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Original article

A novel series of tacrine–selegiline hybrids with cholinesterase and monoamine oxidase inhibition activities for the treatment of Alzheimer's disease

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ABSTRACT

A novel series of tacrine–selegiline hybrids was synthesised and evaluated for application as inhibitors of cholinesterase (AChE/BuChE) and monoamine oxidase (MAO-A/B). The results demonstrate that most of the synthesised compounds exhibit high inhibitory activity. Among these compounds, compound **8g** provided a good balance of activity towards all targets (with IC_{50} values of 22.6 nM, 9.37 nM, 0.3724 μM, and 0.1810 μM for AChE, BuChE, MAO-A and MAO-B, respectively). These results indicated that **8g** has the potential to be a multi-functional candidate for Alzheimer's disease.

1. Introduction

Alzheimer's disease (AD) is an age-related progressive neurodegenerative disorder. It has been estimated that there were 35.6 million people with dementia in 2010, and the number is expected to nearly double every 20 years, resulting in more than 115 million in 2050 [1]. Although the aetiology of AD is not fully understood, several conditions play significant roles in the pathogenesis of AD, including low levels of acetylcholine (ACh), the formation of β-amyloid deposits, τ-protein, oxidative stress and dyshomeostasis of biometals [2].

In the past decade, treatment strategies for AD have mainly been aimed at improving cholinergic neurotransmission in the brain, which were mostly based on the 'cholinergic hypothesis'. Many approaches have been investigated based on this hypothesis [3]. These approaches include the use of cholinesterase inhibitors (ChEIs) to improve the endogenous levels of ACh in the brain of AD patients. ChEIs represent the only species that have demonstrated some promise in the treatment of AD. Tacrine was the first cholinesterase inhibitor approved by the FDA for the treatment of AD patients in clinical trials. Although this compound suffers from therapy-limiting liver toxicity, tacrine has been widely used as a scaffold for the development of new multi-functional agents with additional biological properties beyond AChE inhibition. Recent studies have demonstrated that homo- and hetero-dimers can improve and enlarge the biological profile of tacrine with fewer side effects [4]. The homo- and hetero-dimers that have been investigated include bis-tacrine [5], tacrine-8-hydroxyquinoline hybrids [6], mercapto-tacrine derivatives [7], tacrine–fluorobenzoic acid hybrids [8], and tacrine–multialkoxybenzene hybrids [9], among others [10].

Monoamine oxidases (MAOs) are flavin adenine dinucleotide (FAD)-containing enzymes localised in the outer mitochondrial membrane in various cells found in nerve terminals, liver, intestinal mucosa and other tissues [11]. MAOs are responsible for the oxidative deamination of neurotransmitters. Based on their substrate and inhibitor specificities, MAOs exist in two distinct enzymatic isoforms, MAO-A and MAO-B [12]. Selective inhibitors of MAO-A have been shown to be effective antidepressants, whereas MAO-B inhibitors are useful in several neurodegenerative disorders such as Parkinson's disease (PD), AD, Huntington chorea and amyotrophic lateral sclerosis [13]. It is well known that high expression levels of MAO-B in neuronal tissue could result in an increase in the level of free radicals, which play a major role in the aetiology of AD. Therefore, MAO inhibitors are considered to be potential candidates

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for anti-Alzheimer drugs due to their capacity to inhibit oxidative damage [14]. Among the MAO inhibitors, selegiline, an irreversible and selective MAO-B inhibitor, acts as a neuroprotective agent in cellular and animal models of AD [15].

Numerous highly selective compounds which target a single risk factor have been developed in the past decade. However, very few of them exhibited efficient therapy in clinic trials, which suggests limitations in the traditional "one molecule, one target" paradigm. Given the complex nature of this disease, multi-functional molecules with two or more complementary biological activities may represent an important advance for the treatment of this disease. Because both ChE and MAOs are important targets for the treatment of AD, some studies have been devoted to finding multi-functional agents that target both ChE and MAOs. Ladostigil (Fig. 1), a bifunctional drug developed from the carbamate moiety of rivastigmine and the indolamine moiety of rasagiline, was approved for phase IIb clinical trial recently. This fact suggests that the combination of AChEIs and MAO inhibitors is an effective route for developing new multi-functional anti-AD drugs. Meanwhile, a series of multipotent inhibitors that can simultaneously inhibit MAO and ChE, such as heterocyclic substituted alkyl and cycloalkyl propargyl amines, has been reported in recent years (Fig. 1) [16].

Inspired by these drug design strategies, and our previous work on tacrine–benzylamine hybrids as multi-functional anti-AD agents [17], we combined the tacrine moiety (ChE inhibitory activity) with the selegiline moiety (MAO inhibitory activity) using carbon spacers of different lengths (Fig. 2). Herein, we describe the synthesis and biological evaluation of a series of tacrine–selegiline hybrids as multi-functional anti-AD agents with cholinesterase and MAO inhibition activities.

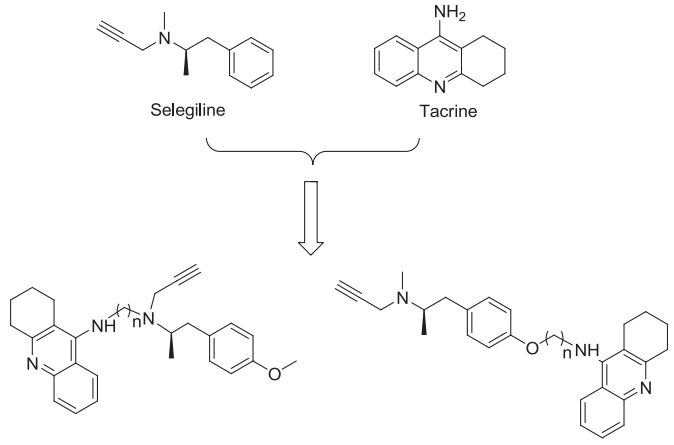


Fig. 2. Design strategy for the new series of tacrine–selegiline hybrids.

2. Results and discussion

2.1. Chemistry

The selegiline analogue **6**, a key intermediate in the synthesis of target compounds **8** and **9**, was prepared according to the pathway described in Scheme 1. Beginning from the commercially available 1-(4-methoxyphenyl)propan-2-one **1**, chiral amine **3** was prepared via a chiral induction followed by debenzylation [18]. Then, **3** was reacted with propargylbromide in the presence of potassium carbonate to yield the chiral propargyl amine **4**, which was converted to **6** by reacting with formaldehyde/formic acid followed the demethylation in high yield.

The synthetic route for the preparation of tacrine–selegiline hybrids is provided in Scheme 2. Briefly, the reaction between compound **6**, an analogue of selegiline, and α,ω-dibromoalkanes in the presence of K_2CO_3 affords compound **7** in good yield. Subsequently, compound **7** was reacted with tacrine or 6-chlorotacrine in the presence of potassium hydroxide and DMSO to obtain target products **8a**–**h** and **9a**–**d**, respectively. For comparative purposes, compound **11**, wherein tacrine is linked to the selegiline moiety via the propargyl amine nitrogen, was also synthesised according to Scheme 3.

2.2. In vitro inhibition studies of AChE and BuChE

To evaluate the potential application of target hybrids in the treatment of AD, the inhibition of AChE and BuChE was evaluated using the spectroscopic method described by Ellman et al. with tacrine as the standard [14]. AChE and BuChE were obtained from electric eel and equine serum, respectively. The results provided in Table 1 show that all target compounds are potent inhibitors of AChE and BuChE with IC_{50} values in the sub-micromolar range. In addition, the length of the alkylene linkage affects the inhibition of both AChE and BuChE. Compounds **8a**–**d** containing two, three, four and five carbon spacers between the tacrine and selegiline moiety provide stronger inhibitory activities of AChE than tacrine (**8a**, IC_{50} = 83.1 nM; **8b**, IC_{50} = 36.1 nM; **8c**, IC_{50} = 53.0 nM; **8d**, IC_{50} = 77.6 nM; Tacrine, IC_{50} = 110.2 nM, respectively). However, compounds **8e** and **8f**, with six and eight-carbon spacers, only exhibited IC_{50} values of 138 and 147 nM, respectively. It is interesting that the inhibitory activities significantly increased when the compounds possess nine and ten methylene spacers (**8g**, IC_{50} = 22.6 nM; **8h**, IC_{50} = 23.2 nM). Generally, the BuChE inhibitory activity increases as the number of methylene groups increases. Compound **8h**, which has ten-carbon spacers, exhibited the best IC_{50} value (2.03 nM) in this series. All target compounds derived from 6-chlorotacrine and selegiline moiety were also potent inhibitors of AChE and BuChE with the same trend, wherein compound **9a**, possessing a three-carbon linker, exhibited the best results for AChE (IC_{50} = 14.2 nM) and good activity for BuChE (IC_{50} = 66.0 nM) in this series. In contrast, compound **11**, wherein the tacrine is linked by a six-carbon spacer to the N position of selegiline moiety, provides the weakest inhibition activity of AChE (IC_{50} = 456 nM) and good activity for BuChE (IC_{50} = 28.7 nM). These results indicate that steric effects may play an important role in the inhibition of AChE. Some evidence suggests that the inhibition of BuChE could raise ACh levels and improve cognition for AD patients [19]. Dual AChE/BuChE inhibitors may have better activity and show clinical efficacy without remarkable side effects [19,20]. Consequently, most of the hybrids, especially **8a**–**c** and **8g**, exhibited a good balance of AChE/BuChE inhibitory activity and may represent good dual AChE/BuChE inhibitors to treat Alzheimer's disease.

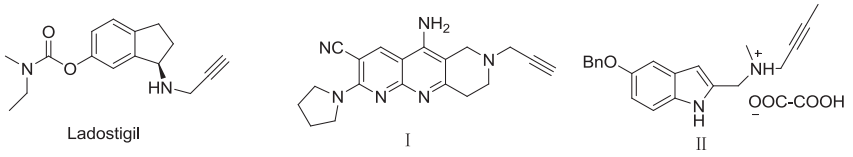
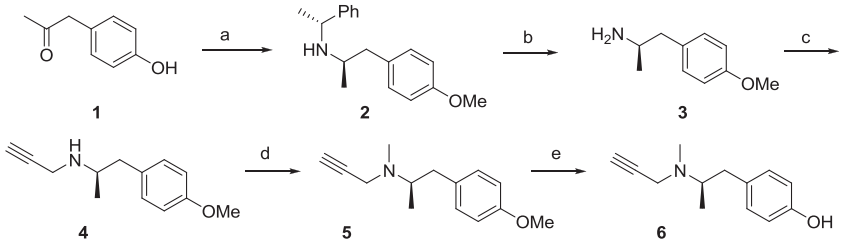


Fig. 1. Chemical structures of Ladostigil and heterocyclic substituted alkyl and cycloalkyl propargyl amine I and II.



Scheme 1. Synthesis of **6**. Reagents and conditions: (a) (i) (*R*)-1-phenylethanamine, Pt/C, H_2; (ii) L-(+)-Tartaric acid, ethanol; (iii) NaOH, H_2O; (b) Pd/C, H_2; (c) Propargylbromide, K_2CO_3, MeCN; (d) HCHO, HCOOH, 90 °C; (e) BBr_3, CH_2Cl_2, −20 °C.

2.3. Kinetic study of AChE

To study the inhibitory mechanism of this class of tacrine–selegiline hybrids, compound **8g**, which provided a good balance of AChE/BuChE inhibitory activity, was chosen for further kinetic studies. The Lineweaver–Burk reciprocal plots of **8g** against AChE (Fig. 3) revealed an increasing slope and an increasing intercept with higher inhibitor concentrations, indicating a mixed-type inhibitory behaviour for compound **8g** in the presence of AChE.

2.4. Molecular modelling studies

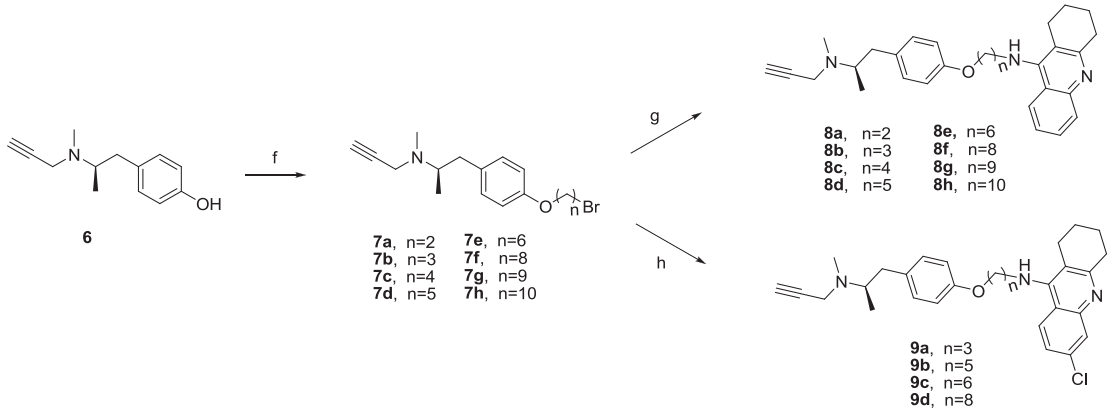
To evaluate the binding modes of this class of tacrine–selegiline hybrids with respect to AChE, docking simulations of compound **8g** were performed using the CDOCKER program in the Discovery studio 2.1 software, based on the structure of the *Torpedo californica* enzyme complex (TcAChE; PDB entry 2CMF). The full image of the interaction between **8g** and TcAChE is provided in Fig. 4. The docking results in Fig. 5 show that the tacrine ring binds to the CAS, which is sandwiched between Trp84 (4.28 Å) and Phe330 (4.15 Å). The carbon linker spans across the aromatic gorge. The selegiline moiety stacks against the indole ring of Trp279 (5.07 Å) through the $\pi-\pi$ interaction bind the PAS. In addition, the O atom links the benzene ring of the selegiline moiety is hydrogen-bonded to the hydroxyl group of Tyr121.

2.5. In vitro inhibition studies of hMAO-A and hMAO-B

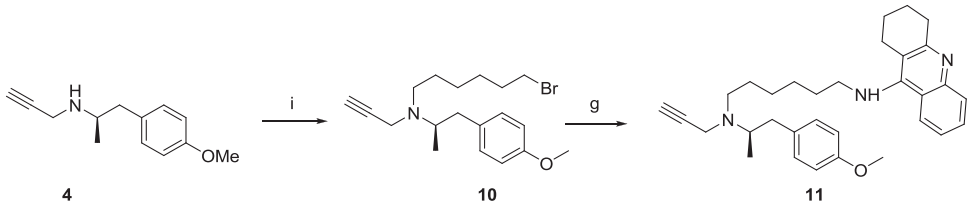
To confirm the multipotent biological profile of tacrine–selegiline hybrids, inhibitory activity against MAO was determined. Because AD patients exhibit depressive symptoms, dual inhibition of MAO-A and MAO-B, rather than inhibition of MAO-B alone, may be of value for AD therapy [21]. Thus, we tested the inhibitory activity against both isoforms. The results in Table 1 show that most of the target compounds were effective in inhibiting MAO-A and MAO-B in the sub-micromolar range. Among the synthesised compounds, **9d**, with an eight-carbon spacer between tacrine and selegiline, gave the best results for both MAO-A (IC_{50} = 0.1926 μM) and MAO-B (IC_{50} = 0.1290 μM). SAR analysis showed that the MAO inhibitory potency was closely related to the length of the alkylene chain. Compounds with 3–5 carbon alkylene chain are poor MAO-A/B inhibitors (**8a**–**c**, **9a** and **9b** with inhibitory activities in the micromolar range). Surprisingly, when the alkylene chain contains six carbons, the compounds are potent MAO inhibitors exhibiting sub-micromolar activities (**8e**, **9c**). Generally, a longer linker resulted in better inhibitory activity (except **8a**). A comparison of compounds **8** and **9**, where the length of the alkylene chain is same, reveals no significant change in the inhibitory activities. Therefore, introducing chlorine in the tacrine ring did not improve the inhibition of MAO as it did for AChE. It should be noted that hybrid **8e** exhibited much better inhibitory activity for MAO-A and MAO-B (IC_{50} = 0.3978 and 0.2090 μM, respectively) than its isomer **11** (IC_{50} = 33.79 μM for MAO-A and 18.11 μM for MAO-B). These results indicate that steric factors may produce the main inhibitory effect. Overall, among the synthesised compounds, **8g** exhibited best balance of inhibition for both ChE and MAO.

2.6. Reversibility and irreversibility study of hMAO-B

The reversibility and irreversibility of inhibitory activities of **8g** was assessed according to a method in the literature [22]. Pargyline, with known irreversibility for the MAO-B inhibitor [23], was used as a reference compound. The results provided in Table 2 demonstrate that **8g** is an irreversible MAO-B inhibitor because no recovery of the enzyme activity was observed even after repeated washing [22].



Scheme 2. Synthesis of **T** series tacrine–selegiline hybrids. Reagents and conditions: (f) $Br(CH_2)_nBr$, K_2CO_3, MeCN, reflux; (g) Tacrine, KOH, DMSO; (h) 6-chlorotacrine, KOH, and DMSO.



Scheme 3. Synthesis of **N** series tacrine–selegiline hybrid. Reagents and conditions: (i) $Br(CH_2)_6Br$, K_2CO_3, MeCN, reflux; (g) Tacrine, KOH, and DMSO.

3. Conclusion

In summary, we have designed, synthesised and evaluated a series of tacrine–selegiline and 6-chlorotacrine–selegiline hybrids as multi-functional anti-AD agents with cholinesterase and MAOs inhibitory activities. Most of these compounds were potent inhibitors of ChE and MAO. The tacrine–selegiline hybrid **8** exhibited better or parallel activity to the reference compound tacrine but slightly lower MAO inhibitory activity than selegiline. Among all the synthesised compounds, **8g** exhibited a good balance of AChE, BuChE, hMAO-A and hMAO-B inhibition activities. Additional experiments on **8g** indicate that it is a mixed-type inhibitor of AChE and an irreversible inhibitor of hMAO-B. Molecular modelling studies demonstrate that **8g** could interact with both CAS and PAS of AChE. Thus, it is expected that this hybrids could enhance patient cognition by increasing levels of acetylcholine and protect neurons by keeping the activities of selegiline. Therefore, this series of tacrine–selegiline hybrids should be considered as novel "one-compound-multi-targets" candidates for anti-Alzheimer therapy.

4. Experimental section

4.1. General

NMR spectra were obtained on a Bruker Avance III spectrometer with TMS as the internal standard. Low-resolution MS spectra were obtained on an Agilent LC–MS 6120 instrument with an ESI mass detector, and the data were obtained in the positive ion mode. High-resolution ESI-MS data were obtained on a Shimadzu LC–MS-IT-TOF mass spectrometer. The purity of the synthesised compounds was evaluated using a high-performance liquid chromatography (HPLC) equipped with an Eclipse Plus C8 column (4.6 mm × 150 mm, 5 μm). IR spectra (KBr disks) were recorded on a Bruker Tensor 27 FT-IR. Compound **2**, tacrine and 6-chlorotacrine were prepared according to the method reported previously [18,24].

4.1.1. Preparation of (R)-1-(4-methoxyphenyl)propan-2-amine (**3**)

To a solution of compound **2** (13.47 g, 50.0 mmol) in methanol (100 mL), 10% Pd/C (1.35 g) was added, and the mixture was stirred under hydrogen atmosphere (1.5 MPa pressure) at 40 °C for 24 h. The catalyst was filtered off and the solvent was evaporated to give compound **3** as a white solid (8.24 g, 99.8%). IR (KBr): 3294, 2958, 2923, 2847, 1610, 1512, 1458, 1246, 1220, 1025 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.10 (d, J = 7.3 Hz, 2H), 6.84 (d, J = 7.1 Hz, 2H), 3.79 (s, 3H), 3.12 (dd, J = 12.4, 6.0 Hz, 1H), 2.66 (dd, J = 13.4, 5.2 Hz, 1H), 2.47 (dd, J = 13.1, 7.8 Hz, 1H), 1.11 (d, J = 7.6 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.06, 131.74, 130.14, 113.43, 55.22, 48.56, 45.71, 23.46; LC–MS (ESI) m/z $[M]^+$ 166.2.

4.1.2. Preparation of (R)-N-(1-(4-methoxyphenyl)propan-2-yl) prop-2-yn-1-amine (**4**)

To a solution of compound **3** (8.24 g, 49.9 mmol), propargylbromide (80% in toluene, 7.42 g, 49.9 mmol) in acetonitrile (150 mL), potassium carbonate (8.48 g, 61.4 mmol) was added. After the mixture was stirred under nitrogen atmosphere at room temperature for 16 h, the solid was filtered, the solvent was evaporated to provide the crude product which was purified on silica gel

Table 1
Cholinesterases and human recombinant MAO isoforms inhibitory activity of tested compounds and reference compounds.

Compd.	n	IC_{50}(nM) ± SD[a]		SI[e]	IC_{50}(μM) ± SD[a]		SI[f]
		AChE[b]	BuChE[c]		hMAO-A	hMAO-B	
8a	2	83.1 ± 1.13	50.6 ± 6.20	0.6	4.86 ± 0.43	0.6930 ± 0.0001	7.0
8b	3	36.1 ± 1.50	41.4 ± 3.04	1.1	43.38 ± 2.53	4.46 ± 0.49	9.7
8c	4	53.0 ± 2.21	39.6 ± 3.10	0.7	7.00 ± 0.52	8.55 ± 0.63	0.8
8d	5	77.6 ± 5.90	23.5 ± 2.31	0.3	1.26 ± 0.20	2.40 ± 0.05	0.5
8e	6	138 ± 9.54	16.1 ± 3.20	0.1	0.3978 ± 0.0338	0.2090 ± 0.0050	1.9
8f	8	147 ± 3.50	35.5 ± 0.28	0.2	0.5910 ± 0.0177	0.1670 ± 0.0007	3.5
8g	9	22.6 ± 3.04	9.37 ± 0.75	0.4	0.3724 ± 0.0249	0.1810 ± 0.0300	2.1
8h	10	23.2 ± 0.23	2.03 ± 0.32	0.1	0.3130 ± 0.1143	0.3460 ± 0.0240	0.9
9a	3	14.2 ± 1.75	66.0 ± 2.90	4.6	9.05 ± 0.67	11.07 ± 1.35	0.8
9b	5	82.0 ± 6.35	18.6 ± 1.83	0.2	2.86 ± 0.35	6.21 ± 0.68	0.5
9c	6	79.3 ± 1.86	18.8 ± 1.20	0.2	0.5214 ± 0.0264	0.5190 ± 0.0109	1.0
9d	8	55.4 ± 3.04	17.6 ± 1.90	0.3	0.1926 ± 0.0269	0.1290 ± 0.0050	1.5
11	6	456 ± 11.00	28.7 ± 0.35	0.1	33.79 ± 3.87	18.11 ± 2.53	1.9
Tacrine	–	110.2 ± 7.30	21.6 ± 1.77	0.2	nt.[d]	nt.	–
Clorgyline	–	nt.	nt.	–	0.0041 ± 0.0002	nt.	–
Pargyline	–	nt.	nt.	–	nt.	0.1880 ± 0.0160	–

[a] Results are the mean of three independent experiments (n = 3) ± SD.
[b] AChE from electric eel was used.
[c] BuChE from equine serum was used.
[d] nt. = not tested.
[e] Selectivity ratio: IC_{50} (BuChE)/IC_{50} (AChE).
[f] Selectivity ratio: IC_{50} (MAO-A)/IC_{50} (MAO-B).

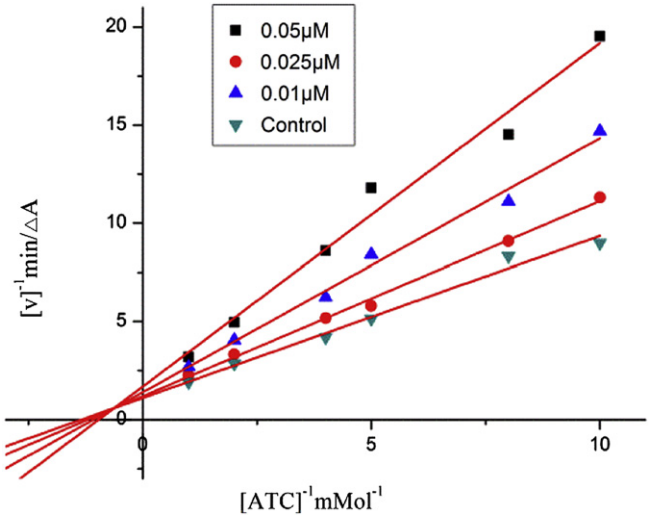


Fig. 3. Steady state inhibition of AChE by **8g** via the hydrolysis of ACh. Reciprocal plots of initial velocity and substrate concentration: the plots show mixed-type inhibition of **8g** on AChE.

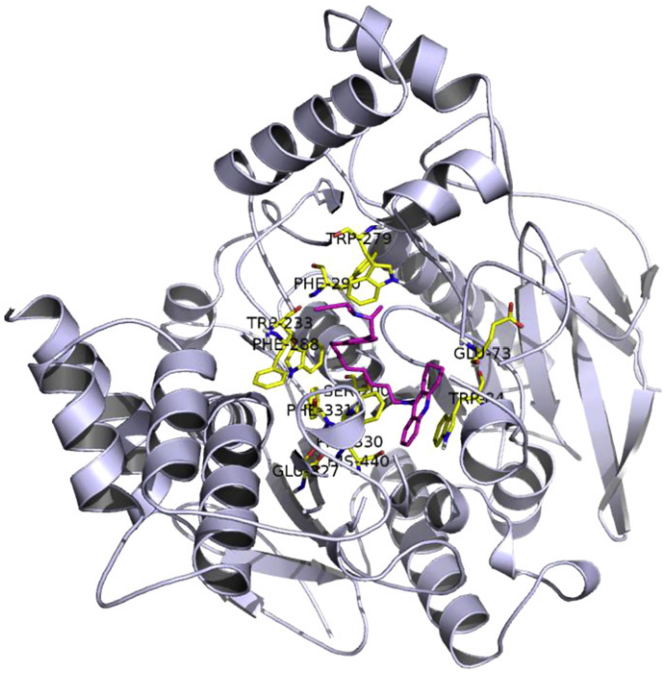


Fig. 4. Binding model for inhibitor **8g** (purple) and *Tc*AChE (grey). Some key residues are coloured yellow. The image was generated using PyMOL.(For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

chromatography to afford compound **4** as a colourless oil (6.15 g, 60.6%). IR (KBr): 3298, 2960, 2925, 2843, 1610, 1512, 1460, 1247, 1220, 1036 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.12 (d, J = 8.4 Hz, 2H), 6.84 (d, J = 8.5 Hz, 2H), 3.79 (s, 3H), 3.43 (q, J = 17.2 Hz, 2H), 3.11 (dd, J = 12.8, 6.4 Hz, 1H), 2.62 (t, J = 6.5 Hz, 2H), 2.17 (s, 1H), 1.05 (d, J = 6.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.12, 131.00, 130.20, 113.8, 82.05, 71.19, 55.22, 52.60, 42.54, 35.56, 19.54; LC–MS (ESI) m/z $[M]^+$ 204.1.

*4.1.3. Preparation of (R)-N-(1-(4-methoxyphenyl)propan-2-yl)-N-methylprop-2-yn-1-amine (**5**)*

A mixture of compound **4** (6.15 g, 30.3 mmol), formalin (7.4 mL), formic acid (7.4 mL) was stirred for 12 h at 90 °C. After the reaction finished (monitored by TLC), the mixture was poured into water (50 mL), basified with aqueous 10 M NaOH and extracted with EtOAc. The organic layer was washed with water and brine, dried with anhydrous Na_2SO_4, filtered, and evaporated. The residue was purified on silica gel chromatography to afford compound **5** as a yellow oil (5.23 g, 79.6%). IR (KBr): 3294, 2958, 2930, 2843, 2791, 1611, 1512, 1457, 1246, 1175, 1038 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.09 (d, J = 8.4 Hz, 2H), 6.82 (d, J = 8.5 Hz, 2H), 3.78 (s, 3H), 3.41 (s, 2H), 3.02–2.87 (m, 2H), 2.41 (s, 3H), 2.34 (dd, J = 12.6, 9.2 Hz, 1H), 2.23 (s, 1H), 0.95 (d, J = 6.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.86, 132.26, 130.14, 113.70, 80.43, 72.49, 59.51, 55.21, 43.14, 38.90, 37.48, 14.99; LC–MS (ESI) m/z $[M]^+$ 218.1.

*4.1.4. Preparation of (R)-4-(2-(methyl(prop-2-ynyl)amino)propyl) phenol (**6**)*

To a solution of compound **5** (5.23 g, 24.1 mmol) in dry CH_2Cl_2 (100 mL) was dropwise added BBr_3 (18.11 g, 72.3 mmol) under nitrogen atmosphere at −20 °C. The mixture was stirred overnight at room temperature and then quenched with water, basified with saturated sodium bicarbonate solution and extracted with CH_2Cl_2. The organic layer was washed with water and brine, dried with anhydrous Na_2SO_4, filtered, and evaporated. The residue was purified on silica gel chromatography to afford compound **6** as a brown solid (4.26 g, 87.0%). IR (KBr): 3676, 3284, 965, 2928, 2857, 2803, 1595, 1515, 1457, 1248, 1138, 1034 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.01 (d, J = 8.3 Hz, 2H), 6.74 (d, J = 8.4 Hz, 2H), 3.46 (s, 2H), 3.02–2.91 (m, 2H), 2.44 (s, 3H), 2.33 (dd, J = 12.8, 9.7 Hz, 1H), 2.26 (s, 1H), 0.98 (d, J = 6.6 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.14, 130.08, 129.86, 114.82, 80.74, 74.93, 58.89, 42.56, 37.88, 36.55, 14.46; LC–MS (ESI) m/z $[M]^+$ 204.1.

*4.1.5. General procedure for preparation of compound **7***

A mixture of compound **6** (0.20 g, 1.0 mmol), α,γ-dibromoethane (2.5 mmol), potassium iodide (16.6 mg, 0.1 mmol) and potassium carbonate (0.41 g, 3.0 mmol) in acetonitrile (5 mL) was refluxed for 16 h under nitrogen atmosphere. After the mixture was filtered, solvent was evaporated and the residue was purified on silica gel chromatography to afford the desired compound **7** respectively.

*4.1.5.1. (R)-N-(1-(4-(2-bromoethoxy)phenyl)propan-2-yl)-N-methylprop-2-yn-1-amine (**7a**).* Colourless oil (96.8 mg, 31.2%). IR (KBr): 3294, 2964, 2928, 2858, 2792, 1609, 1510, 1457, 1240, 1039 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.09 (d, J = 8.5 Hz, 2H), 6.89–6.80 (m, 2H), 4.27 (t, J = 6.3 Hz, 2H), 3.62 (t, J = 6.3 Hz, 2H), 3.42 (s, 2H), 3.02–2.87 (m, 2H), 2.41 (s, 3H), 2.37–2.29 (m, 1H), 2.24 (s, 1H), 0.95 (d, J = 6.5 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.32, 133.10, 130.22, 114.59, 80.39, 72.56, 67.88, 59.38, 43.10, 38.87, 37.41, 29.28, 14.96; LC–MS (ESI) m/z $[M]^+$ 310.1.

*4.1.5.2. (R)-N-(1-(4-(3-bromopropoxy)phenyl)propan-2-yl)-N-methylprop-2-yn-1-amine (**7b**).* Colourless oil (35.4%). IR (KBr): 3295, 2963, 2930, 2869, 2791, 1610, 1510, 1463, 1240, 1036 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.09 (d, J = 8.6 Hz, 2H), 6.82 (d, J = 8.6 Hz, 2H), 4.08 (t, J = 5.8 Hz, 2H), 3.60 (t, J = 6.5 Hz, 2H), 3.42 (s, 2H), 3.00–2.86 (m, 2H), 2.41 (s, 3H), 2.38–2.26 (m, 3H), 2.24 (t, J = 2.4 Hz, 1H), 0.95 (d, J = 6.7 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 132.57, 130.19, 114.36, 80.45, 72.53, 65.29, 59.49, 43.15, 38.91, 37.47, 32.45, 30.11, 15.01; LC–MS (ESI) m/z $[M]^+$ 324.1.

*4.1.5.3. (R)-N-(1-(4-(4-bromobutoxy)phenyl)propan-2-yl)-N-methylprop-2-yn-1-amine (**7c**).* Colourless oil (60.8%). IR (KBr): 3296, 2927, 2861, 2792, 1610, 1511, 1462, 1242, 1040 cm^{-1}; ^1H NMR

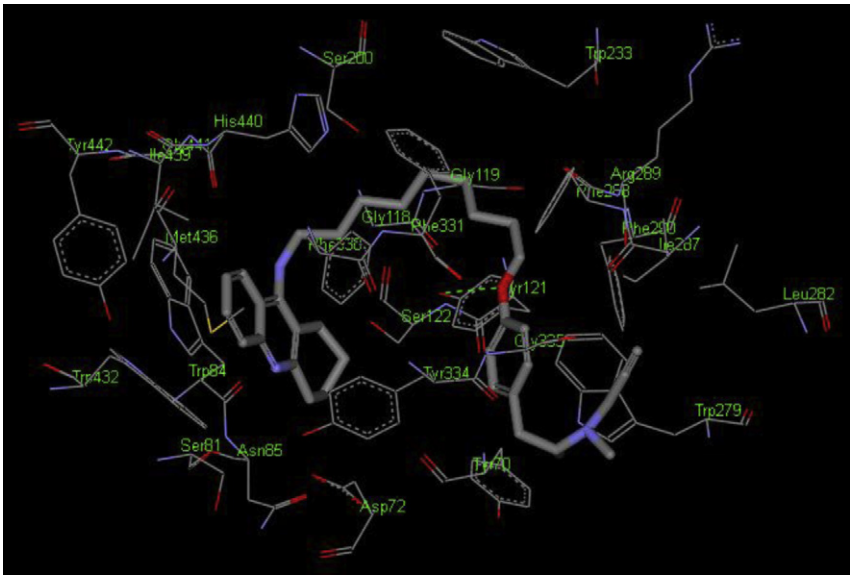


Fig. 5. Compound **8g** (coloured by atom type) docked into the active site of *Tc*AChE (PDB code: 2CMF). Hydrogen bonds are highlighted by green dashed lines.(For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(400 MHz, $CDCl_3$) δ 7.08 (d, J = 8.3 Hz, 2H), 6.80 (d, J = 8.1 Hz, 2H), 3.97 (t, J = 5.9 Hz, 2H), 3.49 (t, J = 6.5 Hz, 2H), 3.42 (s, 2H), 3.02–2.86 (m, 2H), 2.41 (s, 3H), 2.33 (dd, J = 12.6, 9.3 Hz, 1H), 2.23 (s, 1H), 2.12–2.01 (m, 2H), 1.99–1.87 (m, 2H), 0.95 (d, J = 6.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.15, 132.37, 130.16, 114.27, 80.44, 72.49, 66.78, 59.51, 43.15, 38.91, 37.49, 33.51, 29.51, 27.95, 15.01; LC–MS (ESI) m/z $[M]^+$ 338.2.

*4.1.5.4. (R)-N-(1-(4-(5-bromopentyloxy)phenyl)propan-2-yl)-N-methylprop-2-yn-1-amine (**7d**).* Colourless oil (59.8%). IR (KBr): 3297, 2932, 2866, 2792, 1610, 1511, 1463, 1244, 1041 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.07 (d, J = 8.4 Hz, 2H), 6.81 (d, J = 8.4 Hz, 2H), 3.94 (t, J = 6.3 Hz, 2H), 3.46–3.40 (m, 4H), 2.93 (ddt, J = 13.0, 10.5, 5.2 Hz, 2H), 2.41 (s, 3H), 2.33 (dd, J = 12.6, 9.3 Hz, 1H), 2.23 (s, 1H), 1.97–1.90 (m, 2H), 1.84–1.75 (m, 2H), 1.62 (dt, J = 15.2, 7.6 Hz, 2H), 0.95 (d, J = 6.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.26, 132.24, 130.14, 114.29, 80.46, 72.50, 67.52, 59.52, 43.15, 38.91, 37.49, 33.62, 32.51, 28.50, 24.87, 15.02; LC–MS (ESI) m/z $[M]^+$ 352.1.

*4.1.5.5. (R)-N-(1-(4-(6-bromohexyloxy)phenyl)propan-2-yl)-N-methylprop-2-yn-1-amine (**7e**).* Colourless oil (60.3%). IR (KBr): 3299, 2934, 2862, 2793, 1611, 1511, 1464, 1243, 1043 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.07 (d, J = 8.6 Hz, 2H), 6.81 (d, J = 8.6 Hz, 2H), 3.93 (t, J = 6.4 Hz, 2H), 3.42 (dd, J = 7.8, 5.7 Hz, 4H), 3.00–2.86 (m, 2H), 2.41 (s, 3H), 2.33 (dd, J = 12.7, 9.4 Hz, 1H), 2.23 (t, J = 2.4 Hz, 1H), 1.94–1.85 (m, 2H), 1.78 (dd, J = 13.4, 6.6 Hz, 2H), 1.56–1.44 (m, 4H), 0.95 (d, J = 6.7 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.34, 132.13, 130.12, 114.30, 80.37, 72.52, 67.68, 59.54, 43.13, 38.87, 37.47, 33.80, 32.68, 29.13, 27.92, 25.31, 14.99; LC–MS (ESI) m/z $[M]^+$ 366.2.

Table 2
Reversibility and irreversibility of hMAO-B inhibition of **8g** and reference compound pargyline.[a]

Compound	% hMAO-B inhibition	
	Before washing	After repeated washing
8g (200 nM)	70.75 ± 5.00	81.57 ± 5.43
Pargyline (200 nM)	60.30 ± 6.25	56.60 ± 5.56

[a] Each value is a mean ± SD from three experiments.

*4.1.5.6. (R)-N-(1-(4-(8-bromooctyloxy)phenyl)propan-2-yl)-N-methylprop-2-yn-1-amine (**7f**).* Colourless oil (61.8%). IR (KBr): 3300, 2929, 2858, 2792, 1610, 1511, 1463, 1244, 1038 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.07 (d, J = 8.1 Hz, 2H), 6.81 (d, J = 8.2 Hz, 2H), 3.93 (t, J = 6.4 Hz, 2H), 3.41 (t, J = 5.2 Hz, 4H), 2.93 (ddd, J = 16.3, 14.0, 5.1 Hz, 2H), 2.41 (s, 3H), 2.33 (dd, J = 12.5, 9.6 Hz, 1H), 2.24 (s, 1H), 1.86 (dt, J = 13.8, 6.7 Hz, 2H), 1.76 (dt, J = 13.5, 6.6 Hz, 2H), 1.49–1.31 (m, 8H), 0.95 (d, J = 6.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.39, 132.07, 130.11, 114.29, 80.47, 72.52, 67.84, 59.54, 43.15, 38.91, 37.49, 33.95, 32.79, 29.25, 28.70, 28.10, 25.99, 15.04; LC–MS (ESI) m/z $[M]^+$ 394.2.

*4.1.5.7. (R)-N-(1-(4-(9-bromononyloxy)phenyl)propan-2-yl)-N-methylprop-2-yn-1-amine (**7g**).* Colourless oil (60.7%). IR (KBr): 3300, 2930, 2857, 2792, 1611, 1511, 1464, 1244, 1039 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.07 (d, J = 8.5 Hz, 2H), 6.81 (d, J = 8.5 Hz, 2H), 3.92 (t, J = 6.5 Hz, 2H), 3.46–3.36 (m, 4H), 2.94 (ddt, J = 12.9, 10.3, 5.0 Hz, 2H), 2.42 (s, 3H), 2.33 (dd, J = 12.7, 9.4 Hz, 1H), 2.24 (t, J = 2.3 Hz, 1H), 1.89–1.81 (m, 2H), 1.80–1.72 (m, 2H), 1.48–1.31 (m, 10H), 0.96 (d, J = 6.5 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.41, 132.07, 130.10, 114.30, 80.46, 72.46, 67.91, 59.54, 43.14, 38.91, 37.50, 33.99, 32.80, 29.34, 29.31, 29.27, 28.68, 28.14, 26.03, 15.02; LC–MS (ESI) m/z $[M]^+$ 408.2.

*4.1.5.8. (R)-N-(1-(4-(10-bromodecyloxy)phenyl)propan-2-yl)-N-methylprop-2-yn-1-amine (**7h**).* Colourless oil (65.1%). IR (KBr): 3301, 2929, 2856, 2792, 1611, 1511, 1463, 1244, 1039 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.07 (d, J = 8.5 Hz, 2H), 6.81 (d, J = 8.6 Hz, 2H), 3.92 (t, J = 6.5 Hz, 2H), 3.41 (dd, J = 9.0, 4.6 Hz, 4H), 3.01–2.87 (m, 2H), 2.41 (s, 3H), 2.33 (dd, J = 12.7, 9.4 Hz, 1H), 2.23 (t, J = 2.4 Hz, 1H), 1.89–1.81 (m, 2H), 1.77 (dd, J = 14.2, 7.3 Hz, 2H), 1.46–1.29 (m, 12H), 0.95 (d, J = 6.5 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.42, 132.05, 130.09, 114.30, 80.46, 72.47, 67.92, 59.54, 43.15, 38.91, 37.49, 33.99, 32.82, 29.39, 28.74, 28.16, 26.05, 15.03; LC–MS (ESI) m/z $[M]^+$ 422.2.

*4.1.6. Preparation of (R)-6-bromo-N-(1-(4-methoxyphenyl)propan-2-yl)-N-(prop-2-ynyl)hexan-1-amine (**10**)*

A mixture of compound **4** (0.10 g, 0.5 mmol), 1,6-dibromohexane (0.30 g, 1.25 mmol), and potassium carbonate (0.21 g, 1.5 mmol) in

acetonitrile (5 mL) was refluxed for 16 h. After the mixture was filtered, solvent was evaporated and the residue was purified on silica gel chromatography to afford compound **10** as a colourless oil (70.1 mg, 38.3%). IR (KBr): 3296, 2931, 2858, 1608, 1212, 1459, 1245, 1037 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.08 (d, J = 8.5 Hz, 2H), 6.82 (d, J = 8.5 Hz, 2H), 3.79 (s, 3H), 3.44 (d, J = 2.0 Hz, 2H), 3.40 (t, J = 6.8 Hz, 2H), 3.04–2.91 (m, 2H), 2.61 (t, J = 7.2 Hz, 2H), 2.37 (dd, J = 13.1, 9.1 Hz, 1H), 2.18 (s, 1H), 1.90–1.80 (m, 2H), 1.45 (ddd, J = 14.8, 11.0, 7.4 Hz, 4H), 1.35–1.29 (m, 2H), 0.98 (d, J = 6.5 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.79, 132.59, 130.10, 113.61, 81.47, 72.03, 58.99, 55.23, 49.20, 39.37, 39.02, 33.94, 32.80, 28.06, 26.48, 15.56; LC–MS (ESI) m/z $[M]^+$ 366.2.

4.1.7. Preparation of (R)-N1-(1-(4-methoxyphenyl)propan-2-yl)-N1-(prop-2-ynyl)-N6-(1,2,3,4-tetrahydroacridin-9-yl)hexane-1,6-diamine (**11**)

A mixture of tacrine (56.5 mg, 0.29 mmol), KOH (31.9 mg, 0.57 mmol) in DMSO (5 mL) was stirred for 20 min, and then compound **10** (70.1 mg, 0.19 mmol) was added. The mixture was stirred under nitrogen atmosphere at room temperature for 24 h (monitored by TLC) and extracted with *n*-butanol. After the solvent was removed in vacuo, the residue was purified on silica gel chromatography to afford compound **11** as a colourless oil (54.3 mg, 59.1%); Purity 97% (by HPLC); LC–MS (ESI) m/z $[M]^+$ 484.4. IR (KBr): 3303, 2929, 2857, 1728, 1609, 1583, 1508, 1246, 1141, 1035 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, J = 8.4 Hz, 1H), 7.90 (d, J = 8.4 Hz, 1H), 7.57–7.51 (m, 1H), 7.34 (t, J = 7.6 Hz, 1H), 7.07 (d, J = 8.5 Hz, 2H), 6.81 (d, J = 8.5 Hz, 2H), 3.77 (s, 3H), 3.52–3.40 (m, 4H), 3.06 (s, 2H), 3.00–2.88 (m, 2H), 2.71 (s, 2H), 2.60 (dd, J = 13.1, 6.0 Hz, 2H), 2.35 (dd, J = 13.0, 9.2 Hz, 1H), 2.17 (t, J = 2.0 Hz, 1H), 1.96–1.87 (m, 4H), 1.65 (dd, J = 14.6, 7.4 Hz, 2H), 1.49–1.43 (m, 2H), 1.42–1.36 (m, 2H), 1.33 (d, J = 6.7 Hz, 2H), 0.97 (d, J = 6.5 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.50, 157.81, 150.74, 147.55, 132.57, 130.09, 128.80, 128.22, 123.56, 122.82, 120.29, 115.91, 113.63, 81.45, 72.02, 59.00, 55.22, 49.51, 49.23, 39.36, 39.04, 34.08, 31.78, 28.15, 27.10, 26.89, 24.82, 23.09, 22.82, 15.56. HRMS calcd for $C_{32}H_{41}N_3O$ $[M + H]^+$: 484.3322, found: 484.3337.

4.1.8. General procedure for preparation of ***8***

A mixture of 1,2,3,4-tetrahydroacridin-9-amine (89.2 mg, 0.45 mmol), KOH (50.5 mg, 0.90 mmol) in DMSO (5 mL) was stirred for 20 min, then compound **7** (0.30 mmol) was added. The mixture was stirred under nitrogen atmosphere at room temperature for 24 h and then extracted with *n*-butanol. After the solvent was removed in vacuo, the residue was purified on silica gel chromatography.

4.1.8.1. (R)-N-(2-(4-(2-(methyl(prop-2-ynyl)amino)propyl)phenoxy)ethyl)-1,2,3,4-tetrahydroacridin-9-amine (***8a***).

Colourless oil (29.6 mg, 23.1%); Purity 96% (by HPLC); LC–MS (ESI) m/z $[M]^+$ 428.2. IR (KBr): 3296, 2930, 2863, 2792, 1724, 1611, 1583, 1506, 1242, 1126, 1043 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, J = 8.3 Hz, 1H), 7.92 (d, J = 8.3 Hz, 1H), 7.59–7.51 (m, 1H), 7.36 (t, J = 7.3 Hz, 1H), 7.10 (d, J = 7.6 Hz, 2H), 6.82 (d, J = 8.0 Hz, 2H), 4.02 (s, 2H), 3.82 (d, J = 4.0 Hz, 2H), 3.42 (s, 2H), 3.05 (d, J = 5.7 Hz, 2H), 2.95 (dd, J = 17.0, 9.9 Hz, 2H), 2.77 (d, J = 5.0 Hz, 2H), 2.41 (s, 3H), 2.34 (d, J = 9.7 Hz, 1H), 2.24 (s, 1H), 1.94–1.79 (m, 4H), 0.95 (d, J = 5.0 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.82, 156.69, 150.07, 147.42, 133.06, 130.29, 128.85, 128.33, 124.05, 122.67, 120.90, 118.01, 114.44, 80.40, 72.49, 67.40, 59.43, 48.47, 43.15, 38.93, 37.44, 34.02, 24.56, 22.96, 22.78, 14.98. HRMS calcd for $C_{28}H_{33}N_3O$ $[M + H]^+$: 428.2696, found: 428.2695.

4.1.8.2. (R)-N-(3-(4-(2-(methyl(prop-2-ynyl)amino)propyl)phenoxy)propyl)-1,2,3,4-tetrahydroacridin-9-amine (***8b***).

Colourless oil (36.5%); Purity 99% (by HPLC); LC–MS (ESI) m/z $[M]^+$ 442.3. IR (KBr): 3297, 2932, 2865, 2792, 1612, 1582, 1507, 1241, 1123, 1043 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, J = 8.4 Hz, 1H), 7.90 (d, J = 8.4 Hz, 1H), 7.54 (t, J = 7.6 Hz, 1H), 7.30 (t, J = 7.6 Hz, 1H), 7.10 (d, J = 8.5 Hz, 2H), 6.84 (d, J = 8.4 Hz, 2H), 4.11 (t, J = 5.6 Hz, 2H), 3.70 (d, J = 4.7 Hz, 2H), 3.42 (s, 2H), 3.05 (t, J = 5.9 Hz, 2H), 2.95 (ddd, J = 9.8, 9.2, 3.3 Hz, 2H), 2.74 (t, J = 5.7 Hz, 2H), 2.41 (s, 3H), 2.35 (dd, J = 12.6, 9.2 Hz, 1H), 2.24 (s, 1H), 2.15–2.08 (m, 2H), 1.94–1.85 (m, 4H), 0.96 (d, J = 6.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.61, 156.91, 150.55, 147.58, 132.77, 130.26, 128.86, 128.20, 123.66, 122.74, 120.39, 116.40, 114.26, 80.44, 72.48, 66.44, 59.47, 47.24, 43.15, 38.94, 37.46, 34.11, 30.83, 25.00, 23.07, 22.82, 15.00. HRMS calcd for $C_{29}H_{35}N_3O$ $[M + H]^+$: 442.2853, found: 442.2863.

4.1.8.3. (R)-N-(4-(4-(2-(methyl(prop-2-ynyl)amino)propyl)phenoxy)butyl)-1,2,3,4-tetrahydroacridin-9-amine (***8c***).

Colourless oil (52.6%); Purity 96% (by HPLC); LC–MS (ESI) m/z $[M]^+$ 456.2; IR (KBr): 3300, 2930, 2864, 2792, 1725, 1609, 1582, 1507, 1244, 1128, 1038 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, J = 8.4 Hz, 1H), 7.90 (d, J = 8.4 Hz, 1H), 7.54 (t, J = 7.6 Hz, 1H), 7.33 (t, J = 7.6 Hz, 1H), 7.08 (d, J = 8.5 Hz, 2H), 6.80 (d, J = 8.5 Hz, 2H), 3.97 (t, J = 5.4 Hz, 2H), 3.56 (s, 2H), 3.41 (s, 2H), 3.05 (d, J = 6.0 Hz, 2H), 3.00–2.89 (m, 2H), 2.72 (t, J = 5.3 Hz, 2H), 2.41 (s, 3H), 2.35–2.30 (m, 1H), 2.24 (s, 1H), 1.95–1.88 (m, 4H), 1.88–1.83 (m, 4H), 0.95 (d, J = 6.6 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.56, 157.12, 150.57, 147.53, 132.45, 130.18, 128.82, 128.25, 123.67, 122.71, 120.31, 116.14, 114.30, 80.42, 72.47, 67.41, 59.48, 49.12, 43.14, 38.92, 37.46, 34.06, 28.57, 26.78, 24.87, 23.06, 22.79, 14.99. HRMS calcd for $C_{30}H_{37}N_3O$ $[M + H]^+$: 456.3009, found: 456.3003.

4.1.8.4. (R)-N-(5-(4-(2-(methyl(prop-2-ynyl)amino)propyl)phenoxy)pentyl)-1,2,3,4-tetrahydroacridin-9-amine (***8d***).

Colourless oil (47.8%); Purity 99% (by HPLC); LC–MS (ESI) m/z $[M]^+$ 470.3. IR (KBr): 3299, 2930, 2861, 2793, 1722, 1611, 1581, 1508, 1243, 1133, 1038 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, J = 8.5 Hz, 1H), 7.90 (d, J = 8.4 Hz, 1H), 7.55 (dd, J = 8.2, 7.0 Hz, 1H), 7.34 (t, J = 7.6 Hz, 1H), 7.07 (d, J = 8.4 Hz, 2H), 6.79 (d, J = 8.5 Hz, 2H), 3.93 (t, J = 6.2 Hz, 2H), 3.51 (t, J = 6.9 Hz, 2H), 3.41 (s, 2H), 3.06 (s, 2H), 2.98–2.88 (m, 2H), 2.72 (s, 2H), 2.41 (s, 3H), 2.33 (dd, J = 12.6, 9.4 Hz, 1H), 2.23 (s, 1H), 1.95–1.86 (m, 4H), 1.80 (dd, J = 14.2, 6.7 Hz, 2H), 1.76–1.70 (m, 2H), 1.59 (dd, J = 15.0, 7.9 Hz, 2H), 0.95 (d, J = 6.7 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.55, 157.26, 150.63, 147.55, 132.29, 130.14, 128.83, 128.23, 123.64, 122.74, 120.35, 116.13, 114.28, 80.44, 72.44, 67.52, 59.50, 49.37, 43.14, 38.92, 37.46, 34.09, 31.47, 29.05, 24.82, 23.59, 23.07, 22.80, 15.00. HRMS calcd for $C_{31}H_{39}N_3O$ $[M + H]^+$: 470.3166, found: 470.3176.

4.1.8.5. (R)-N-(6-(4-(2-(methyl(prop-2-ynyl)amino)propyl)phenoxy)hexyl)-1,2,3,4-tetrahydroacridin-9-amine (***8e***).

Colourless oil (42.7%); Purity 98% (by HPLC); LC–MS (ESI) m/z $[M]^+$ 484.2. IR (KBr): 3308, 2929, 2857, 2795, 1612, 1582, 1511, 1219, 1134, 1037 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, J = 8.5 Hz, 1H), 7.90 (d, J = 8.5 Hz, 1H), 7.54 (t, J = 7.6 Hz, 1H), 7.33 (t, J = 7.6 Hz, 1H), 7.07 (d, J = 8.3 Hz, 2H), 6.83–6.75 (m, 2H), 3.92 (t, J = 6.3 Hz, 2H), 3.48 (t, J = 6.7 Hz, 2H), 3.41 (s, 2H), 3.06 (s, 2H), 2.93 (ddd, J = 11.6, 10.7, 3.8 Hz, 2H), 2.71 (s, 2H), 2.40 (s, 3H), 2.33 (dd, J = 12.5, 9.4 Hz, 1H), 2.23 (s, 1H), 1.95–1.86 (m, 4H), 1.76 (dd, J = 13.0, 6.5 Hz, 2H), 1.72–1.65 (m, 2H), 1.48 (d, J = 3.2 Hz, 4H), 0.95 (d, J = 6.3 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.49, 157.32, 150.69, 147.52, 132.20, 130.12, 128.78, 128.23, 123.60, 122.79, 120.30, 115.99, 114.29, 80.45, 72.47, 67.67, 59.50, 49.41, 43.14, 38.91, 37.46, 34.07, 31.70, 29.21, 26.72, 25.91, 24.81, 23.07, 22.80, 15.00. HRMS calcd for $C_{32}H_{41}N_3O$ $[M + H]^+$: 484.3322, found: 484.3342.

4.1.8.6. (R)-N-(8-(4-(2-(methyl(prop-2-ynyl)amino)propyl)phenoxy)octyl)-1,2,3,4-tetrahydroacridin-9-amine (***8f***).

Colourless oil (44.6%);

Purity 99% (by HPLC); LC–MS (ESI) *m/z* $[M]^+$ 512.3. IR (KBr): 3302, 2926, 2855, 1727, 1611, 1580, 1508, 1244, 1135, 1034 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, J = 8.5 Hz, 1H), 7.91 (d, J = 8.5 Hz, 1H), 7.58–7.51 (m, 1H), 7.34 (t, J = 7.6 Hz, 1H), 7.07 (d, J = 8.4 Hz, 2H), 6.80 (d, J = 8.5 Hz, 2H), 3.91 (t, J = 6.4 Hz, 2H), 3.49 (d, J = 6.8 Hz, 2H), 3.42 (s, 2H), 3.06 (s, 2H), 2.99–2.91 (m, 2H), 2.71 (s, 2H), 2.41 (s, 3H), 2.35–2.30 (m, 1H), 2.23 (s, 1H), 1.96–1.87 (m, 4H), 1.78–1.72 (m, 2H), 1.67 (dd, J = 13.6, 6.8 Hz, 2H), 1.44–1.34 (m, 8H), 0.95 (d, J = 6.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.40, 157.40, 150.80, 147.44, 132.12, 130.10, 128.69, 128.27, 123.57, 122.82, 120.22, 115.83, 114.3, 80.44, 72.44, 67.87, 59.51, 49.51, 43.13, 38.91, 37.46, 33.99, 31.76, 29.28, 29.25, 26.86, 25.98, 24.79, 23.06, 22.78, 15.00. HRMS calcd for $C_{34}H_{45}N_3O$ $[M + H]^+$: 512.3635, found: 512.3644.

*4.1.8.7. (R)-N-(9-(4-(2-(methyl(prop-2-ynyl)amino)propyl)phenoxy) nonyl)-1,2,3,4-tetrahydroacridin-9-amine (**8g**).* Colourless oil (41.8%); Purity 97% (by HPLC); LC–MS (ESI) *m/z* $[M]^+$ 526.3. IR (KBr): 3304, 2927, 2856, 2794, 1610, 1584, 1507, 1219, 1134, 1037 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, J = 8.5 Hz, 1H), 7.90 (d, J = 8.5 Hz, 1H), 7.54 (t, J = 7.6 Hz, 1H), 7.34 (t, J = 7.6 Hz, 1H), 7.07 (d, J = 8.4 Hz, 2H), 6.81 (d, J = 8.5 Hz, 2H), 3.92 (t, J = 6.5 Hz, 2H), 3.48 (t, J = 7.2 Hz, 2H), 3.41 (s, 2H), 3.06 (s, 2H), 2.99–2.89 (m, 2H), 2.71 (s, 2H), 2.41 (s, 3H), 2.33 (dd, J = 12.7, 9.3 Hz, 1H), 2.23 (s, 1H), 1.95–1.87 (m, 4H), 1.75 (dd, J = 14.5, 6.8 Hz, 2H), 1.65 (dd, J = 14.5, 7.4 Hz, 2H), 1.45–1.29 (m, 10H), 0.95 (d, J = 6.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.49, 157.42, 150.74, 147.57, 132.10, 130.10, 128.82, 128.20, 123.54, 122.82, 120.29, 115.89, 114.31, 80.46, 72.44, 67.92, 59.52, 49.53, 43.14, 38.92, 37.47, 34.10, 31.78, 29.42, 29.31, 29.28, 29.27, 26.92, 26.03, 24.81, 23.09, 22.82, 15.01. HRMS calcd for $C_{35}H_{47}N_3O$ $[M + H]^+$: 526.3792, found: 526.3793.

*4.1.8.8. (R)-N-(10-(4-(2-(methyl(prop-2-ynyl)amino)propyl)phenoxy)decyl)-1,2,3,4-tetrahydroacridin-9-amine (**8h**).* Colourless oil (40.7%); Purity 96% (by HPLC); LC–MS (ESI) *m/z* $[M]^+$ 540.3. IR (KBr): 3303, 2927, 2855, 2793, 1727, 1611, 1581, 1507, 1243, 1125, 1039 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, J = 8.4 Hz, 1H), 7.91 (d, J = 8.3 Hz, 1H), 7.54 (t, J = 7.5 Hz, 1H), 7.34 (t, J = 7.5 Hz, 1H), 7.07 (d, J = 7.6 Hz, 2H), 6.81 (d, J = 7.6 Hz, 2H), 3.92 (t, J = 6.2 Hz, 2H), 3.48 (t, J = 6.7 Hz, 2H), 3.41 (s, 2H), 3.07 (s, 2H), 2.94 (dd, J = 18.0, 9.9 Hz, 2H), 2.71 (s, 2H), 2.41 (s, 3H), 2.36–2.29 (m, 1H), 2.23 (s, 1H), 1.97–1.86 (m, 4H), 1.79–1.72 (m, 2H), 1.69–1.62 (m, 2H), 1.47–1.28 (m, 12H), 0.95 (d, J = 6.0 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.33, 157.42, 150.86, 147.38, 132.08, 130.09, 128.62, 128.29, 123.56, 122.87, 120.18, 115.75, 114.31, 80.45, 72.45, 67.94, 59.52, 49.52, 43.13, 38.91, 37.46, 33.96, 31.77, 29.43, 29.33, 26.92, 26.05, 24.77, 23.06, 22.77, 15.00. HRMS calcd for $C_{36}H_{49}N_3O$ $[M + H]^+$: 540.3948, found: 540.3953.

*4.1.9. General procedure for preparation of **9***

A mixture of 6-chloro-1,2,3,4-tetrahydroacridin-9-amine (104.7 mg, 0.45 mmol), KOH (50.5 mg, 0.90 mmol) in DMSO (5 mL) was stirred for 20 min, then compound **7** (0.30 mmol) was added. The mixture was stirred under nitrogen atmosphere at room temperature for 24 h, and then was extracted with *n*-butanol. After the solvent was removed in vacuo, the residue was purified on silica gel chromatography.

*4.1.9.1. (R)-6-chloro-N-(3-(4-(2-(methyl(prop-2-ynyl)amino)propyl) phenoxy)propyl)-1,2,3,4-tetrahydroacridin-9-amine (**9a**).* Colourless oil (45.1 mg, 31.6%); Purity 95% (by HPLC); LC–MS (ESI) *m/z* $[M]^+$ 476.2. IR (KBr): 3303, 2932, 2866, 2793, 1607, 1581, 1509, 1240, 1222, 1124, 1043 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.89 (dd, J = 7.6, 5.5 Hz, 2H), 7.22 (dd, J = 9.0, 1.5 Hz, 1H), 7.11 (d, J = 8.3 Hz, 2H), 6.83 (d, J = 8.3 Hz, 2H), 4.12 (t, J = 5.4 Hz, 2H), 3.71 (dd, J = 11.6, 5.8 Hz, 2H), 3.42 (s, 2H), 3.05–2.89 (m, 4H), 2.70 (s, 2H), 2.41 (s, 3H), 2.36 (dd, J = 12.4, 9.0 Hz, 1H), 2.24 (d, J = 1.6 Hz, 1H), 2.12 (dt, J = 11.8, 5.9 Hz, 2H), 1.94–1.82 (m, 4H), 0.96 (d, J = 6.3 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.67, 156.81, 150.67, 148.20, 133.91, 132.88, 130.29, 127.64, 124.48, 124.26, 118.54, 116.23, 114.23, 80.42, 72.49, 66.48, 59.44, 47.48, 43.15, 38.94, 37.44, 34.05, 30.78, 24.79, 22.92, 22.66, 14.99. HRMS calcd for $C_{29}H_{34}N_3OCl$ $[M + H]^+$: 476.2463, found: 476.2454.

*4.1.9.2. (R)-6-chloro-N-(5-(4-(2-(methyl(prop-2-ynyl)amino) propyl)phenoxy)pentyl)-1,2,3,4-tetrahydroacridin-9-amine (**9b**).* Colourless oil (40.5%); Purity 97% (by HPLC); LC–MS (ESI) *m/z* $[M]^+$ 504.2. IR (KBr): 3306, 2934, 2864, 2796, 1723, 1609, 1582, 1510, 1243, 1219, 1125, 1041 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.89 (dd, J = 5.5, 3.2 Hz, 2H), 7.29–7.23 (m, 1H), 7.07 (d, J = 8.5 Hz, 2H), 6.79 (d, J = 8.6 Hz, 2H), 3.94 (t, J = 6.2 Hz, 2H), 3.51 (t, J = 7.1 Hz, 2H), 3.42 (s, 2H), 3.03 (s, 2H), 2.94 (ddd, J = 9.9, 9.4, 3.2 Hz, 2H), 2.67 (s, 2H), 2.41 (s, 3H), 2.33 (dd, J = 12.6, 9.3 Hz, 1H), 2.24 (s, 1H), 1.94–1.86 (m, 4H), 1.83–1.78 (m, 2H), 1.76–1.70 (m, 2H), 1.63–1.54 (m, 2H), 0.95 (d, J = 6.6 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.64, 157.23, 150.70, 148.21, 133.94, 132.33, 130.15, 127.67, 124.48, 124.27, 118.52, 115.99, 114.27, 80.44, 72.46, 67.46, 59.49, 49.47, 43.14, 38.92, 37.45, 34.07, 31.46, 29.00, 24.60, 23.56, 22.93, 22.65, 14.99. HRMS calcd for $C_{31}H_{38}N_3OCl$ $[M + H]^+$: 504.2776, found: 504.2792.

*4.1.9.3. (R)-6-chloro-N-(6-(4-(2-(methyl(prop-2-ynyl)amino)propyl) phenoxy)hexyl)-1,2,3,4-tetrahydroacridin-9-amine (**9c**).* Colourless oil (35.1%); Purity 98% (by HPLC); LC–MS (ESI) *m/z* $[M]^+$ 518.2. IR (KBr): 3304, 2932, 2860, 2794, 1607, 1580, 1510, 1243, 1126, 1039 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.89 (d, J = 7.6 Hz, 2H), 7.26 (d, J = 6.4 Hz, 1H), 7.07 (d, J = 7.8 Hz, 2H), 6.79 (d, J = 7.8 Hz, 2H), 3.93 (d, J = 5.9 Hz, 2H), 3.49 (s, 2H), 3.41 (s, 2H), 3.02 (s, 2H), 2.94 (dd, J = 17.9, 8.9 Hz, 2H), 2.66 (s, 2H), 2.41 (s, 3H), 2.37–2.29 (m, 1H), 2.23 (s, 1H), 1.95–1.86 (m, 4H), 1.82–1.74 (m, 2H), 1.72–1.65 (m, 2H), 1.57–1.41 (m, 4H), 0.95 (d, J = 5.9 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.61, 157.31, 150.75, 148.21, 133.95, 132.24, 130.13, 127.65, 124.51, 124.24, 118.49, 115.87, 114.28, 80.44, 72.45, 67.64, 59.50, 49.52, 43.13, 38.91, 37.46, 34.06, 31.71, 29.19, 26.68, 25.90, 24.59, 22.93, 22.66, 14.99. HRMS calcd for $C_{32}H_{40}N_3OCl$ $[M + H]^+$: 518.2933, found: 518.2959.

*4.1.9.4. (R)-6-chloro-N-(8-(4-(2-(methyl(prop-2-ynyl)amino)propyl) phenoxy)octyl)-1,2,3,4-tetrahydroacridin-9-amine (**9d**).* Colourless oil (33.6%); Purity 97% (by HPLC); LC–MS (ESI) *m/z* $[M]^+$ 546.2. IR (KBr): 3306, 2930, 2858, 2796, 1725, 1608, 1580, 1510, 1244, 1219, 1123, 1040 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.95–7.85 (m, 2H), 7.29–7.24 (m, 1H), 7.07 (d, J = 8.4 Hz, 2H), 6.80 (d, J = 8.5 Hz, 2H), 3.92 (t, J = 6.4 Hz, 2H), 3.48 (t, J = 7.1 Hz, 2H), 3.41 (s, 2H), 3.03 (s, 2H), 2.93 (ddd, J = 11.6, 10.5, 3.9 Hz, 2H), 2.66 (s, 2H), 2.41 (s, 3H), 2.33 (dd, J = 12.6, 9.3 Hz, 1H), 2.23 (s, 1H), 1.96–1.86 (m, 4H), 1.79–1.72 (m, 2H), 1.65 (dd, J = 14.1, 7.2 Hz, 2H), 1.47–1.33 (m, 8H), 0.95 (d, J = 6.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.44, 157.40, 150.88, 148.04, 134.02, 132.15, 130.10, 127.49, 124.58, 124.22, 118.38, 115.66, 114.31, 80.46, 72.43, 67.86, 59.51, 49.58, 43.14, 38.92, 37.45, 33.95, 31.75, 29.28, 29.24, 26.82, 25.97, 24.56, 22.92, 22.62, 15.01. HRMS calcd for $C_{34}H_{44}N_3OCl$ $[M + H]^+$: 546.3246, found: 546.3244.

4.2. Biological assay

4.2.1. In vitro inhibition of AChE and BuChE

Acetylcholinesterase (AChE, from the electric eel), butylcholinesterase (BuChE, from equine serum), 5,5′-dithiobis-(2-nitrobenzoic acid) (Ellman's reagent, DTNB), acetylthiocholinechloride (ATC) and butylthiocholine chloride (BTC) were purchased from Sigma–Aldrich. All *in vitro* AChE assays were performed in 0.1 M KH_2PO_4/K_2HPO_4 buffer (pH 8.0), using a Shimadzu UV-2450 Spectrophotometer. The ChE solutions with a concentration of 2.0

units/mL were prepared 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. Test compounds were added to the assay solution and pre-incubated at 37 °C with the enzyme for 15 min, followed by the addition of substrate. Inhibitory activity was determined at 37 °C by measuring the increase in absorbance at 412 nm at 1 min intervals. Calculations were performed according to the method of Ellman et al. [25]. Each concentration was assayed in triplicate.

4.2.2. Determination of MAO activity

Adequate amounts of recombinant hMAO-A or hMAO-B (Sigma–Aldrich) were acquired and adjusted to 12.5 and 75 μg/mL for hMAO-A and hMAO-B, respectively. Test drugs (20 μL) and MAO (80 μL) were incubated for 15 min at 37 °C in a flat-black-bottom 96-well microtest plate in the dark. The reaction was initiated by adding 200 μM of Amplex Red reagent, 2 U/mL of horseradish peroxidase, and 2 mM of *p*-tyramine for hMAO-A or 2 mM benzylamine for hMAO-B, and the reactions were incubated at 37 °C for 20 min. The results were quantified in a multidetection microplate fluorescence reader based on the fluorescence generated (excitation, 545 nm; emission, 590 nm).

The specific fluorescence emission was calculated after subtracting the background, which was determined by using a solution containing all the components except hMAO.

4.2.3. Kinetic characterisation of AChE inhibition

The kinetic characterisation of AChE was performed using a reported method [26]. Test compounds and AChE were pre-incubated at 37 °C for 15 min, followed by the addition of substrate. Kinetic characterisation of the hydrolysis of ATC catalysed by AChE was recorded by spectrometrically at 412 nm. A parallel control was made for an assay solution with no inhibitor. 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. The reciprocal plot slopes were plotted as a function of the concentration of the inhibitors in a weighted analysis.

4.2.4. Reversibility and irreversibility study

The experiment was performed accordance to a previously reported method [27]. Adequate amounts of recombinant monoamine oxidase with or without test drugs were incubated for 15 min at 37 °C. An aliquot of this incubated solution was stored at 4 °C for the subsequent measurement of MAO activity. Another aliquot was transferred to an Amicon Ultra-0.5 Centrifugal Filter Unit with Ultracel-30 membrane (Millipore) and centrifuged (4 °C, 9000*g*, 20 min) 3 times. The enzyme was obtained and then used for the subsequent measurement of activity using a similar method to the one described above (in section Determination of MAO Activity). The corresponding values of the percent (%) hMAO-B inhibition were separately calculated for samples with and without repeated washing.

4.2.5. Molecular modelling

The molecular modelling was performed according to the previously reported method [26b]. The simulation system was built on the X-ray structure of the bis-tacrine–AChE complex, which was obtained from the Protein Data Bank (PDB entry 2CMF). The original ligand was removed, while the water molecules present in the PDB file were maintained in their native positions. The 3D structures of the derivatives were generated and optimised by the Discovery studio 2.1 package (Accelrys Inc., San Diego, CA). The CDOCKER program in the Discovery studio 2.1 software was used to perform docking simulations, which provided full flexibility for the ligand.

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