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RESEARCH ARTICLE

Tacrine–propargylamine derivatives with improved acetylcholinesterase inhibitory activity and lower hepatotoxicity as a potential lead compound for the treatment of Alzheimer's disease

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Abstract

A series of tacrine–propargylamine derivatives were synthesised and evaluated as possible anti-Alzheimer's disease (AD) agents. Among these derivatives, compounds **3a** and **3b** exhibited superior activities and a favourable balance of AChE and BuChE activities (**3a**: IC_{50} values of 51.3 and 77.6 nM; **3b**: IC_{50} values of 11.2 and 83.5 nM). Compounds **3a** and **3b** also exhibited increased hAChE inhibitory activity compared with tacrine by approximately 5- and 28-fold, respectively, and low neurotoxicity. Importantly, these compounds also had lower hepatotoxicity than tacrine. Based on these results, compounds **3a** and **3b** could be considered as potential lead compounds for the treatment of AD and other AChE related diseases, such as schizophrenia, glaucoma and myasthenia gravis.

Keywords

Alzheimer's disease, cholinesterase inhibitor, hepatotoxicity, neurotoxicity

History

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Introduction

Alzheimer's disease (AD) is a progressive neuro-degenerative disorder which was first described by German psychiatrist and neuropathologist Alois Alzheimer in 1906. AD is the fourth leading cause of death in people over 65 years old worldwide. This disease is characterised by the atrophy of cholinergic neurons, memory loss, deterioration of cognition, loss of speech, behavioural abnormalities and, eventually, death[1]. Although the aetiology of AD is still elusive, several hallmarks, such as low levels of acetylcholine (ACh), β-amyloid (Aβ) deposits, τ-protein aggregation, oxidative stress and the dyshomeostasis of biometals, are thought to play important roles in the development of AD[2–4]. Due to the complex pathogenesis of AD, to date, there is no ideal drug for the prevention or treatment of AD. Thus, the treatment of AD remains a challenge in the pharmaceutical community.

Based on the ''cholinergic hypothesis''[5], the primary pharmacological strategies for the treatment of AD are to improve cholinergic neurotransmission by decreasing the rate of decomposition of ACh at synapses in the brain with the use of acetylcholinesterase inhibitors (AChEIs). It is well-known that there are two major forms of cholinesterases in mammalian tissues, acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). In general, AChE displays a greater affinity for ACh and thus has greater activity for its hydrolysis as compared to BuChE. Studies indicate that in the case of AD, the level of AChE in certain brain regions is significantly reduced and the BuChE level is progressively increased, which is responsible for the level of ACh[6,7]. In fact, a portion of evidence suggests that the inhibition of BuChE can raise ACh levels and improves cognition in AD[8]. Currently, the most efficacious treatment approaches for AD approved by the FDA are four cholinesterase inhibitors (ChEIs)[9–11] (Figure 1), tacrine, donepezil, rivastigmine and galantamine, and a *N*-methyl-D-aspartate (NMDA) receptor antagonist, memantine, which was approved for the treatment of moderate to severe AD in 2003[12,13]. Considering that these drugs exhibit modest improvements in memory and cognitive function but do not appear to prevent or slow the progressive neuro-degeneration, it is still an important and urgent task for pharmaceutical chemists to find more appropriate chemical entities for the treatment of AD.

Tacrine, the first dual inhibitor of both ChEs approved by FDA in 1993, was withdrawn from the pharmaceutical market shortly after its approval due to its side effects such as hepatotoxicity[14]. Nevertheless, medicinal chemists remain interested in researching tacrine analogues or related new candidates[15–21]. In our previous work, we designed and synthesised a series of tacrine–ebselen derivatives as multifunctional anti-AD agents[22]. In this study, inspired by the compounds possessing a propargylamine group which have neuroprotective effects such as rasagiline, TVP-1022 and M30 (Figure 2)[23–27], we describe the design, synthesis and evaluation of a series of tacrine–propargylamine derivatives as anti-AD agents.

Materials and methods

Materials

The ^{1}H NMR and ^{13}C NMR spectra were recorded using TMS as the internal standard on a Bruker BioSpin GmbH spectrometer

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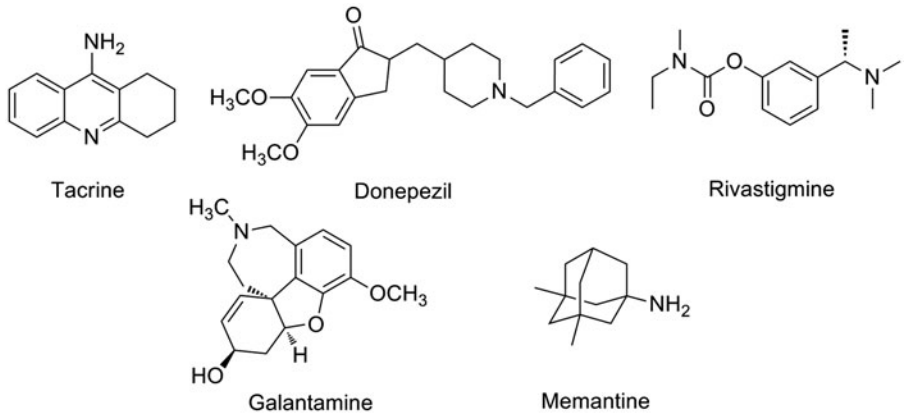
Figure 1. Tacrine, donepezil, rivastigmine, galantamine and memantine.



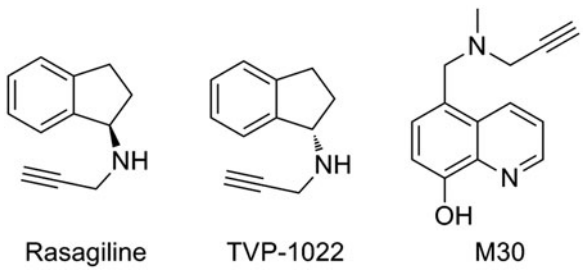


Figure 2. Structures of compounds with a propargylamine group (Rasagiline, TVP-1022 and M30).

(Östliche Rheinbrückenstr, Germany) at 400.132 MHz and 100.614 MHz, respectively. Coupling constants are given in Hz. MS spectra were recorded on an Agilent LC–MS 6120 instrument (California, USA) with an ESI mass selective detector. Flash column chromatography was performed with silica gel (200–300 mesh) purchased from Qingdao Haiyang Chemical Co. Ltd. (Qingdao, China). All the reactions were monitored by thin-layer chromatography on silica gel. The chemicals used in the chemistry section were procured from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) and all of the chemicals are analytical reagents. The purities of the synthesised compounds were confirmed to be higher than 95% by analytical HPLC (Agilent technologies 1200 series system) with a TC-C8 column (4.6×150 mm, 5 μm) and the compounds were eluted with CH_3OH/water (1% CH_3COONH_4, w/v) in ratios of 70:30–40:60 at a flow rate of 1.0 mL/min.

Chemistry

The synthetic pathway of the tacrine–propargylamine derivatives is shown in Scheme 1. First, tacrine (**2a**) and 6-chlorotacrine (**2b**) were synthesised following literature reports[28]. Then compound **2** was reacted with propargyl bromide in the presence of potassium hydroxide to give **3** (**3a**: 1.0 eq propargyl bromide, 1.2 eq potassium hydroxide, acetonitrile as the solvent; **3b**: 1.0 eq propargyl bromide, 1.2 eq potassium hydroxide, dimethyl sulphoxide/acetonitrile (1:3) as the mixed solvents; **3c**: 2.1 eq propargyl bromide, 2.4 eq potassium hydroxide, acetonitrile as the solvent).

To investigate the influence of the chain length between tacrine and the propargylamine moiety, compounds **7a** and **7b** were also synthesised from the known intermediate **6**[29], which was reacted with propargyl bromide in the presence of potassium hydroxide to yield the target compound **7** (**7a**: 1.0 eq propargyl bromide, 1.0 eq potassium carbonate, dichloromethane/acetonitrile (1:1) the mixed solvents; **7b**: 2.1 eq propargyl bromide, 2.1 eq potassium carbonate, acetonitrile as the solvent).

*General procedure for the synthesis of compound **2***

To a mixture of 2-aminobenzonitrile or 2-amino-4-chlorobenzonitrile (30 mmol) and zinc chloride (4.1 g, 30 mmol), cyclohexanone (36 mL) was added. After heating to 140 °C for 3 h, the mixture was cooled to room temperature and filtered. The filter cake was dispersed in water (50 mL) and stirred for 10 min. After adjusting the pH of the mixture to 12 with 10% sodium hydroxide, it was filtered. The filter cake was dispersed in ethanol (50 mL) and stirred for 10 min under reflux, then the mixture was filtered. The filtrate was concentrated and purified by recrystallised from toluene/water to produce the desired product.

*1,2,3,4-Tetrahydroacridin-9-amine (**2a**)*

White solid. 88% yield. ^{1}H NMR (400 MHz, DMSO) δ 8.12 (d, $J = 8.4$ Hz, 1H), 7.62 (d, $J = 8.4$ Hz, 1H), 7.51–7.43 (m, 1H), 7.26 (ddd, $J = 8.1$, 6.8, 1.2 Hz, 1H), 6.29 (s, 2H), 2.81 (t, $J = 5.7$ Hz, 2H), 2.54 (t, $J = 5.9$ Hz, 2H), 1.87–1.75 (m, 4H). LC/MS (ESI): m/z 199.1 $[M+H]^+$.

*6-Chloro-1,2,3,4-tetrahydroacridin-9-amine (**2b**)*

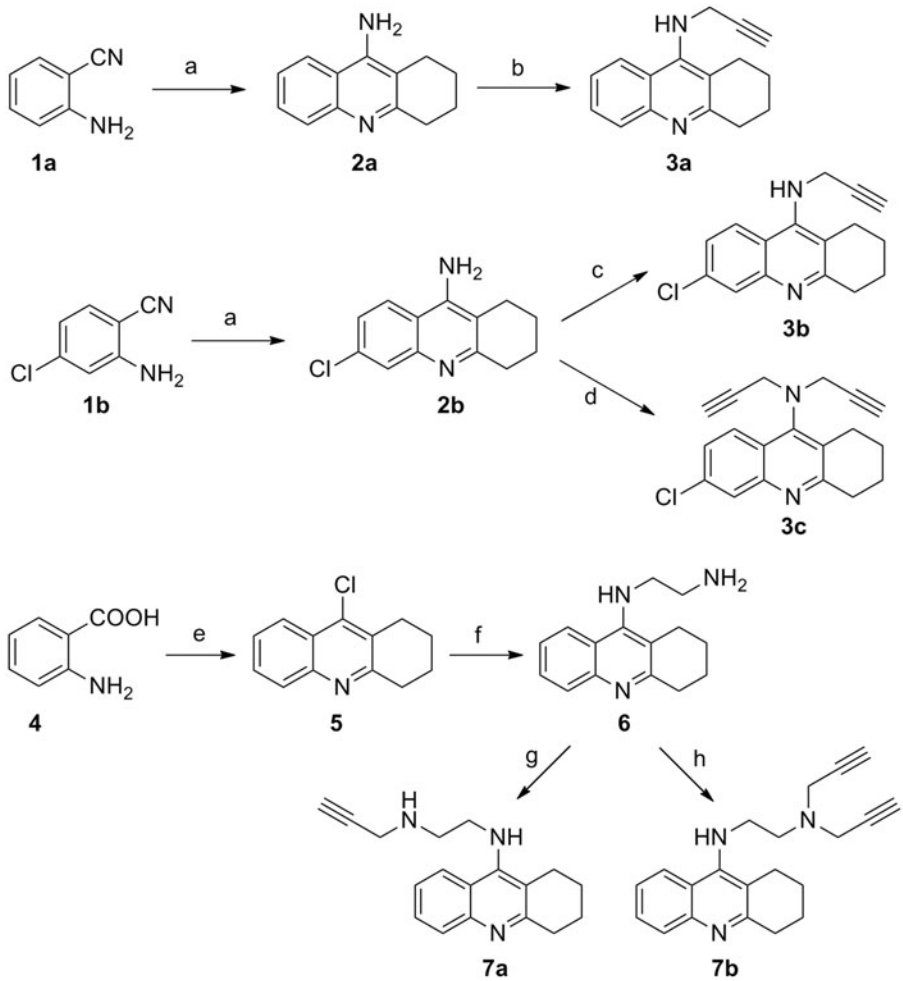
White solid. 69% yield. ^{1}H NMR (400 MHz, DMSO) δ 8.17 (d, $J = 9.0$ Hz, 1H), 7.62 (d, $J = 2.0$ Hz, 1H), 7.28 (s, 1H), 6.46 (s, 2H), 2.81 (d, $J = 5.5$ Hz, 2H), 2.52 (s, 2H), 1.80 (d, $J = 4.7$ Hz, 4H). LC/MS (ESI): m/z 233.2 $[M+H]^+$.

General procedure for the synthesis of compound 3

To a solution of compound **2a** or **2b** (2 mmol) in the corresponding solvents, potassium hydroxide was added. After the solution was stirred for 10 min at room temperature, propargyl bromide was added to the mixture. After stirring for 36 h, the mixture was filtered and the filtrate was evaporated under reduced pressure to provide crude **3a** and **3c**, which were purified by flash chromatography with dichloromethane/methanol (30:1, for **3a**) or petroleum ether/ethyl acetate (5:1, for **3c**) as the eluent. Simultaneously, compound **3b** was obtained by the addition of water to the reaction mixture, extraction with ethyl acetate, concentration and purification by flash chromatography.

*N-(prop-2-yn-1-yl)-1,2,3,4-tetrahydroacridin-9-amine (**3a**)*

Compound **2a** was treated with 1.0 eq propargyl bromide and 1.2 eq potassium hydroxide in acetonitrile according to the general procedure to give the desired product as a grey yellow solid (62% yield). m.p. 67.4–68.7 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (t, $J = 8.2$ Hz, 2H), 7.62–7.55 (m, 1H), 7.44–7.38 (m, 1H), 4.16 (s, 3H), 3.10 (t, $J = 6.1$ Hz, 2H), 2.85 (t, $J = 6.0$ Hz, 2H), 2.28



Scheme 1. Synthesis of tacrine–propargylamine derivatives. Reagents and conditions: (a) cyclohexanone, $ZnCl_2$, 140 °C; (b) propargyl bromide (1.0 eq), KOH (1.2 eq), CH_3CN, rt; (c) propargyl bromide (1.0 eq), KOH (1.2 eq), DMSO/CH_3CN = 1:3, rt; (d) propargyl bromide (2.1 eq), KOH (2.4 eq), CH_3CN, rt; (e) cyclohexanone, $POCl_3$, 0 °C reflux; (f) ethylenediamine, KI, 1-pentanol, 160 °C; (g) propargyl bromide (1.0 eq), K_2CO_3 (1.0 eq), DCM/CH_3CN = 1:1, rt; (h) propargyl bromide (2.1 eq), K_2CO_3 (2.1 eq), CH_3CN, rt.

(s, 1H), 1.99–1.87 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.98, 149.24, 147.40, 128.97, 128.42, 124.45, 122.32, 120.97, 118.92, 81.23, 72.53, 38.44, 34.07, 24.75, 22.94, 22.74. Purity: 99.6% by HPLC; LC/MS (ESI) *m/z*: $[M+H]^+$ 237.1.

*6-Chloro-N-(prop-2-yn-1-yl)-1,2,3,4-tetrahydroacridin-9-amine (**3b**)*

Compound **2b** was treated with 1.0 eq propargyl bromide and 1.2 eq potassium hydroxide in the mixed solvent of sulphoxide/acetonitrile (1:3) according to the general procedure to give the desired product as a grey yellow solid (42% yield). m.p. 67.4–68.7 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (d, J = 2.1 Hz, 1H), 7.85 (d, J = 9.0 Hz, 1H), 7.33 (dd, J = 9.0, 2.1 Hz, 1H), 4.15 (s, 3H), 3.06 (t, J = 5.6 Hz, 2H), 2.80 (t, J = 5.7 Hz, 2H), 2.29 (t, J = 1.6 Hz, 1H), 1.96–1.88 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.25, 149.37, 147.96, 134.19, 127.87, 125.24, 123.95, 119.31, 119.08, 80.93, 72.77, 38.54, 34.07, 24.62, 22.82, 22.60. Purity: 99.8% by HPLC; LC/MS (ESI) *m/z*: $[M+H]^+$ 271.1.

*6-Chloro-N,N-di(prop-2-yn-1-yl)-1,2,3,4-tetrahydroacridin-9-amine (**3c**)*

Compound **2b** was treated with 2.1 eq propargyl bromide and 2.4 eq potassium hydroxide in acetonitrile according to the general procedure to give the desired product as a grey yellow solid (71% yield). m.p. 67.4–68.7 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, J = 2.0 Hz, 1H), 7.93 (d, J = 9.0 Hz, 1H), 7.38 (dd, J = 9.0, 2.0 Hz, 1H), 4.16 (d, J = 2.2 Hz, 4H), 3.11 (t, J = 6.5 Hz, 2H), 2.96 (t, J = 6.4 Hz, 2H), 2.29 (d, J = 2.1 Hz, 2H), 1.99–1.92 (m, 2H), 1.90–1.80 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.86, 151.22, 148.31, 134.25, 129.32, 127.73, 126.30, 125.60, 124.46, 79.81, 72.80, 41.55, 33.94, 26.43, 22.69, 22.67. Purity: 98.9% by HPLC; LC/MS (ESI) *m/z*: $[M+H]^+$ 309.1.

*General procedure for the synthesis of compound **7***

To a solution of compound **6** (1 mmol) in the corresponding solvent, potassium carbonate was added. After the solution was stirred for 10 min at room temperature, propargyl bromide was added. The mixture was filtered after stirring for 12–16 h. The filtrate was evaporated under reduced pressure to provide the crude product, which was purified by flash chromatography with dichloromethane/methanol (20:1) as the eluent.

*N^1-(prop-2-yn-1-yl)-N^2-(1,2,3,4-tetrahydroacridin-9-yl)ethane-1,2-diamine (**7a**)*

Compound **6** was treated with 1.0 eq propargyl bromide and 1.0 eq potassium carbonate in a mixed solvent of dichloromethane/acetonitrile (1:1) according to the general procedure to give the desired product as a grey yellow solid (35% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, J = 8.5 Hz, 1H), 7.97 (d, J = 8.4 Hz, 1H), 7.55 (t, J = 7.6 Hz, 1H), 7.34 (t, J = 7.6 Hz, 1H), 3.63–3.56 (m, 2H), 3.49 (d, J = 2.3 Hz, 2H), 3.08 (s, 2H), 3.01–2.94 (m, 2H), 2.73 (s, 2H), 2.25 (t, J = 2.3 Hz, 1H), 1.96–1.80 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.61, 150.40, 145.33, 127.71, 126.71, 122.79, 121.97, 118.86, 114.78, 80.71, 70.78, 47.12, 46.67, 36.49, 32.28, 23.64, 21.91, 21.56. Purity: 98.8% by HPLC; LC/MS (ESI) *m/z*: $[M+H]^+$ 280.2.

*N^1,N^1-di(prop-2-yn-1-yl)-N^2-(1,2,3,4-tetrahydroacridin-9-yl)ethane-1,2-diamine (**7b**)*

Compound **6** was treated with 2.1 eq propargyl bromide and 2.1 eq potassium carbonate in acetonitrile according to the general procedure to give the desired product as a grey yellow solid (82% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (d, $J = 8.4$ Hz, 1H), 8.08 (d, $J = 8.5$ Hz, 1H), 7.62 (t, $J = 7.6$ Hz, 1H), 7.39 (t, $J = 7.6$ Hz, 1H), 3.76 (s, 2H), 3.53 (d, $J = 2.1$ Hz, 4H), 3.18 (s, 2H), 2.99–2.88 (m, 2H), 2.71 (s, 2H), 2.30 (s, 2H), 1.92 (s, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.74, 151.59, 142.88, 128.93, 124.68, 123.22, 122.25, 117.47, 113.12, 77.23 (2C), 72.72 (2C), 50.64, 44.11, 41.19 (2C), 28.68, 23.20, 21.58, 20.99. Purity: 98.9% by HPLC; LC/MS (ESI) *m/z*: $[M+H]^+$ 318.2.

Biological activity

Enzyme inhibition assays

Acetylcholinesterase (AChE, E.C. 3.1.1.7, from the electric eel), human acetylcholinesterase (hAChE, E.C. 3.1.1.7, from human erythrocytes), butyrylcholinesterase (BuChE, E.C. 3.1.1.8, from equine serum), 5,5′-dithiobis-(2-nitrobenzoic acid) (Ellman's reagent, DTNB), acetylthiocholine chloride (ATC) and butylthiocholine chloride (BTC) were purchased from Sigma Aldrich. Tacrine–propargylamine derivative hybrids were dissolved in DMSO and then diluted in PBS (0.1 M KH_2PO_4/K_2HPO_4 buffer solution, pH 8.0) to the final concentration.

All *in vitro* AChE assays were carried out in PBS, using a multifunctional microplate reader (Molecular Devices, Flex Station 3). The assay medium (PBS, 200 µL) consisted of the diluted compound (90 µL), enzyme (10 µL), 0.002 M substrate (50 µL) (ACh chloride solution) and 0.002 M DTNB (50 µL). The test compounds were added to the assay solution and preincubated at 37 °C with the enzyme for 15 min, followed by the addition of the mixture of substrate and DTNB. The inhibitory activity was determined by measuring the increase in absorbance at 412 nm at 1 min intervals at 37 °C. Calculations were performed according to the method of Ellman et al[30]. Each concentration was assayed in triplicate. Data from concentration-inhibition experiments with the inhibitors were subjected to nonlinear regression analysis using GraphPad Prism 5.0 Software, which gave estimates of the IC_{50} (concentration of drug resulting in 50% inhibition of the enzyme activity).

The *in vitro* BuChE (BuCh as the enzyme substrate) and hAChE assay (hAChE from human erythrocytes as the enzyme) was performed according to a method similar to that described above.

Kinetic characterisation of AChE inhibition

The kinetic characterisation of the AChE inhibitory activity was performed according to a published protocol[30]. Briefly, the test compound was added to the assay solution and incubated with the enzyme at 37 °C for 15 min, and then the mixture of substrate (the final concertrations of substrate were 0.05 mM, 0.0625 mM, 0.10 mM, 0.125 mM, 0.25 mM, 0.50 mM, respectively) and DTNB was added. The kinetic characterisation of the hydrolysis of ATC catalysed by AChE was performed spectrophotometrically at 412 nm. The parallel control experiments were carried out without inhibitor in the mixture.

Determination of neurotoxicity

Cell viability in the human neuroblastoma cell line, SH-SY5Y, was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay according to previously described procedures[31]. SH-SY5Y cells were routinely cultured at 37 °C in a humidified incubator with 5% CO_2 in Dulbecco's modified Eagle's medium/Nutrient Mixture F-12 (DMEM/F12 = 1:1, GIBCO) supplemented with 10% fetal calf serum (FCS, GIBCO), 1 mM glutamine, 100 U/mL penicillin and 100 µg/mL streptomycin. SH-SY5Y cells were seeded at a density of 3×10^3 cells/well in 96-well plates. After 24 h, the medium was removed and replaced with the test compounds at different concentrations for another 24 h at 37 °C. All compounds were dissolved in DMSO and diluted with fresh medium. The DMSO concentration of every well in final dilutions was 1% (including the controls). The cells were then incubated with MTT (0.5 mg/mL, final concentration) in PBS for 4 h. After the removal of MTT, the formazan crystals were dissolved in DMSO. The amount of formazan was measured at 570 nm. Cell viability was expressed as the percentage of viable cells compared with untreated control cells.

Determination of hepatotoxicity

Cell viability of human hepatic stellate cells (HSCs) was determined by the MTT assay. HSC were routinely cultured at 37 °C in a humidified incubator with 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM, GIBCO) supplemented with 10% foetal calf serum (FCS, GIBCO), 1 mM glutamine, 100 U/mL penicillin and 100 µg/mL streptomycin. HSC cells were seeded at a density of 3×10^3 cells/well in 96-well plates. After 24 h, the medium was removed and replaced with the test compounds at different concentrations for another 24 h at 37 °C. All compounds were dissolved in DMSO and diluted with fresh medium. The DMSO concentration of every well in final dilutions was 1% (including the controls). The cells were then incubated with MTT (0.5 mg/mL, final concentration) in PBS for 4 h. After the removal of MTT, the formazan crystals were dissolved in DMSO. The amount of formazan was measured at 570 nm. Cell viability was expressed as the percentage of viable cells compared with untreated control cells.

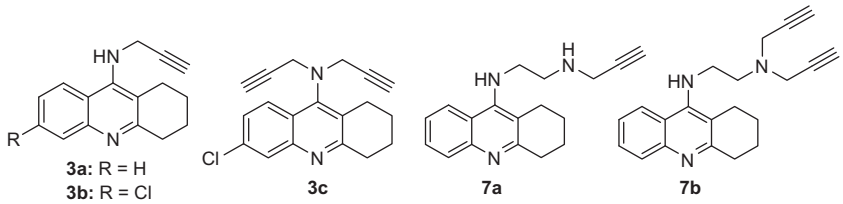
Results and discussion

In vitro inhibition studies of AChE and BuChE

To evaluate the potential application of target compounds for the treatment of AD, the AChE (electric eel) and BuChE (equine serum) inhibitory activities of the tacrine–propargylamine derivatives **3a–3c**, **7a** and **7b** were examined with tacrine as the reference standard by Ellman et al.'s spectroscopic method[29]. The IC_{50} values of eeAChE and BuChE inhibition are summarised in Table 1. The results of eeAChE inhibition of compounds showed that a propargyl substituted amino group in tacrine was beneficial to the inhibitory activities of eeAChE, for example, compound **3a** ($IC_{50} = 51.3$ nM) was more potent than tacrine ($IC_{50} = 105.8$ nM) by 2-fold improvement and **3b** ($IC_{50} = 11.2$ nM) was also more potent than 6-chlorotacrine ($IC_{50} = 23.5$ nM). However, di-propargyl group tacrine derivatives, such as **3c** and **7b**, provided relatively poor inhibitory activities (**3c**, $IC_{50} = 883.7$ nM; **7b**, $IC_{50} = 339.4$ nM, respectively). Compared with the IC_{50} values of compounds **3a** and **7a** (51.3 and 225.6 nM), we realised that the chain between tacrine and the propargylamine moiety decreased the inhibitory activities.

Most of the tacrine-propargyl derivatives substantially inhibited BChE. Compounds **3a** and **3b**, which had excellent AChE inhibitory activities, also had very good BuChE inhibitory activities (IC_{50} values: 77.6 and 83.5 nM, respectively). Similar to AChE inhibitory activities, compounds **3c** and **7b** also had relatively weaker BuChE inhibitory activities (**3c**: $IC_{50} = 5375.3$ nM; **7b**: $IC_{50} = 352.7$ nM) when compared with the mono-propargyl substituted tacrine derivatives **3a** and **3b**.

Table 1. *In vitro* inhibition and selectivity of eeAChE, BuChE and hAChE by tacrine and tacrine–propargylamine derivatives **3a–3c**, **7a** and **7b**.



	IC_{50}*(nM)		
Compound	eeAChE†	BuChE‡	Selectivity for eeAChE¶
3a	51.3 ± 2.8 (50.7 ± 1.9)§	77.6 ± 0.5	1.51
3b	11.2 ± 0.8 (9.4 ± 0.7)§	83.5 ± 1.7	7.46
3c	883.7 ± 79.1	5375.3 ± 345.0	6.08
7a	225.6 ± 14.5	104.3 ± 9.1	0.46
7b	339.4 ± 3.7	352.7 ± 15.9	1.04
Tacrine	105.8 ± 7.0 (265.2 ± 29.1)§	14.8 ± 0.9	0.14
6-Chlorotacrine	23.5 ± 9.0 (41.0 ± 1.2)§	539.1 ± 31.4	23.0

*Mean ± SD of at least three independent measurements.
†eeAChE from the electric eel.
‡BuChE from equine serum.
¶Selectivity for eeAChE = IC_{50} (BuChE)/IC_{50} (eeAChE).
§Values in parentheses are IC_{50} against human AChE(hAChE) of human erythrocytes.

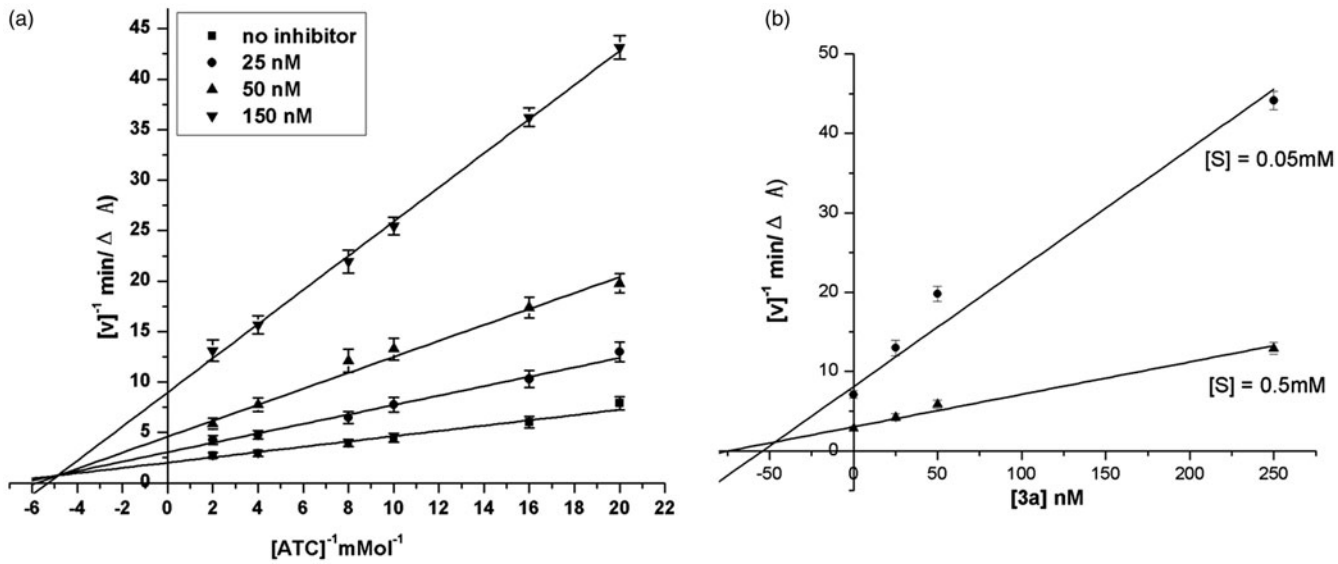


Figure 3. Lineweaver–Burk plot (a) and Dixon plot (b) for the inhibition of AChE by compound **3a**. Values are expressed as the mean ± SD ($n = 3$).

Compounds **3a** and **3b**, which exhibited very good inhibitory activities for eeAChE, were selected for the evaluation in human AChE (human erythrocytes). As shown in Table 1, compounds **3a** and **3b** had nearly the same inhibition activity (IC_{50} values of 50.7 and 9.4 nM, respectively) as that of eeAChE (IC_{50} values of 51.3 and 11.2 nM). Comparatively, tacrine (IC_{50} values of 265.2 nM for hAChE) exhibited inhibition that was one-third weaker than eeAChE (105.8 nM), which could be attributed to a better bonding of tacrine to the active sites of eeAChE compared with hAChE. The hAChE inhibitory activities of **3a** and **3b** were more potent than tacrine by approximately 5- and 28-fold, respectively.

Kinetics of AChE inhibition

We also carried out kinetic studies with the most potent ChEIs, **3a** and **3b**, by graphical analysis of steady state inhibition data to study the inhibitory mechanism for this class of tacrine–propargylamine derivatives[31]. As shown in Figures 3(a) and 4(a), both of the Lineweaver–Burk plots of **3a** and **3b** against eeAChE revealed an increasing slope (decreased V_{max}) and an increasing intercept (increased K_m) with higher inhibitor concentrations, indicating a mixed-type inhibitory behaviour. From the Dixon plot (Figures 3(b) and 4(b), we estimated that the inhibition constants K_i values of **3a** and **3b** are 47.27 and 3.26 nM, respectively.

Determination of neurotoxicity

To evaluate the neurotoxicity of the tacrine derivatives, we tested the cytotoxic effects of these compounds on the human neuroblastoma cell line, SH-SY5Y, using the colorimetric MTT assay[32] as summarised in Figure 5. The results indicated that compound

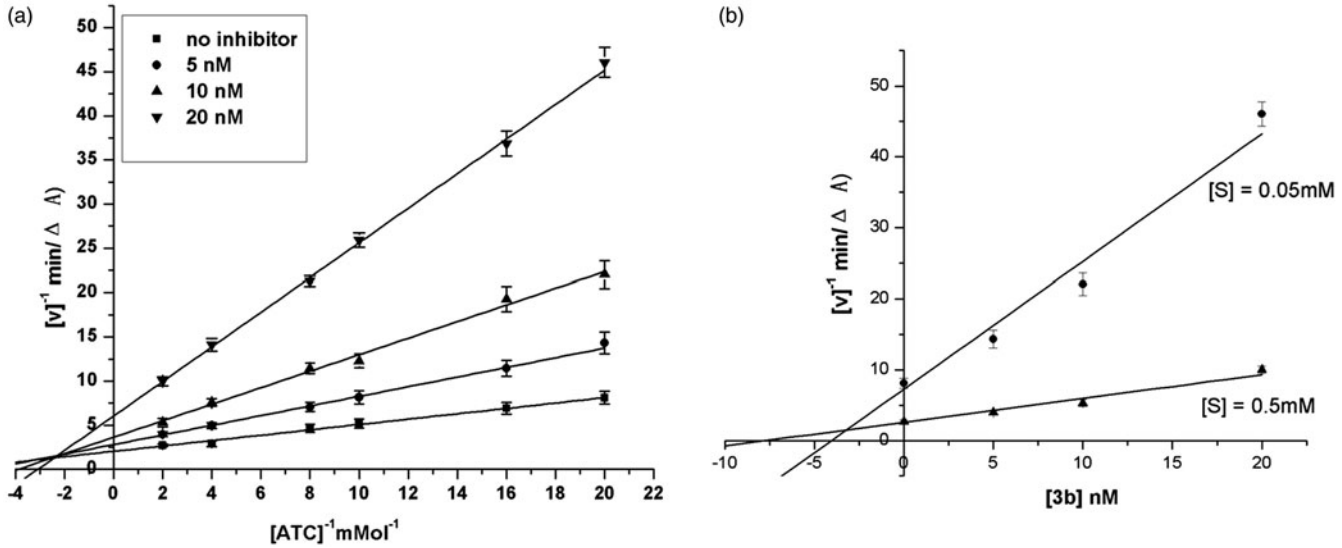


Figure 4. Lineweaver–Burk plot (a) and Dixon plot (b) for the inhibition of AChE by compound **3b**. Values are expressed as the mean ± SD ($n = 3$).

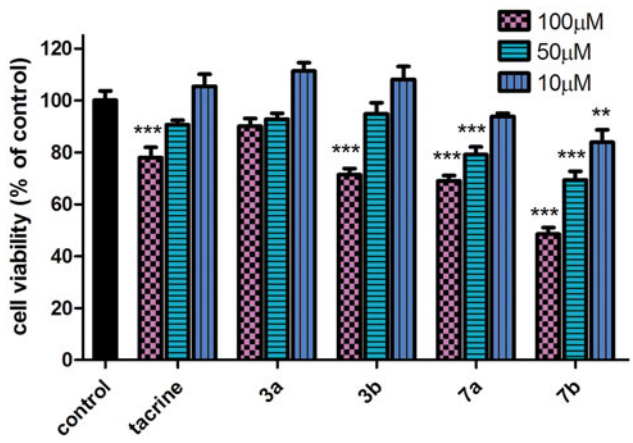


Figure 5. Effects of compounds on the cell viability of human neuroblastoma cell line SH-SY5Y. Values are expressed as the mean ± SD ($n = 3$). Data were subjected to one-way ANOVA followed by Tukey's multiple comparison test using GraphPad Prism 5.0 Software (levels of significance $^{**}p < 0.01$; $^{***}p < 0.001$).

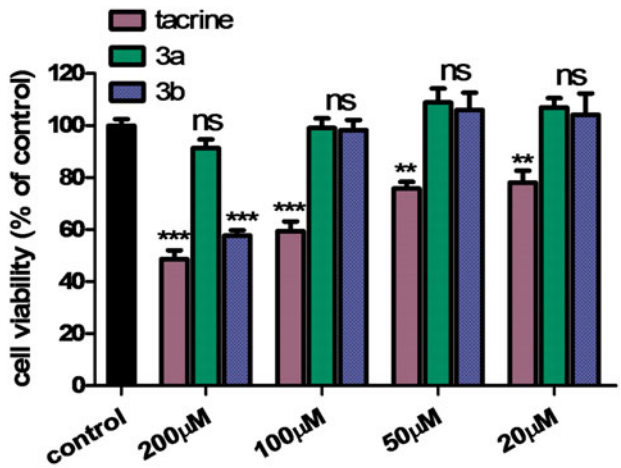


Figure 6. Cell viability determined by MTT after the treatment of HSC with different concentrations of tacrine, compound **3a** and **3b**. Values are expressed as the mean ± SD ($n = 3$). Data were subjected to one-way ANOVA followed by Dunnett's multiple comparison test using GraphPad Prism 5.0 Software (levels of significance $^{**}p < 0.01$; $^{***}p < 0.001$; ns = not significant).

3a had nearly no effect on the viability of SH-SY5Y cells at the concentrations of 10, 50 and 100 μM, which is a lower cytotoxicity than tacrine. Compound **3b** and tacrine had similar effects on the viability of SH-SY5Y cells at all of the tested concentrations. However, compounds **7a** and **7b** exhibited higher cytotoxicity than tacrine, which may be attributed to the high lipophilicity of the molecules.

Determination of hepatotoxicity

To evaluate the ability of tacrine–propargylamine derivatives to reduce the side effects of tacrine, **3a** and **3b** were investigated in human hepatic stellate cells (HSC)[33] using the colorimetric MTT assay. The results shown in Figure 6 indicate that at all of the tested concentrations (20, 50, 100 and 200 μM), both **3a** and **3b** exhibited higher cell viability (lower hepatotoxicity) compared with tacrine. At the concentration of 100 μM, **3a** and **3b** exhibited nearly no hepatotoxicity while tacrine had a 59.49% cell survival rate at the same concentration. At a higher concentration of 200 μM, compound **3a** still had a 91.38% cell survival rate (tacrine and **3b** gave 48.65% and 57.78%, respectively), which indicated that **3a** almost eliminated the hepatotoxicity of tacrine and is a potential lead compound for the treatment of AD.

Conclusion

In conclusion, our study involved the synthesis of a new series of tacrine–propargylamine derivatives, the evaluation of the inhibitory activities of ChE and the cytotoxic effects of these compounds in SH-SY5Y and HSC cells. Most of these compounds were potent inhibitors of AChE and BuChE and compounds **3a** and **3b** exhibited superior inhibition, a better balance of AChE and BuChE activities and low neurotoxicity. Importantly, these compounds also had a lower hepatotoxicity than tacrine. Based on these results, compounds **3a** and **3b** could be considered as potential lead compounds for the treatment of AD and the further *in vivo* experiments are in progress.

Declaration of interest

The authors declare no conflicts of interest.

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