 3, 4.44; **6b**, n = 4, 4.31; **7b**, n = 5, 2.93; **8b**, n = 6, 1.98). However, a different trend was observed for the compounds linked with dipropylamine (**4c**, **5c**, **6c**, **7c**, **8c**) and dibutylamine (**4d**, **5d**, **6d**, **7d**, **8d**). Clearly, compounds **6c** and **6d**, which combined dipropylamine or dibutylamine group with chalcone backbone by a four methylene spacer, showed better inhibition activity against AChE (**6c**, 16.92; **6d**, 4.56). Moreover, for compounds containing the same terminal group, the one with higher inhibition against AChE showed higher selectivity for AChE in the meantime (**4a**, ratio: 4.35; **4b**, ratio: 4.14; **6c**, ratio: 1.50; **6d**, ratio: 1.91).

2.4. Kinetic studies

Compound **4a** was selected for further kinetic measurement since it is the most active one against AChE. The linear Lineweaver–Burk equation which is a double reciprocal form of the Michaelis–Menten equation was converted to evaluate the inhibition mechanism. The graphical analysis of the steady-state inhibition data of compound **4a** was shown in Figure 3. These results showed that increasing the concentration of compound **4a** resulted in different slopes and intercepts. According to Table 2, higher concentrations of compound **4a** resulted in increases of K_m, while V_{max} decreased, which was conformed to the characteristics of mixed-type inhibition. It was therefore concluded that a mixed-type inhibition could be attributed to the compound **4a**. This behavior showed that this compound can bind both with the catalytic site and the non-catalytic site of the enzyme, with different equilibrium constants. The inhibition constants for the inhibitor binding with the catalytic site, K_i, and with the non-catalytic site,

Table 1
In vitro inhibition of AChE/BuChE and log*P* values

Compound	R	*n*	IC_{50}[a] (μM)		Selectivity for AChE[b]	Log*P*[c]
			AChE	BuChE		
2			.>500	>500		
4a		2	4.68 ± 0.13	20.36 ± 1.84	4.35	1.49
5a		3	10.47 ± 1.22	32.29 ± 2.41	3.08	1.59
6a		4	8.95 ± 0.59	11.75 ± 1.18	1.32	1.64
7a		5	13.07 ± 1.23	17.42 ± 1.38	1.33	1.73
8a		6	14.24 ± 1.35	10.42 ± 0.82	0.73	1.80
4b		2	7.63 ± 0.82	31.59 ± 3.33	4.14	1.61
5b		3	22.51 ± 2.15	20.12 ± 1.81	0.89	1.71
6b		4	23.21 ± 2.47	10.24 ± 0.76	0.44	1.74
7b		5	34.14 ± 2.81	26.33 ± 3.06	0.77	1.84
8b		6	50.44 ± 5.86	18.47 ± 1.53	0.37	1.91
4c		2	64.55 ± 5.39	52.58 ± 4.37	0.81	1.86
5c		3	24.63 ± 3.02	10.13 ± 0.53	0.41	1.91
6c		4	5.91 ± 0.28	8.84 ± 0.55	1.50	1.95
7c		5	29.48 ± 2.31	39.65 ± 6.57	2.09	2.01
8c		6	44.05 ± 4.19	40.74 ± 4.47	0.92	2.07
4d		2	252.96 ± 35.76	279.11 ± 32.52	1.10	2.00
5d		3	151.83 ± 14.53	68.25 ± 6.25	0.45	2.06
6d		4	21.94 ± 2.64	41.91 ± 3.66	1.91	2.09
7d		5	76.87 ± 6.53	96.92 ± 21.49	3.86	2.14
8d		6	162.59 ± 40.87	75.09 ± 7.85	0.46	2.19
Rivastigmine			10.54 ± 0.86	0.26 ± 0.081	0.03	

[a] IC_{50}: 50% inhibitory concentration (means ± SD of three experiments).
[b] Selectivity for AChE is defined as IC_{50} (BuChE)/IC_{50} (AChE).
[c] The partition coefficients of in the octanol/buffer solution at pH 7.4 were determined by the classical shake-flask method.

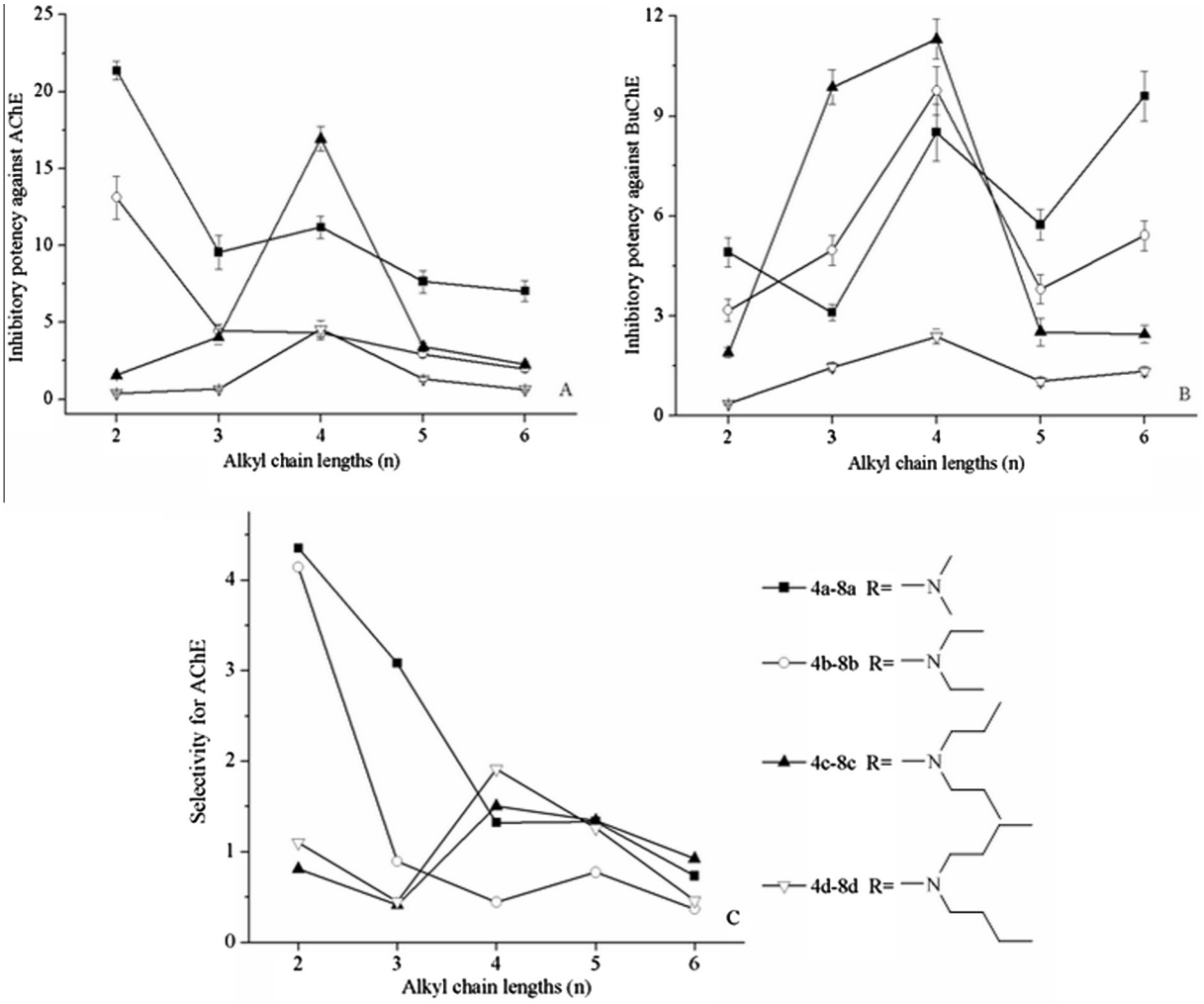


Figure 2. (A) Effects of alkyl chain lengths on anti-AChE activities; (B) Effects of alkyl chain lengths on anti-BuChE activities; (C) Effects of alkyl chain lengths on selectivity for AChE.

Table 2
Kinetic parameters of AChE inhibited by compound **4a**

4a (μM)	Michaelis–Menten equation	K_m (mM)	$v_{max}/\Delta A_{min}^{-1}$	K_i (μM)	K_i' (μM)
0	$1/v = 125.89/[S] + 14.10$	8.92	0.0709	7.99	3.52
1.5	$1/v = 187.08/[S] + 34.42$	5.44	0.029		
3	$1/v = 208.00/[S] + 40.78$	5.10	0.024		
6	$1/v = 241.88/[S] + 51.25$	4.72	0.020		

K_i', are obtained from the replots of the slope and intercept versus concentration of compound **4a**, respectively, which are both linear in Figure 3. The K_i and K_i' value are 7.99 μmol/L and 3.52 μmol/L, respectively, which indicated that the inhibition mechanism of compound **4a** against AChE includes competitive and uncompetitive inhibition.

2.5. Molecular modeling

The X-ray crystallographic structure of AChE showed that it contains two separate ligand binding sites—a catalytic active site (CAS) and a peripheral cationic site (PAS), located at the entrance and the bottom of the active-site gorge, respectively.[28] The simultaneous binding to both the CAS and PAS has been suggested to be important in designing powerful and selective AChE inhibitors.

In order to study the binding mode of our compounds with two cholinesterases, molecular modeling was carried out using the docking program MOE2008 and the results were shown in Figure 4. Compound **4a**, with strong inhibitory activity and high selectivity against AChE, exhibited multiple points binding modes with AChE. The aromatic moiety adopted an appropriate orientation for its binding to PAS, via the π–π stacking interaction with Trp279 and Phe330, and the ring-to-ring distance was 4.02 Å and 4.17 Å. Moreover, the conformation of the side chain could fit well with the shape of the gorge and the positively-charged nitrogen atom of dimethylamine made a cation–π interaction (3.85 Å) with Trp84 in the CAS. In contrast, Compound **4a** could only have a cation–π interaction between Trp82 (4.26 Å) and the nitrogen atom of dimethylamine with BuChE. Meanwhile, the calculated binding free energy of compound **4a** with AChE is lower than that with BuChE (−30.7299 J/(K*mol/l and −25.8991 J/(K*mol/l, respectively). These results may rationalize the potent inhibition of compound **4a** for AChE and the high selectivity for AChE over BuChE.

We also performed the docking study of compound **2** (IC_{50} >500 μmol/L) with AChE. From the docking result, only the π–π stacking interactions between the chalcone backbone and Trp279, Phe330 were observed, and no cation–π interaction between chalcone backbone and the CAS was found. This result supported that a tertiary amino group on the alkyl side chain is the key requirement for the potent AChE inhibition, because it is an important functional group which can made interactions with

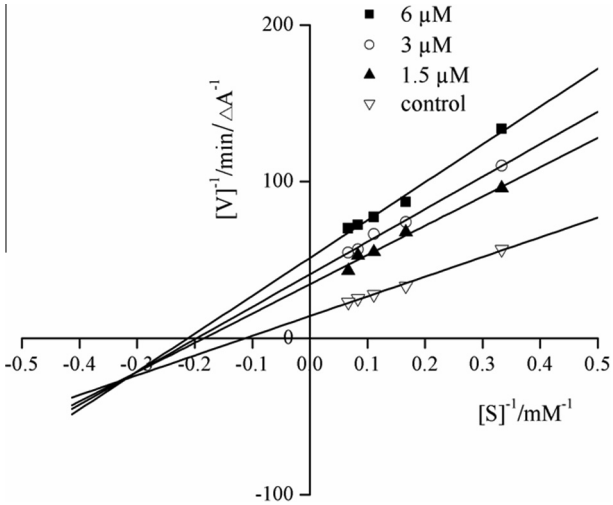


Figure 3. Lineaweaver–Burk plot for the inhibition of AChE by compound **4a**.

the amino acids in the CAS. Additionally, molecular docking study of compound **4d** (IC_{50} = 252.96 μmol/L) with AChE indicated that compound 4d could bind to PAS via the π–π stacking interaction with Trp279. However, the positively-charged nitrogen atom of dibutylamine did not bind to CAS via the cation–π interaction with Trp84, but revealing a cation–π interaction with Phe330. Due to the lack of interaction with CAS, this binding mode caused its lower inhibitory activities against AChE.

3. Conclusion

In summary, a series of chalcone derivatives were designed, synthesized and tested for their biological activity. Some of them exhibited AChE inhibition activity compared with chalcone in the preliminary bioassay. Among them, compound **4a**, which contained a dimethylamine group linked to chalcone backbone by a two-carbon spacer, exhibited the most potent AChE inhibitory activity and high selectivity for AChE over BuChE. The kinetic study on compound **4a** suggested that it conducted its effect as a mixed-type inhibition including competitive and uncompetitive inhibition against AChE. Molecular docking study on compound **4a** indicated that it could bind to both the CAS and PAS of AChE simultaneously. Overall, Compound **4a** might serve as a potential agent for the treatment of AD.

4. Experimental

4.1. Chemistry

All chemicals and reagents were of analytical reagent grade and were used without further purification. ^1H NMR spectra were recorded on an INORA400 MHz instrument in $CDCl_3$ with TMS as the internal reference. Infrared spectrum was obtained by Shimadzu Infinity-1 infrared spectrometer. Mass spectra were obtained on Finnigan LCQ Advantage MAX by electrospray ionization (ESI-MS). The purity of the compounds was checked on Shimadzu LC-20A high performance liquid chromatography.

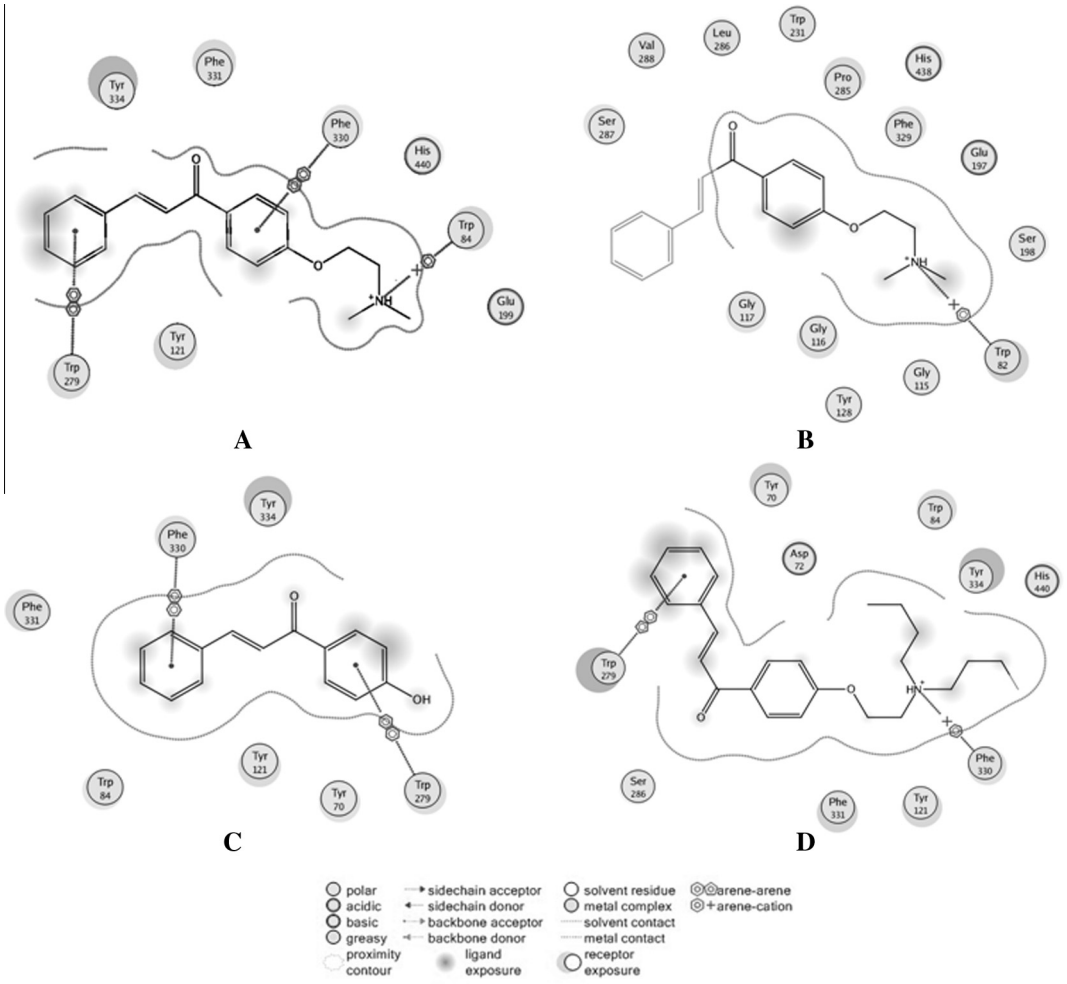


Figure 4. Molecular modeling of compound **4a** with AChE (A) and BuChE; (B) compound **2** with AChE; (C) and compound **4d** with AChE; (D) generated with MOE2008.

4.2. Synthesis of 4′-hydroxychalcone (2)

A mixture of 4-hydroxyacetophenone (1.36 g, 10 mmol), benzaldehyde (1.3 mL, 11 mmol) and sodium hydroxide (1 g, 25 mmol) were dissolved in ethanol/water (4:1 v/v, 20 mL) and stirred at room temperature for a period of 24 h. 10% HCl was added to adjust the solution to pH = 3 and the product precipitated. Filtration of the reaction mixture gave a light yellow solid product with a yield of 85.3%. Mp 173–175 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 6.95 (2H, d, J = 8.8 Hz, 3′-H and 5′-H), 7.41–7.44 (3H, m, 3-H and 4-H and 5-H), 7.55 (1H, d, J = 16.0 Hz, α-H), 7.64–7.66 (2H, m, 2-H and 4-H), 7.82 (1H, d, J = 16.0 Hz, β-H), 8.01 (2H, d, J = 8.0 Hz, 2′-H and 6′-H). MS m/z (ESI): 225 $[M+H]^+$. IR (KBr) ν/cm^{-1}: 3319, 3038, 2968, 1658, 1604, 1576, 1449, 1337, 1222, 1171, 835, 766.

4.3. General procedure for the synthesis of 3a–3e

To a solution of compound **2** (1.12 g, 5 mmol) and K_2CO_3 (2.07 g, 15 mmol) in DMF (15 mL), an appropriate amount of α,ω-dibromoalkane was added. After stirring at 80 °C for 2–10 h until the material 2 disappeared, the solvent was poured into ice water (100 mL), extracted with EtOAc (2 × 70 mL) and washed with saturated aq NaCl solution (2 × 80 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuum. Then the residue was purified by a silica-gel column chromatography to afford product.

4.3.1. (*E*)-1-(4-(2-Bromoethyl)phenyl)-3-phenylprop-2-en-1-one (3a)

It was synthesized from compound **2** (1.12 g, 5 mmol) with K_2CO_3 (2.07 g, 15 mmol) in DMF (15 mL) and 1,2-dibromoethane (2.6 mL, 30 mmol), and purified using silica-gel column chromatography with ethyl acetate/petroleum ether (1:10, v/v) as elution to give a white solid product with a yield with a yield of 45.1%. Mp 89–91 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 3.72 (2H, t, J = 6.0 Hz, BrCH_2), 4.39 (2H, t, J = 6.0 Hz, OCH_2), 7.01 (2H, d, J = 8.8 Hz, 3′-H and 5′-H), 7.41–7.44 (3H, m, 3-H and 4-H and 5-H), 7.55 (1H, d, J = 16.0 Hz, α-H), 7.64–7.67 (2H, m, 2-H and 4-H), 7.82 (1H, d, J = 16.0 Hz, β-H), 8.05 (2H, d, J = 8.8 Hz, 2′-H and 6′-H). MS m/z (ESI): 331 $[M+H]^+$. IR (KBr) ν/cm^{-1}: 3028, 2952, 2846, 1659, 1601, 1576, 1341, 1221, 1182, 833, 764.

4.3.2. (*E*)-1-(4-(3-Bromopropyl)phenyl)-3-phenylprop-2-en-1-one (3b)

It was synthesized from compound **2** (1.12 g, 5 mmol) with K_2CO_3 (2.07 g, 15 mmol) in DMF (15 mL) and 1,3-dibromopropane (3.1 mL, 30 mmol), and purified using silica-gel column chromatography with ethyl acetate/petroleum ether (1:12, v/v) as elution to give a white solid product with a yield of 56.3%. Mp 84–86 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.33–2.56 (2H, m, CH_2CH$_2$Br), 3.63 (2H, t, J = 6.0 Hz, CH$_2$CH_2Br), 4.20 (2H, t, J = 6.0, OCH_2CH$_2$), 6.99 (2H, d, J = 8.8 Hz, 3′-H and 5′-H), 7.41–7.43 (3H, m, 3-H and 4-H and 5-H), 7.56 (1H, d, J = 16.0 Hz, α-H), 7.64–7.67 (2H, m, 2-H and 6-H), 7.81 (1H, d, J = 16.0 Hz, β-H), 8.04 (2H, d, J = 8.8 Hz, 2′-H and 6′-H). MS m/z (ESI): 345 $[M+H]^+$. IR (KBr) ν/cm^{-1}: 3031, 2956, 2843, 2781, 1655, 1603, 1589, 1508, 1344, 1221, 1173, 833, 767.

4.3.3. (*E*)-1-(4-(4-Bromobutyl)phenyl)-3-phenylprop-2-en-1-one (3c)

It was synthesized from compound **2** (1.12 g, 5 mmol) with K_2CO_3 (2.07 g, 15 mmol) in DMF (15 mL) and 1,4-dibromobutane (3.6 mL, 30 mmol), and purified using silica-gel column chromatography with ethyl acetate/petroleum ether (1:14, v/v) as elution to give a white solid product with a yield of 67.7%. Mp 78–80 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.96–2.04 (2H, m, OCH$_2$CH_2), 2.07–2.14 (2H, m, CH_2CH$_2$Br), 3.51 (2H, t, J = 6.0 Hz, CH$_2$CH_2Br), 4.09 (2H, t, J = 6.0, OCH_2CH$_2$), 6.98 (2H, d, J = 8.0 Hz, 3′-H and 5′-H), 7.40–7.43 (3H, m, 3-H and 4-H and 5-H), 7.55 (1H, d, J = 16.0 Hz, α-H), 7.64–7.66 (2H, m, 2-H and 6-H), 7.81 (1H, d, J = 16.0 Hz, β-H), 8.05 (2H, d, J = 8.0 Hz, 2′-H and 6′-H). MS m/z (ESI): 359 $[M+H]^+$. IR (KBr) ν/cm^{-1}: 3029, 2951, 2848, 2786, 1653, 1601, 1586, 1505, 1340, 1219, 1175, 835, 768.

4.3.4. (*E*)-1-(4-(5-Bromopentyloxy)phenyl)-3-phenylprop-2-en-1-one (3d)

It was synthesized from compound **2** (1.12 g, 5 mmol) with K_2CO_3 (2.07 g, 15 mmol) in DMF (15 mL) and 1,5-dibromopentane (4.1 mL, 30 mmol), and purified using silica-gel column chromatography with ethyl acetate/petroleum ether (1:16, v/v) as elution to give a white solid product with a yield of 71.5%. Mp 73–75 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.63–1.70 (2H, m, CH$_2$CH_2CH$_2$), 1.82–1.88 (2H, m, CH_2CH$_2$Br), 1.92–1.99 (2H, m, OCH$_2$CH_2), 3.45 (2H, t, J = 6.0 Hz, CH$_2$CH_2Br), 4.06 (2H, t, J = 6.0, OCH_2CH$_2$), 6.98 (2H, d, J = 8.0 Hz, 3′-H and 5′-H), 7.39–7.44 (3H, m, 3-H and 4-H and 5-H), 7.55 (1H, d, J = 16.0 Hz, α-H), 7.63–7.67 (2H, m, 2-H and 6-H), 7.81 (1H, d, J = 16.0 Hz, β-H), 8.05 (2H, d, J = 8.0 Hz, 2′-H and 6′-H). MS m/z (ESI): 373 $[M+H]^+$. IR (KBr) ν/cm^{-1}: 3030, 2953, 2850, 2784, 1656, 1602, 1588, 1507, 1343, 1221, 1177, 834, 767.

4.3.5. (*E*)-1-(4-(6-Bromohexyloxy)phenyl)-3-phenylprop-2-en-1-one (3e)

It was synthesized from compound **2** (1.12 g, 5 mmol) with K_2CO_3 (2.07 g, 15 mmol) in DMF (15 mL) and 1,6-dibromohexane (4.6 mL, 30 mmol), and purified using silica-gel column chromatography with ethyl acetate/petroleum ether (1:18, v/v) as elution to give a white solid product with a yield of 75.8%. Mp 68–70 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.50–1.58 (4H, m, CH$_2$CH$_2$CH_2CH$_2$ and CH$_2$CH_2CH$_2$CH$_2$), 1.81–1.95 (4H, m, CH_2CH$_2$Br and OCH$_2$CH_2), 3.43 (2H, t, J = 6.0 Hz, CH$_2$CH_2Br), 4.05 (2H, t, J = 6.0, OCH_2CH$_2$), 6.97 (2H, d, J = 8.0 Hz, 3′-H and 5′-H), 7.40–7.44 (3H, m, 3-H and 4-H and 5-H), 7.55 (1H, d, J = 16.0 Hz, α-H), 7.62–7.66 (2H, m, 2-H and 6-H), 7.80 (1H, d, J = 16.0 Hz, β-H), 8.03 (2H, d, J = 8.8 Hz, 2′-H and 6′-H). MS m/z (ESI): 387 $[M+H]^+$. IR (KBr) ν/cm^{-1}: 3031, 2958, 2851, 2782, 1655, 1603, 1588, 1506, 1342, 1220, 1176, 836, 765.

4.4. General procedure for the synthesis of 4a–8d

A mixture of compounds **3a**–**3c** (1 mmol), secondary amines (dimethylamine, diethylamine, dipropylamine and dibutylamine), K_2CO_3 (0.414 g, 3 mmol), NaI (0.008 g, 0.05 mmol), acetone (20 mL) was heated under reflux for 8 h. The solvent was removed and the residue was dissolved with EtOAc (30 mL). Then the organic extracts was washed with saturated aq NaCl solution (2 × 20 mL), dried over anhydrous Na_2SO_4 and evaporated in vacuum, giving a crude product which was chromatographed on silica gel with different ratio of methanol/dichloromethane as elution to afford the compounds **4a**–**8d**.

4.4.1. (*E*)-1-(4-(2-(Dimethylamino)ethoxy)phenyl)-3-phenylprop-2-en-1-one (4a)

According to the general method, the reaction of compound **3a** (0.33 g, 1 mmol) with dimethylamine (0.34 mL, 3 mmol) produced the crude product. Then it was purified using silica-gel column chromatography with methanol/dichloromethane (1:35, v/v) as elution to give a light yellow solid product with a yield of 77.3%. Mp 73–75 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.37 (6H, s, 2 × NCH_3), 2.79 (2H, t, J = 6.0 Hz, NCH_2CH$_2$), 4.14 (2H, t, J = 6.0 Hz, OCH_2CH$_2$), 7.01 (2H, d, J = 8.8 Hz, 3′-H and 5′-H), 7.41–7.44 (3H, m, 3-H and 4-H and 5-H), 7.56 (1H, d, J = 16.0 Hz, α-H), 7.63–7.66

(2H, m, 2-H and 4-H), 7.81 (1H, d, J = 16.0 Hz, β-H), 8.04 (2H, d, J = 8.0 Hz, 2′-H and 6′-H). MS m/z (ESI): 296 $[M+H]^+$. IR (KBr) ν/cm^{-1}: 3031, 2951, 2801, 2769, 1660, 1607, 1588, 1512, 1339, 1219, 1173, 836, 764. Purity: 98.8% by HPLC (MeOH/0.1% triethylamine (TEA) 85:15 (v/v); t_R 3.28 min).

4.4.2. (*E*)-1-(4-(2-(Diethylamino)ethoxy)phenyl)-3-phenylprop-2-en-1-one (4b)

According to the general method, the reaction of compound **3a** (0.33 g, 1 mmol) with diethylamine (0.31 mL, 3 mmol) produced the crude product. Then it was purified using silica-gel column chromatography with methanol/dichloromethane (1:40, v/v) as elution to give a light yellow oil product with a yield of 67.8%. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.12 (6H, t, J = 6.8 Hz, 2 × NCH_2CH_3), 2.68–2.74 (4H, m, 2 × NCH_2CH_3), 2.96 (2H, t, J = 6.0 Hz, NCH_2CH_2), 4.17 (2H, t, J = 6.0 Hz, OCH_2CH_2), 6.99 (2H, d, J = 8.0 Hz, 3′-H and 5′-H), 7.41–7.44 (3H, m, 3-H and 4-H and 5-H), 7.56 (1H, d, J = 16.0 Hz, α-H), 7.64–7.66 (2H, m, 2-H and 4-H), 7.81 (1H, d, J = 16.0 Hz, β-H), 8.04 (2H, d, J = 8.8 Hz, 2′-H and 6′-H). MS m/z (ESI): 324 $[M+H]^+$. IR (KBr) ν/cm^{-1}: 3028, 2956, 2816, 2789, 1656, 1605, 1586, 1509, 1337, 1221, 1175, 833, 768. Purity: 98.6% by HPLC (MeOH/0.1% TEA 85:15 (v/v); t_R 4.63 min).

4.4.3. (*E*)-1-(4-(2-(Dipropylamino)ethoxy)phenyl)-3-phenylprop-2-en-1-one (4c)

According to the general method, the reaction of compound **3a** (0.33 g, 1 mmol) with dipropylamine (0.41 mL, 3 mmol) produced the crude product. Then it was purified using silica-gel column chromatography with methanol/dichloromethane (1:45, v/v) as elution to give a light yellow oil product with a yield of 71.3%. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.91 (6H, t, J = 6.8 Hz, 2 × $NCH_2CH_2CH_3$), 1.48–1.54 (4H, m, 2 × $NCH_2CH_2CH_3$), 2.51 (4H, t, J = 6.0 Hz, 2 × $NCH_2CH_2CH_3$), 2.91 (2H, t, J = 6.0 Hz, NCH_2CH_2), 4.12 (2H, t, J = 6.0 Hz, OCH_2CH_2), 6.99 (2H, d, J = 8.0 Hz, 3′-H and 5′-H), 7.41–7.45 (3H, m, 3-H and 4-H and 5-H), 7.56 (1H, d, J = 16.0 Hz, α-H), 7.64–7.67 (2H, m, 2-H and 4-H), 7.81 (1H, d, J = 16.0 Hz, β-H), 8.04 (2H, d, J = 8.8 Hz, 2′-H and 6′-H). MS m/z (ESI): 352 $[M+H]^+$. IR (KBr) ν/cm^{-1}: 3031, 2959, 2872, 2776, 1657, 1607, 1589, 1508, 1339, 1220, 1175, 832, 770. Purity: 97.8% by HPLC (MeOH/0.1% TEA 85:15 (v/v); t_R 8.81 min).

4.4.4. (*E*)-1-(4-(2-(Dibutylamino)ethoxy)phenyl)-3-phenylprop-2-en-1-one (4d)

According to the general method, the reaction of compound **3a** (0.33 g, 1 mmol) with dibutylamine (0.5 mL, 3 mmol) produced the crude product. Then it was purified using silica-gel column chromatography with methanol/dichloromethane (1:45, v/v) as elution to give a light yellow oil product with a yield of 59.2%. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.93 (6H, t, J = 6.8 Hz, 2 × $NCH_2CH_2CH_2CH_3$), 1.26–1.33 (4H, m, 2 × $NCH_2CH_2CH_2CH_3$), 1.35–1.49 (4H, m, 2 × $NCH_2CH_2CH_2CH_3$), 2.54 (4H, t, J = 6.0 Hz, 2 × $NCH_2CH_2CH_2CH_3$), 2.89 (2H, t, J = 6.0 Hz, NCH_2CH_2), 4.11 (2H, t, J = 6.0 Hz, OCH_2CH_2), 6.98 (2H, d, J = 8.0 Hz, 3′-H and 5′-H), 7.41–7.44 (3H, m, 3-H and 4-H and 5-H), 7.56 (1H, d, J = 16.0 Hz, α-H), 7.64–7.67 (2H, m, 2-H and 4-H), 7.81 (1H, d, J = 16.0 Hz, β-H), 8.04 (2H, d, J = 8.8 Hz, 2′-H and 6′-H). MS m/z (ESI): 380 $[M+H]^+$. IR (KBr) ν/cm^{-1}: 3035, 2955, 2870, 2781, 1655, 1607, 1589, 1508, 1340, 1219, 1176, 831, 769. Purity: 96.9% by HPLC (MeOH/0.1% TEA 85:15 (v/v); t_R 14.63 min).

4.4.5. (*E*)-1-(4-(3-(Dimethylamino)propoxy)phenyl)-3-phenylprop-2-en-1-one (5a)

According to the general method, the reaction of compound **3b** (0.344 g, 1 mmol) with dimethylamine (0.34 mL, 3 mmol) produced the crude product. Then it was purified using silica-gel column chromatography with methanol/dichloromethane (1:40, v/v) as elution to give a light yellow solid product with a yield of 63.3%. Mp 68–70 °C. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.01–2.05 (2H, m, $NCH_2CH_2CH_2$), 2.32 (6H, s, 2 × NCH_3), 2.53 (2H, t, J = 6.0 Hz, NCH_2CH_2), 4.12 (2H, t, J = 6.0 Hz, OCH_2CH_2), 6.99 (2H, d, J = 8.0 Hz, 3′-H and 5′-H), 7.42–7.44 (3H, m, 3-H and 4-H and 5-H), 7.56 (1H, d, J = 16.0 Hz, α-H), 7.64–7.67 (2H, m, 2-H and 4-H), 7.81 (1H, d, J = 16.0 Hz, β-H), 8.04 (2H, d, J = 8.8 Hz, 2′-H and 6′-H). MS m/z (ESI): 310 $[M+H]^+$. IR (KBr) ν/cm^{-1}: 3032, 2951, 2870, 2772, 1655, 1609, 1589, 1503, 1346, 1223, 1175, 831, 773. Purity: 98.3% by HPLC (MeOH/0.1% TEA 85:15 (v/v); t_R 3.96 min).

4.4.6. (*E*)-1-(4-(3-(Diethylamino)propoxy)phenyl)-3-phenylprop-2-en-1-one (5b)

According to the general method, the reaction of compound **3b** (0.344 g, 1 mmol) with diethylamine (0.31 mL, 3 mmol) produced the crude product. Then it was purified using silica-gel column chromatography with methanol/dichloromethane (1:40, v/v) as elution to give a light yellow solid product with a yield of 70.7%. Mp 55–57 °C. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.11 (6H, t, J = 6.8 Hz, 2 × NCH_2CH_3), 2.04–2.06 (2H, m, $NCH_2CH_2CH_2$), 2.63–2.74 (6H, m, 3 × NCH_2CH_2), 4.12 (2H, t, J = 6.0 Hz, OCH_2CH_2), 6.98 (2H, d, J = 8.0 Hz, 3′-H and 5′-H), 7.41–7.44 (3H, m, 3-H and 4-H and 5-H), 7.56 (1H, d, J = 16.0 Hz, α-H), 7.64–7.67 (2H, m, 2-H and 4-H), 7.81 (1H, d, J = 16.0 Hz, β-H), 8.04 (2H, d, J = 8.8 Hz, 2′-H and 6′-H). MS m/z (ESI): 338 $[M+H]^+$. IR (KBr) ν/cm^{-1}: 3033, 2952, 2873, 2769, 1656, 1607, 1587, 1505, 1337, 1221, 1171, 829, 769. Purity: 97.8% by HPLC (MeOH/0.1% TEA 85:15 (v/v); t_R 5.52 min).

4.4.7. (*E*)-1-(4-(3-(Dipropylamino)propoxy)phenyl)-3-phenylprop-2-en-1-one (5c)

According to the general method, the reaction of compound **3b** (0.344 g, 1 mmol) with dipropylamine (0.41 mL, 3 mmol) produced the crude product. Then it was purified using silica-gel column chromatography with methanol/dichloromethane (1:45, v/v) as elution to give a light yellow solid product with a yield of 61.9%. Mp 43–45 °C. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.88 (6H, t, J = 6.8 Hz, 2 × $NCH_2CH_2CH_3$), 1.45–1.51 (4H, m, 2 × $NCH_2CH_2CH_3$), 1.95–1.98 (2H, m, $NCH_2CH_2CH_2$), 2.42 (4H, t, J = 7.2 Hz, 2 × $NCH_2CH_2CH_3$), 2.63 (2H, t, J = 6.0 Hz, NCH_2CH_2), 4.11 (2H, t, J = 6.0 Hz, OCH_2CH_2), 6.98 (2H, d, J = 8.8 Hz, 3′-H and 5′-H), 7.41–7.44 (3H, m, 3-H and 4-H and 5-H), 7.57 (1H, d, J = 16.0 Hz, α-H), 7.64–7.67 (2H, m, 2-H and 4-H), 7.81 (1H, d, J = 16.0 Hz, β-H), 8.04 (2H, d, J = 8.8 Hz, 2′-H and 6′-H). MS m/z (ESI): 366 $[M+H]^+$. IR (KBr) ν/cm^{-1}: 3034, 2956, 2868, 2776, 1655, 1605, 1591, 1507, 1342, 1225, 1173, 831, 770. Purity: 98.1% by HPLC (MeOH/0.1% TEA 85:15 (v/v); t_R 10.64 min).

4.4.8. (*E*)-1-(4-(3-(Dibutylamino)propoxy)phenyl)-3-phenylprop-2-en-1-one (5d)

According to the general method, the reaction of compound **3b** (0.344 g, 1 mmol) with dibutylamine (0.5 mL, 3 mmol) produced the crude product. Then it was purified using silica-gel column chromatography with methanol/dichloromethane (1:45, v/v) as elution to give a light yellow oil product with a yield of 72.5%. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.89 (6H, t, J = 6.8 Hz, 2 × $NCH_2CH_2CH_2CH_3$), 1.25–1.34 (4H, m, 2 × $NCH_2CH_2CH_2CH_3$), 1.39–1.46 (4H, m, 2 × $NCH_2CH_2CH_2CH_3$), 1.94–1.98 (2H, m, $NCH_2CH_2CH_2$), 2.44 (4H, t, J = 6.0 Hz, 2 × $NCH_2CH_2CH_2CH_3$), 2.62 (2H, t, J = 6.0 Hz, NCH_2CH_2), 4.11 (2H, t, J = 6.0 Hz, OCH_2CH_2), 6.98 (2H, d, J = 8.8 Hz, 3′-H and 5′-H), 7.41–7.43 (3H, m, 3-H and 4-H and 5-H), 7.57 (1H, d, J = 16.0 Hz, α-H), 7.64–7.67 (2H, m, 2-H and 4-H), 7.81 (1H, d, J = 16.0 Hz, β-H), 8.04 (2H, d, J = 8.8 Hz, 2′-H and 6′-H). MS m/z (ESI): 394 $[M+H]^+$. IR (KBr) ν/cm^{-1}: 3033, 2955, 2870, 2772, 1661, 1607, 1586, 1508, 1337, 1219, 1169,

831, 766. Purity: 97.9% by HPLC (MeOH/0.1% TEA 85:15 (v/v); t_R 17.89 min).

4.4.9. (*E*)-1-(4-(4-(Dimethylamino)butoxy)phenyl)-3-phenylprop-2-en-1-one (6a)

According to the general method, the reaction of compound **3c** (0.358 g, 1 mmol) with dimethylamine (0.34 mL, 3 mmol) produced the crude product. Then it was purified using silica-gel column chromatography with methanol/dichloromethane (1:40, v/v) as elution to give a light yellow solid product with a yield of 73.5%. Mp 81–83 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.70–1.75 (2H, m, $NCH_2CH_2CH_2$), 1.84–1.88 (2H, m, $OCH_2CH_2CH_2$), 2.29 (6H, s, 2 × NCH_3), 2.40 (2H, t, J = 6.0 Hz, NCH_2CH_2), 4.07 (2H, t, J = 6.0 Hz, OCH_2CH_2), 6.98 (2H, d, J = 8.8 Hz, 3′-H and 5′-H), 7.41–7.44 (3H, m, 3-H and 4-H and 5-H), 7.56 (1H, d, J = 16.0 Hz, α-H), 7.64–7.67 (2H, m, 2-H and 4-H), 7.81 (1H, d, J = 16.0 Hz, β-H), 8.04 (2H, d, J = 8.8 Hz, 2′-H and 6′-H). MS m/z (ESI): 324 $[M+H]^+$. IR (KBr) ν/cm^{-1}: 3031, 2951, 2808, 2756, 1655, 1603, 1588, 1339, 1260, 1175, 835, 768. Purity: 98.5% by HPLC (MeOH/0.1% TEA 85:15 (v/v); t_R 4.52 min).

4.4.10. (*E*)-1-(4-(4-(diethylamino)butoxy)phenyl)-3-phenylprop-2-en-1-one (6b)

According to the general method, the reaction of compound **3c** (0.358 g, 1 mmol) with diethylamine (0.31 mL, 3 mmol) produced the crude product. Then it was purified using silica-gel column chromatography with methanol/dichloromethane (1:45, v/v) as elution to give a light yellow solid product with a yield of 75.8%. Mp 57–59 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.22 (6H, t, J = 6.8 Hz, 2 × NCH_2CH_3), 2.85–2.88 (4H, m, NCH_2CH_2 and OCH_2CH_2), 2.79–2.86 (6H, m, 3 × NCH_2CH_2), 4.09 (2H, t, J = 6.0 Hz, OCH_2CH_2), 6.97 (2H, d, J = 8.8 Hz, 3′-H and 5′-H), 7.41–7.44 (3H, m, 3-H and 4-H and 5-H), 7.56 (1H, d, J = 16.0 Hz, α-H), 7.64–7.67 (2H, m, 2-H and 4-H), 7.82 (1H, d, J = 16.0 Hz, β-H), 8.04 (2H, d, J = 8.8 Hz, 2′-H and 6′-H). MS m/z (ESI): 352 $[M+H]^+$. IR (KBr) ν/cm^{-1}: 3029, 2957, 2872, 2785, 1658, 1605, 1589, 1511, 1341, 1227, 1169, 831, 766. Purity: 98.2% by HPLC (MeOH/0.1% TEA 85:15 (v/v); t_R 6.43 min).

4.4.11. (*E*)-1-(4-(4-(Dipropylamino)butoxy)phenyl)-3-phenylprop-2-en-1-one (6c)

According to the general method, the reaction of compound **3c** (0.358 g, 1 mmol) with dipropylamine (0.41 mL, 3 mmol) produced the crude product. Then it was purified using silica-gel column chromatography with methanol/dichloromethane (1:45, v/v) as elution to give a light yellow oil product with a yield of 67.2%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.89 (6H, t, J = 6.8 Hz, 2 × $NCH_2CH_2CH_3$), 1.46–1.52 (4H, m, 2 × $NCH_2CH_2CH_3$), 1.66–1.68 (2H, m, NCH_2CH_2), 1.82–1.86 (2H, m, OCH_2CH_2), 2.41–2.43 (4H, t, J = 6.0 Hz, 2 × $NCH_2CH_2CH_3$), 2.53 (2H, t, J = 6.0 Hz, NCH_2CH_2), 4.07 (2H, t, J = 6.0 Hz, OCH_2CH_2), 6.98 (2H, d, J = 8.8 Hz, 3′-H and 5′-H), 7.41–7.43 (3H, m, 3-H and 4-H and 5-H), 7.56 (1H, d, J = 16.0 Hz, α-H), 7.64–7.67 (2H, m, 2-H and 4-H), 7.81 (1H, d, J = 16.0 Hz, β-H), 8.04 (2H, d, J = 8.8 Hz, 2′-H and 6′-H). MS m/z (ESI): 380 $[M+H]^+$. IR (KBr) ν/cm^{-1}: 3030, 2953, 2872, 2789, 1656, 1607, 1590, 1508, 1341, 1228, 1169, 829, 768. Purity: 97.6% by HPLC (MeOH/0.1% TEA 85:15 (v/v); t_R 11.91 min).

4.4.12. (*E*)-1-(4-(4-(Dibutylamino)butoxy)phenyl)-3-phenylprop-2-en-1-one (6d)

According to the general method, the reaction of compound **3c** (0.358 g, 1 mmol) with dibutylamine (0.5 mL, 3 mmol) produced the crude product. Then it was purified using silica-gel column chromatography with methanol/dichloromethane (1:50, v/v) as elution to give a light yellow oil product with a yield of 61.9%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.92 (6H, t, J = 6.8 Hz, 2 × $NCH_2CH_2CH_2CH_3$), 1.25–1.31 (4H, m, 2 × $NCH_2CH_2CH_2CH_3$), 1.33–1.45 (4H, m, 2 × $NCH_2CH_2CH_2CH_3$), 1.62–1.65 (2H, m, NCH_2CH_2), 1.81–1.84 (2H, m, OCH_2CH_2), 2.43–2.46 (6H, m, 3 × NCH_2CH_2), 4.07 (2H, t, J = 6.0 Hz, OCH_2CH_2), 6.98 (2H, d, J = 8.8 Hz, 3′-H and 5′-H), 7.41–7.43 (3H, m, 3-H and 4-H and 5-H), 7.57 (1H, d, J = 16.0 Hz, α-H), 7.64–7.67 (2H, m, 2-H and 4-H), 7.81 (1H, d, J = 16.0 Hz, β-H), 8.04 (2H, d, J = 8.8 Hz, 2′-H and 6′-H). MS m/z (ESI): 408 $[M+H]^+$. IR (KBr) ν/cm^{-1}: 3032, 2955, 2870, 2782, 1658, 1607, 1587, 1511, 1339, 1228, 1173, 835, 771. Purity: 97.7% by HPLC (MeOH/0.1% TEA 85:15 (v/v); t_R 19.81 min).

4.4.13. (*E*)-1-(4-(5-(Dimethylamino)pentyloxy)phenyl)-3-phenylprop-2-en-1-one (7a)

According to the general method, the reaction of compound **3d** (0.372 g, 1 mmol) with dimethylamine (0.34 mL, 3 mmol) produced the crude product. Then it was purified using silica-gel column chromatography with methanol/dichloromethane (1:45, v/v) as elution to give a light yellow solid product with a yield of 74.2%. Mp 186–188 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.60–1.66 (2H, m, $CH_2CH_2CH_2$), 1.87–1.92 (2H, m, NCH_2CH_2), 1.95–2.01 (2H, m, OCH_2CH_2), 2.86 (6H, s, 2 × NCH_3), 3.11 (2H, t, J = 6.0 Hz, NCH_2CH_2), 4.08 (2H, t, J = 6.0 Hz, OCH_2CH_2), 6.97 (2H, d, J = 8.0 Hz, 3′-H and 5′-H), 7.40–7.44 (3H, m, 3-H and 4-H and 5-H), 7.55 (1H, d, J = 16.0 Hz, α-H), 7.62–7.67 (2H, m, 2-H and 4-H), 7.80 (1H, d, J = 16.0 Hz, β-H), 8.03 (2H, d, J = 8.0 Hz, 2′-H and 6′-H). MS m/z (ESI): 338 $[M+H]^+$. IR (KBr) ν/cm^{-1}: 3030, 2954, 2867, 2779, 1657, 1605, 1589, 1505, 1340, 1225, 1175, 833, 768. Purity: 98.4% by HPLC (MeOH/0.1% TEA 85:15 (v/v); t_R 5.74 min).

4.4.14. (*E*)-1-(4-(5-(Diethylamino)pentyloxy)phenyl)-3-phenylprop-2-en-1-one (7b)

According to the general method, the reaction of compound **3d** (0.372 g, 1 mmol) with diethylamine (0.31 mL, 3 mmol) produced the crude product. Then it was purified using silica-gel column chromatography with methanol/dichloromethane (1:50, v/v) as elution to give a white solid product with a yield of 78.7%. Mp 143–145 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.44 (6H, t, J = 6.8 Hz, 2 × NCH_2CH_3), 1.58–1.66 (2H, m, $CH_2CH_2CH_2$), 1.86–1.99 (4H, m, NCH_2CH_2 and OCH_2CH_2), 3.07 (2H, t, J = 6.0 Hz, NCH_2CH_2), 3.20 (4H, m, 2 × NCH_2CH_3), 4.08 (2H, t, J = 6.0 Hz, OCH_2CH_2), 6.97 (2H, d, J = 8.8 Hz, 3′-H and 5′-H), 7.39–7.43 (3H, m, 3-H and 4-H and 5-H), 7.54 (1H, d, J = 16.0 Hz, α-H), 7.63–7.67 (2H, m, 2-H and 4-H), 7.79 (1H, d, J = 16.0 Hz, β-H), 8.03 (2H, d, J = 8.0 Hz, 2′-H and 6′-H). MS m/z (ESI): 366 $[M+H]^+$. IR (KBr) ν/cm^{-1}: 3032, 2958, 2871, 2776, 1656, 1605, 1587, 1508, 1342, 1223, 1175, 835, 767. Purity: 98.7% by HPLC (MeOH/0.1% TEA 85:15 (v/v); t_R 8.09 min).

4.4.15. (*E*)-1-(4-(5-(Dipropylamino)pentyloxy)phenyl)-3-phenylprop-2-en-1-one (7c)

According to the general method, the reaction of compound **3d** (0.372 g, 1 mmol) with dipropylamine (0.41 mL, 3 mmol) produced the crude product. Then it was purified using silica-gel column chromatography with methanol/dichloromethane (1:50, v/v) as elution to give a white solid product with a yield of 65.9%. Mp 129–131 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.02 (6H, t, J = 6.0 Hz, 2 × $NCH_2CH_2CH_3$), 1.56–1.63 (2H, m, $CH_2CH_2CH_2$), 1.83–2.01 (8H, m, NCH_2CH_2 and OCH_2CH_2 and 2 × $NCH_2CH_2CH_3$), 2.99–3.12 (6H, m, 3 × NCH_2CH_2), 4.07 (2H, t, J = 6.0 Hz, OCH_2CH_2), 6.96 (2H, d, J = 8.0 Hz, 3′-H and 5′-H), 7.40–7.45 (3H, m, 3-H and 4-H and 5-H), 7.54 (1H, d, J = 16.0 Hz, α-H), 7.63–7.68 (2H, m, 2-H and 4-H), 7.79 (1H, d, J = 16.0 Hz, β-H), 8.02 (2H, d, J = 8.0 Hz, 2′-H and 6′-H). MS m/z (ESI): 394 $[M+H]^+$. IR (KBr) ν/cm^{-1}: 3033, 2961, 2875, 2778, 1656, 1603, 1586, 1507, 1340, 1225, 1173, 836, 768. Purity: 97.2% by HPLC (MeOH/0.1% TEA 85:15 (v/v); t_R 14.42 min).

4.4.16. (*E*)-1-(4-(5-(Dibutylamino)pentyloxy)phenyl)-3-phenylprop-2-en-1-one (7d)

According to the general method, the reaction of compound **3d** (0.372 g, 1 mmol) with dibutylamine (0.5 mL, 3 mmol) produced the crude product. Then it was purified using silica-gel column chromatography with methanol/dichloromethane (1:55, v/v) as elution to give a light yellow oil product with a yield of 60.8%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.93 (6H, t, J = 6.0 Hz, 2 × $NCH_2CH_2CH_2CH_3$), 1.22–1.32 (4H, m, 2 × $NCH_2CH_2CH_2CH_3$), 1.45–1.52 (2H, m, $CH_2CH_2CH_2$), 1.72–1.89 (8H, m, NCH_2CH_2 and OCH_2CH_2 and 2 × $NCH_2CH_2CH_2CH_3$), 2.66–2.74 (6H, m, 3 × NCH_2CH_2), 4.05 (2H, t, J = 6.0 Hz, OCH_2CH_2), 6.96 (2H, d, J = 8.0 Hz, 3′-H and 5′-H), 7.41–7.45 (3H, m, 3-H and 4-H and 5-H), 7.55 (1H, d, J = 16.0 Hz, α-H), 7.62–7.66 (2H, m, 2-H and 4-H), 7.79 (1H, d, J = 16.0 Hz, β-H), 8.03 (2H, d, J = 8.0 Hz, 2′-H and 6′-H). MS m/z (ESI): 422 $[M+H]^+$. IR (KBr) ν/cm^{-1}: 3032, 2958, 2873, 2779, 1656, 1608, 1589, 1508, 1341, 1226, 1172, 836, 770. Purity: 97.8 % by HPLC (MeOH/0.1% TEA 85:15 (v/v); t_R 23.98 min).

4.4.17. (*E*)-1-(4-(6-(Dimethylamino)hexyloxy)phenyl)-3-phenylprop-2-en-1-one (8a)

According to the general method, the reaction of compound **3e** (0.386 g, 1 mmol) with diethylamine (0.31 mL, 3 mmol) produced the crude product. Then it was purified using silica-gel column chromatography with methanol/dichloromethane (1:50, v/v) as elution to give a white solid product with a yield of 75.6%. Mp 139–141 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.48–1.61 (4H, m, $CH_2CH_2CH_2CH_2$ and $CH_2CH_2CH_2CH_2$), 1.80–1.96 (4H, m, NCH_2CH_2 and OCH_2CH_2), 2.86 (6H, s, 3 × NCH_3), 3.09 (2H, t, J = 6.0 Hz, NCH_2CH_2), 4.05 (2H, t, J = 6.0, OCH_2CH_2), 6.97 (2H, d, J = 8.0 Hz, 3′-H and 5′-H), 7.40–7.44 (3H, m, 3-H and 4-H and 5-H), 7.55 (1H, d, J = 16.0 Hz, α-H), 7.61–7.66 (2H, m, 2-H and 4-H), 7.79 (1H, d, J = 16.0 Hz, β-H), 8.03 (2H, d, J = 8.0 Hz, 2′-H and 6′-H). MS m/z (ESI): 352 $[M+H]^+$. IR (KBr) ν/cm^{-1}: 3033, 2962, 2877, 2781, 1655, 1606, 1587, 1510, 1340, 1224, 1173, 836, 771. Purity: 98.3% by HPLC (MeOH/0.1% TEA 85:15 (v/v); t_R 7.13 min).

4.4.18. (*E*)-1-(4-(6-(Diethylamino)hexyloxy)phenyl)-3-phenylprop-2-en-1-one (8b)

According to the general method, the reaction of compound **3e** (0.386 g, 1 mmol) with dimethylamine (0.34 mL, 3 mmol) produced the crude product. Then it was purified using silica-gel column chromatography with methanol/dichloromethane (1:50, v/v) as elution to give a white solid product with a yield of 68.3%. Mp 165–167 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.44 (6H, t, J = 6.8 Hz, 2 × NCH_2CH_3), 1.49–1.60 (4H, m, $CH_2CH_2CH_2CH_2$ and $CH_2CH_2CH_2CH_2$), 1.81–1.95 (4H, m, NCH_2CH_2 and OCH_2CH_2), 3.05 (2H, t, J = 6.0 Hz, NCH_2CH_2), 3.19 (4H, m, 2 × NCH_2CH_3), 4.04 (2H, t, J = 6.0, OCH_2CH_2), 6.96 (2H, d, J = 8.80 Hz, 3′-H and 5′-H), 7.40–7.44 (3H, m, 3-H and 4-H and 5-H), 7.54 (1H, d, J = 16.0 Hz, α-H), 7.61–7.66 (2H, m, 2-H and 4-H), 7.79 (1H, d, J = 16.0 Hz, β-H), 8.03 (2H, d, J = 8.0 Hz, 2′-H and 6′-H). MS m/z (ESI): 380 $[M+H]^+$. IR (KBr) ν/cm^{-1}: 3031, 2960, 2875, 2778, 1657, 1605, 1589, 1508/, 1342, 1225, 1172, 837, 769. Purity: 98.1% by HPLC (MeOH/0.1% TEA 85:15 (v/v); t_R 10.17 min).

4.4.19. (*E*)-1-(4-(6-(Dipropylamino)hexyloxy)phenyl)-3-phenylprop-2-en-1-one (8c)

According to the general method, the reaction of compound **3e** (0.386 g, 1 mmol) with dipropylamine (0.41 mL, 3 mmol) produced the crude product. Then it was purified using silica-gel column chromatography with methanol/dichloromethane (1:55, v/v) as elution to give a white solid product with a yield of 71.5%. Mp 120–122 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.97 (6H, t, J = 6.0 Hz, 2 × $NCH_2CH_2CH_3$), 1.46–1.58 (4H, m, $CH_2CH_2CH_2CH_2$ and $CH_2CH_2CH_2CH_2$), 1.68–1.79 (6H, m, 2 × $NCH_2CH_2CH_3$ and NCH_2CH_2), 1.80–1.87 (2H, m, OCH_2CH_2), 2.76–2.84 (6H, m, 3×NCH_2CH_2), 4.05 (2H, t, J = 6.0 Hz, OCH_2CH_2), 6.96 (2H, d, J = 8.0 Hz, 3′-H and 5′-H), 7.40–7.45 (3H, m, 3-H and 4-H and 5-H), 7.54 (1H, d, J = 16.0 Hz, α-H), 7.63–7.68 (2H, m, 2-H and 4-H), 7.79 (1H, d, J = 16.0 Hz, β-H), 8.02 (2H, d, J = 8.0 Hz, 2′-H and 6′-H). MS m/z (ESI): 408 $[M+H]^+$. IR (KBr) ν/cm^{-1}: 3031, 2957, 2877, 2776, 1656, 1606, 1588, 1509, 1341, 1223, 1171, 838, 769. Purity: 97.7% by HPLC (MeOH/0.1% TEA 85:15 (v/v); t_R 18.51 min).

4.4.20. (*E*)-1-(4-(6-(Dibutylamino)hexyloxy)phenyl)-3-phenylprop-2-en-1-one (8d)

According to the general method, the reaction of compound **3e** (0.386 g, 1 mmol) with dibutylamine (0.5 mL, 3 mmol) produced the crude product. Then it was purified using silica-gel column chromatography with methanol/dichloromethane (1:55, v/v) as elution to give a light yellow oil product with a yield of 63.7%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.93 (6H, t, J = 6.0 Hz, 2 × $NCH_2CH_2CH_3$), 1.25–1.31 (4H, m, 2 × $NCH_2CH_2CH_2CH_3$), 1.45–1.57 (4H, m, $CH_2CH_2CH_2CH_2$ and $CH_2CH_2CH_2CH_2$), 1.62–1.78 (6H, m, 2 × $NCH_2CH_2CH_2CH_3$ and NCH_2CH_2), 1.83–1.90 (2H, m, OCH_2CH_2), 2.70–2.77 (6H, m, 3 × NCH_2CH_2), 4.05 (2H, t, J = 6.0 Hz, OCH_2CH_2), 6.96 (2H, d, J = 8.0 Hz, 3′-H and 5′-H), 7.41–7.45 (3H, m, 3-H and 4-H and 5-H), 7.55 (1H, d, J = 16.0 Hz, α-H), 7.62–7.66 (2H, m, 2-H and 4-H), 7.79 (1H, d, J = 16.0 Hz, β-H), 8.03 (2H, d, J = 8.0 Hz, 2′-H and 6′-H). MS m/z (ESI): 436 $[M+H]^+$. IR (KBr) ν/cm^{-1}: 3032, 2955, 2876, 2778, 1654, 1607, 1586, 1507, 1343, 1221, 1169, 836, 770. Purity: 97.3% by HPLC (MeOH/0.1% TEA 85:15 (v/v); t_R 28.37 min).

4.5. Enzyme inhibition assays

AChE/BuChE inhibitory activities of the synthesized compounds were assayed by the spectrophotometric method developed by Ellman et al. with slight modification.[29] The tested compounds were dissolved in tween 80 (final concentration was 0.06% in each reaction) and followed by dilution in 2% tween 80-water solution to obtain final assay concentrations. Five different concentrations were tested for each compound in triplicate to obtain the range of 20–80% inhibition for AChE and BuChE. The reaction mixture containing 40 μL AChE or BuChE, 100 μL acetylthiocholine iodide or butyrylthiocholine iodide, 2.76 mL Na_2HPO_4/NaH_2PO_4 buffer (pH 8.0), 100 μL different concentrations of tested compounds was incubated at 30 °C for 25 min. Then the reaction was terminated by adding 100 μL 20% Sodium dodecyl sulfate (SDS) and 100 μL 10 mmol/L 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) was added to generate the yellow anion 5-thio-2-nitro-benzoic acid. The absorbance of each tested mixture was measured at 412 nm by UV spectroscopy. The IC_{50} values were calculated by Bliss method and expressed as mean ± SD of the replicates.

4.6. Kinetic assay

Kinetic characterization of AChE was performed using a reported method.[30] Compound **4a** was added into the assay solution and preincubated with the enzyme at 30 °C for 25 min, followed by the addition of Acetylthiocholine iodide. The assay solution contained 100 μL compound **4a**, 100 μL DTNB, 2.76 mL Na_2HPO_4/NaH_2PO_4 buffer (pH 8.0) and 100 μL Acetylthiocholine iodide. Kinetic characterization of the hydrolysis of Acetylthiocholine iodide catalyzed by AChE was done spectrometrically at 412 nm. The parallel control experiment was carried out without compound 4a in the mixture.

4.7. Molecular docking

Molecular docking was carried out by means of Molecular Operating Environment (MOE) software package. The crystal structure of Donepezil in complex with AChE (code: 1EVE) and the human butyrylcholinesterase complexed with echothiophate (code: 1POI) were obtained from protein data bank. Then, the inhibitor and water molecules in the PDB file were removed and hydrogen atoms were added to the protein. After the preparation of the protein, 3D structure of the most potent inhibitor **4a** was built using the builder interface of MOE program, and docked into the active site of the protein after energy minimized, using the ASE scoring function as the Dock scoring. Finally, the geometry of resulting complex was studied using the MOE's pose viewer utility.

4.8. Log*P* measurement

Octanol–water partition coefficients of compounds **4a**–**8d** were measured by the shake flask method with slight modification[31] and PBS (pH = 7.4) was used as the aqueous phase. The mobile phase was methanol: 0.1% TEA /85:15(v/v), at a flow rate of 1.0 mL min^{-1} through a C_{18} column (250 nm × 4.6 mm, 5 μm) at 32 °C with detect wavelength 319 nm . Experiments were conducted in triplicate and log P values were calculated.

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Supplementary data

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