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Design, synthesis and biological evaluation of tacrine-1,2,3-triazole derivatives as potent cholinesterase inhibitors†

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We report herein the design and synthesis of a series of 11 novel tacrine-1,2,3-triazole derivatives via a Cu(I)-catalyzed alkyne-azide 1,3-dipolar cycloaddition (CuAAC) reaction. The newly synthesized compounds were evaluated for their inhibition activity against *Electrophorus electricus* acetylcholinesterase (AChE) and horse serum butyrylcholinesterase (BChE) as potential drug targets for Alzheimer's disease (AD). Among the designed compounds, compound **8a2** exhibited potent inhibition against AChE and BChE with IC₅₀ values of 4.89 μM and 3.61 μM, respectively. Further structure-activity relationship (SAR) and molecular modeling studies may provide valuable insights into the design of better tacrine-triazole analogues with potential therapeutic applications for AD.

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Introduction

Alzheimer's disease (AD) characterized by a progressive loss of memory and other cognitive function impairments is the most common form of senile dementia.¹ Environmental, genetic and some endogenous factors play a role during the development of AD. A recent study estimates that over 36 million people are living with AD worldwide with an estimated global economic impact of approximately \$605 billion in 2010;² however, the figure has changed to \$818 billion in 2015,³ which indicates an increase of 35%, since 2010. It has been predicted that if an efficient treatment isn't developed by 2050, the number of patients with AD will rise to 70 million.⁴ The impact of this pathology is tremendous for the patients and their families. According to the Alzheimer's Association,⁵ AD is the only one without a means of preventing, curing, or slowing its progression among the top 10 causes of death in the United States. Although there has been considerable improvement in the management of AD, due to its complex pathogenesis, a successful AD chemotherapy is still demanding and challenging for medicinal chemists. Given the

background, several hypotheses have been proposed which include the cholinergic hypothesis.^{6–8} This hypothesis suggests that the loss of cholinergic activity is related to the severity of AD.⁷ Studies have shown that human acetylcholinesterase (hAChE) or 'pseudocholinesterase' (also called human butyrylcholinesterase (hBChE)) can quickly break down acetylcholine in the body, produce inactive acetic acid and choline, and thereby terminate nerve impulses,^{9,10} causing the loss of learning ability and cognition. Thus, reducing acetylcholinesterase (AChE) so as to increase the level of acetylcholine (ACh) could be beneficial to alleviating the symptoms of AD. Meanwhile, there was evidence showing that butyrylcholinesterase (BChE), a serine hydrolase related to AChE, catalyzed the hydrolysis of esters of choline, including ACh, and played important roles in cholinergic neurotransmission.¹¹ That is to say, the inhibition of butyrylcholinesterase (BChE) can raise ACh levels. Consequently, dual inhibition of AChE/BChE may be helpful to improve AD symptoms without remarkable side effects.¹² Both hAChE and hBChE structures are very well characterized from X-ray crystallography studies as being small and narrow gorge structures, which are mainly composed of the catalytically active site (CAS) at the bottom of the gorge and the peripheral anionic site (PAS) at the entrance of the gorge.¹³ In hAChE, the CAS region is mainly composed of Ser203, Glu334, Trp86, Pro446, Phe295, Phe297, Gly120, Gly121, Ala204 and His447 residues.¹⁴ Among them, a catalytic triad (Ser203, Glu334, and His447) is mainly involved in the hydrolysis of choline, and the PAS region is composed of Asp74, Tyr72, Tyr124, Trp286 and Arg298 residues, which stabilize the substrate binding. Although hAChE and hBChE have high homology (54% identity and 81% similarity), the

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hAChE catalytic gorge is lined with fourteen aromatic residues, *e.g.*, Tyr72, Tyr124, Trp286, Phe295, Phe297, and Tyr337, whereas six of these positions were replaced by aliphatic residues (Asn68, Gln119, Ala277, Leu286, Val288, and Ala328) in hBChE.¹⁴ Importantly, π -stacking interaction is the main force in the substrate and inhibitor binding, *e.g.* tacrine's aromatic ring.

So far, five cholinergic drugs have been approved for clinical use, namely tacrine, donepezil, rivastigmine, galantamine, and huperzine A, and many others are in various stages of clinical trials. Among them, tacrine, the first drug approved by FDA, which binds at the CAS region,¹⁵ is a potent non-selective inhibitor of both hAChE and hBChE and shows large modifiable chemical space. Nonetheless, tacrine suffers from severe side effects, including liver toxicity.¹⁶ On the other hand, quinoline derivatives (I–IV)^{17–20} were reported to possess the ability to specifically bind with the PAS and displayed favorable anti-AD activity (Fig. 1). The purpose of this study was to investigate the effect of dual binding site inhibitors on the PAS and CAS of ChE and to find more effective ChE inhibitors.

Meanwhile, conjugation of functional molecules through 1,2,3-triazole has received great attention in drug discovery

for the reason that the 1,2,3-triazole structural motif is a neo-classical bioisostere of amide, which is a widely employed functional group in approved drugs.²¹ In general, 1,2,3-triazole has several advantages in drug design.^{22,23} For instance, the triazole has two H-bond acceptors, which is capable of interacting with the biomolecular targets through H-bonding, π - π stacking, and dipole interaction. Besides, the triazole ring exhibits better chemical stability in biological environments, and this improves the pharmacokinetic and toxicity properties. All these advantages make 1,2,3-triazole a remarkable fragment in drug design. Besides, the formation of 1,2,3-triazole compounds *via* a Cu(I)-catalyzed alkyne-azide 1,3-dipolar cycloaddition (CuAAC) reaction, often referred to as click chemistry, is widely used for the rapid assembly of heterocyclic molecules that may be subsequently used as candidate leads in drug development.²⁴

Considering the above benefits and in continuation of our interest in searching for the anti-AChE and anti-BChE pharmacological effects of tacrine-based PAS–CAS dual binders, in the present study, we describe the synthesis and evaluation of a set of diverse tacrine-quinolines (see Fig. 1) as PAS–CAS dual inhibitors for electric eel AChE and horse serum BChE.

Fig. 1 Tacrine and quinoline derivatives with favorable inhibitory activity and the newly designed tacrine derivatives.

Results and discussion

Chemistry

The synthetic route towards the target tacrine-1,2,3-triazole derivatives is outlined in Schemes 1–3. Initially, the commercially available starting material indoline-2,3-dione (**1**) yielded intermediate 2-aminobenzoic acid (**2a**) in the presence of sodium hydroxide and 30% hydrogen peroxide. Under the presence of POCl₃, compound **2a** (or **2b**) reacted with cyclohexanone to obtain 9-chloro-1,2,3,4-tetrahydroacridine (**3a**) or 6,9-dichloro-1,2,3,4-tetrahydroacridine (**3b**), which were used to yield intermediate **4a** (or **4b** or **4c**) by a nucleophilic substitution reaction. Then, **4a** (or **4b** or **4c**) reacted with different fragments of alkynes giving the key intermediates **5a1–5a5** and **5b1–5b6**.^{25–29} In the meantime, compound **6** was reacted with NaN₃ in the presence of NaI to prepare compound **7**, followed by its reaction with **5a1–5a5** and **5b1–5b6** to give the target products **8a** and **8b** via the CuAAC reaction.^{30,31} The newly synthesized compounds were characterized by physicochemical and spectral means, and the MS and NMR spectral data were found to be in agreement with the assigned molecular structures.

Cholinesterase inhibition evaluation

Based on the reported protocol,^{32–36} all the newly synthesized compounds (**8a1–8a5**, **8b1–8b6**) were evaluated for their inhibitory activities toward electric eel AChE (Sigma-Aldrich, USA; EC: 3.1.1.7; product information file code: GRO, JWM, RBG, MAM 01/08-1, the download website is shown in the ESI†) and horse serum BChE (Sigma-Aldrich, USA; EC: 3.1.1.8; product information file code: TMG/AJH 10/07, the download website is shown in the ESI†). The authors declare that all procedures were performed strictly in compliance with Chinese relevant laws and institutional guidelines. Tacrine was selected as a reference drug. The initial *in vitro* screening was performed using a single concentration of 100 μM (see Table 1). Among all the tested compounds, com-

pound **8a2** exhibited the most potent inhibitory activity against AChE and BChE with an inhibition ratio of 78.69% and 91.80% at 100 μM. In addition, compounds **8a3**, **8a4**, **8b2** and **8b3** showed inhibitory percentages for AChE more than 60% at 100 μM, which is comparable to that of **8a2**. Interestingly, compound **8b6** had better selectivity for BChE and displayed an inhibitory percentage of 82.73% against BChE, which is about two times that for AChE (49.37%). After the preliminary screening, compounds **8a2–8a5**, **8b2**, **8b3**, and **8b6** were chosen to determine their IC₅₀ values for cholinesterase. Among them, **8a2** demonstrated the best anti-AChE (IC₅₀ = 4.89 μM) and anti-BChE activity (IC₅₀ = 3.61 μM). Besides, the inhibitory efficacy of **8a3**, **8a4**, **8b3**, and **8b2** against AChE demonstrated IC₅₀ values of 10, 11.07, 18.66 and 19.59 μM, respectively. As for the anti-BChE activity, **8b6** was inferior to **8a2**, and its IC₅₀ value was 6.06 μM, followed by **8a5** (IC₅₀ = 61.13 μM) and **8a3** (IC₅₀ = 66.68 μM). However, all the newly synthesized compounds showed weaker inhibitory activity compared to the reference drug tacrine.

Preliminary structure–activity relationship (SAR) analysis indicated that the size and length of the linker between the piperazine and triazole motifs are major determinants for their inhibitory activity. An alkane chain with three carbons was the most active one (**8a2**); whether increasing or shortening the length of the carbon chain would decrease their inhibitory effect on AChE and BChE (inhibition activity: **8a2** > **8a3** > **8a1**). Comparing series **a** with series **b**, the introduction of a chlorine atom at the C₆ position of tacrine would decrease the inhibitory activity (inhibition activity: **8a1** > **8b1**, **8a3** > **8b2**, **8a4** > **8b3**). The current SAR will provide additional information to help in the design of inhibitors with better potency.

Molecular modeling analysis

To provide better insight into the binding mode of compound **8a2** to hAChE and hBChE, molecular docking followed

Scheme 1 The synthetic route to intermediates **4a**, **4b** and **4c**. Reagents and conditions: (i) 30% H₂O₂, NaOH, 0 °C, 3 h; (ii) cyclohexanone, POCl₃, 100 °C, 3 h; (iii) piperazine, NaI, PhOH, 180 °C, 3 h; (iv) ethane-1,2-diamine, NaI, PhOH, 180 °C, 3 h.

Scheme 2 The synthetic route to intermediates **5a1–5a5** and **5b1–5b6**. Reagents and conditions: (v) **5a1**: 3-bromoprop-1-yne, Et_3N ; **5a2**: 5-chloropent-1-yne, K_2CO_3 ; **5a3**: 6-chlorohex-1-yne, K_2CO_3 ; **5a4**: hept-6-ynoic acid, EDCI, HOBT, Et_3N ; **5a5**: 1) 4-chlorobutanoyl chloride, Et_3N ; 2) *N*-methylprop-2-yn-1-amine, K_2CO_3 ; (vi) **5b1**: 3-bromoprop-1-yne, Et_3N ; **5b2**: 6-chlorohex-1-yne, K_2CO_3 ; **5b3**: hept-6-ynoic acid, EDCI, HOBT, Et_3N ; **5b4**: 1) 2-chloroacetyl chloride, Et_3N ; 2) *N*-methylprop-2-yn-1-amine, K_2CO_3 ; **5b5**: 1) 5-bromopentanoyl chloride, Et_3N ; 2) *N*-methylprop-2-yn-1-amine, K_2CO_3 ; (vii) **5b6**: 1) ethane-1,2-diamine, NaI, PhOH; 2) hept-6-ynoic acid, EDCI, HOBT, Et_3N .

Scheme 3 The synthetic route to tacrine-1,2,3-triazole derivatives. Reagents and conditions: (viii) NaI, NaN_3 , DMF/ H_2O (v/v = 1:1), 80 °C, 8 h; (ix) sodium ascorbate, CuSO_4 , Et_3N , *n*-butanol/ H_2O (v/v = 1:1).

by MM-GBSA studies was performed. To this end, compound **8a2** was docked into the hAChE and hBChE ligand-binding

site (see the Experimental section for details). The active site of hAChE has previously been well characterized as having a

Table 1 Inhibition activity of tacrine derivatives against *Electrophorus electricus* AChE and horse serum BChE

Compound	R	Linker	Inhibition (%) at 100 μ M		IC ₅₀ (μ M)	
			AChE	BChE	AChE	BChE
8a1	H		39.32	38.56	—	—
8a2	H		78.69	91.80	4.89	3.61
8a3	H		69.31	55.64	10	66.68
8a4	H		65.69	46.37	11.07	>100
8a5	H		47.56	57.90	>100	61.13
8b1	Cl		30.38	22.12	—	—
8b2	Cl		66.02	46.68	19.59	>100
8b3	Cl		64.19	49.28	18.66	>100
8b4	Cl		41.47	27.97	—	—
8b5	Cl		42.31	39.04	—	—
8b6	Cl		49.37	82.73	>100	6.06
Tacrine	—	—	86.22	99.63	0.316	0.066

catalytic site (CAS) composed of Trp86, Glu334, Tyr336, Trp439, Met443, Pro446 and His447 residues and a peripheral site or anionic subsite (PAS) composed of Asp74, Tyr72, Tyr124, Trp286 and Arg298 residues. It is shown in the crystal structure of the hAChE complex with tacrine that the acridine ring is involved in the π - π interaction with the indole ring of Trp84. In addition to the acridine ring surrounded by hydrophobic residues, such as Phe330, Tyr334, Tyr442 and Trp434,³⁷ the active site of hBChE is very similar to that of hAChE as having a similar set of residues in both CAS and PAS regions.³⁸ In this study, the binding mode of compound 8a2 is compared in both hAChE and hBChE active sites with tacrine. The MM-GBSA method was employed to rank the best binding poses from the docking solutions, and the bind-

ing pose that showed the highest binding affinity in the MM-GBSA calculation for hAChE and hBChE was further discussed. It is clear from Fig. 2 that the binding mode of 8a2 is similar in both cases and that the acridine ring is involved in the π - π interaction with Trp86 (hAChE) and Trp82 (hBChE) as has previously been shown.³⁷

Moreover, the piperazine and triazole rings of compound 8a2 showed interactions with Tyr124 and Trp286 in hAChE. However, compound 8a2 binds slightly better in the hBChE structure, for instance, the quinoline ring strongly binds to Ser287 and Pro285 residues through π - π interactions, and importantly, the electronegative chlorine atom also shows a strong interaction with the neighboring residue *e.g.*, Gln71. Besides, the tacrine structure was also prepared in the same

Fig. 2 Comparison of the binding mode of compound **8a2** (cyan) at the active site of hAChE (A) and hBChE (B). Important residues belonging to CAS (green) and PAS (orange) are highlighted. (C) Schematic representation of the binding poses of **8a2** with compounds **III**, **IV** and tacrine (for clarity, only the ligand pose is shown).

manner as compound **8a2** and re-docked into hAChE and hBChE active sites to assess the docking protocol and compare the binding pose to the X-ray crystal structure. Overall, the docking simulation is able to reproduce the same binding pose as was observed in the X-ray crystallography studies with an RMSD of 0.05 Å (*cf.* Fig. 2 and ESI†). In addition to the comparison of **8a2** with tacrine, the binding mode of compounds **III** and **IV** (*cf.* Fig. 1) was compared to understand the impact of the linker fragment. Compounds **III** and **IV** were also docked in the same manner as tacrine into the hAChE and hBChE structures. As clearly seen from Fig. 2C, the binding mode of **8a2** was found to be similar to those of compounds **III** and **IV** (see the protein–ligand interaction map in the ESI†) but showed lesser potency as compared to tacrine or compound **III/IV**. The higher IC_{50} of **8a2** could be due to not only the rigid triazole and piperazine rings, but also its feature of having more polar atoms, which may lead to an unfavorable contribution to the hydrophobicity of active sites, particularly PAS as reported previously.^{17,18}

Conclusion

To summarize, a concise and efficient synthetic approach has been established to readily access two novel series of tacrine-1,2,3-triazole derivatives in moderate to high yields.

Subsequently, the synthesized compounds were screened for electric eel AChE and horse serum BChE inhibition. Among these compounds, compound **8a2** inhibited the AChE and horse serum BChE activity by more than 75% (IC_{50} : 4.89 μ M) and 90% (IC_{50} : 3.61 μ M) at a concentration of 100 μ M, respectively. However, the cholinesterase inhibition activity of compound **8a2** is slightly lower as compared to that of the reference drug tacrine, with its unique binding mode at both CAS and PAS, making it a particularly promising lead for the development of new dual inhibitors of AChE and BChE. Further, molecular docking studies were also performed to understand its binding mode in the AChE and BChE active sites, particularly revealing the importance of quinoline substituents at the C-1 position of the 1,2,3-triazole ring involved in the π – π interaction with Trp286 (hAChE), Ser287 (hBChE) and Pro285 (hBChE) in the PAS region. Meanwhile, the acridine ring is involved in the π – π interaction with Trp86 (hAChE) and Trp82 (hBChE) in the CAS region, the same as the reference compound tacrine.

Several works have reported the design of tacrine-based dimers with different linkers (*e.g.* disulfide bond, alkane, piperazine, amide, *etc.*), including dual binding site inhibitors on the PAS and CAS against AChE and BChE, which is a promising trend deserving to be explored and studied.³⁹ In this work, we took advantage of CuAAC click chemistry in drug

design and the unique physiochemical properties of 1,2,3-triazole, and thus, adapted a linker including the 1,2,3-triazole group introduced by the CuAAC reaction but maintained its unique binding mode like tacrine-based dimers. Although the most potent compound from this series is significantly weaker as compared to tacrine or previously known compounds, *e.g.*, **III** and **IV**, a non-aliphatic linker design provides further opportunities to design a smarter linker which facilitates the binding of molecules to CAS and PAS. With this understanding, efforts are further expanded in our laboratory for further lead optimization of tacrine-based triazole analogues as potent cholinesterase inhibitors with the hope to develop promising candidates for AD chemotherapy.

Experimental section

General

All melting points of the compounds were determined using micro melting point apparatus and were uncorrected. ^1H -NMR and ^{13}C -NMR spectra were obtained *via* a Bruker Avance-400 NMR spectrometer in CDCl_3 . Chemical shifts were expressed in δ units and TMS was used as an internal reference. Mass spectrometry was conducted *via* a LC Autosampler Device: Standard G1313A instrument. Flash column chromatography was performed using a column packed with Silica Gel 60 (200–300 mesh). Meanwhile, TLC was conducted using Silica Gel GF254, and spots were visualized by irradiation with UV light ($\lambda = 254 \text{ nm}$). Solvents were of reagent grade, which were purified and dried by standard methods when necessary. The reaction solutions were concentrated using a rotary evaporator under reduced pressure conditions.

General procedure for the synthesis of compound 7 (4-azido-7-chloroquinoline). 4,7-Dichloroquinoline (**6**, 3.0 g, 11.4 mmol), NaN_3 (2.0 g, 30.0 mmol), and NaI (0.2 g, 1.5 mmol) were dissolved in a solvent mixture of DMF/ H_2O (v:v = 1:1, 60 mL). The resulting mixture was stirred at 80 °C for 8 h. After the reaction was cooled to room temperature, water (30 mL) was added to the reaction system. Then, the resulting mixture was extracted with ethyl acetate ($3 \times 40 \text{ mL}$). The combined organic phase was washed with saturated salt water ($3 \times 40 \text{ mL}$), dried over anhydrous Na_2SO_4 , filtered, and concentrated under reduced pressure to give the corresponding crude target product, which was purified by flash column chromatography with petroleum ether/ethyl acetate to afford compound **7** as a white solid with a yield of 91%.

General procedure for the synthesis of 3a (9-chloro-1,2,3,4-tetrahydroacridine) and 3b (6,9-dichloro-1,2,3,4-tetrahydroacridine). Indoline-2,3-dione (**1**, 5.0 g, 34.0 mmol) was dissolved in NaOH solvent (67 mL, 1 mol L^{-1}). After hydrogen peroxide (7.3 mL, 30%) was added slowly into the above solution at 0 °C, the resulting mixture was stirred at 30–40 °C for 3 h. Then, the cooling solution was neutralized to pH = 7.5 with hydrochloric acid (3 mol L^{-1}). Subsequently, moderate activated carbon was added and the mixture was stirred for another 0.5 h. Afterwards, the resulting precipitate was fil-

tered and the filtrate was further acidified until pH = 4–5 and stirring was continued for another 1 h. The resulting mixture was filtered again and combined with the filter cake, which was dried in a vacuum drying oven to obtain the crude product **2a**. The crude product **2a** was purified by flash column chromatography with dichloromethane/methanol to afford compound **2a** as a white hairy solid with a yield of 84%.

2-Aminobenzoic acid (**2a**, 1.0 g, 7.3 mmol) and cyclohexanone (0.9 g, 8.8 mmol) were mixed in a flask. Then, POCl_3 (10 mL) was added dropwise into the flask at 0 °C. The above resulting mixture was refluxed at 100 °C for 3 h. Then, the mixture was quenched with ice water and neutralized to neutral pH with K_2CO_3 solution (1 mol L^{-1}). Subsequently, the neutralized solution was extracted with ethyl acetate ($3 \times 15 \text{ mL}$), and the combined organic phase was washed with saturated salt water ($3 \times 15 \text{ mL}$), dried over anhydrous MgSO_4 , filtered, and concentrated under reduced pressure to give the corresponding crude target product. Finally, the crude product was purified by flash column chromatography with petroleum ether/ethyl acetate to afford the product compound **3a** as a yellow solid. Yield: 88%. Notably, the synthetic procedure for compound **3b** (yellow solid; yield: 81.5%) was also the same as that for compound **3a**.

General procedure for the synthesis of compounds 4a (9-(piperazin-1-yl)-1,2,3,4-tetrahydroacridine), 4b (6-chloro-9-(piperazin-1-yl)-1,2,3,4-tetrahydroacridine) and 4c (N1-(6-chloro-1,2,3,4-tetrahydroacridin-9-yl)ethane-1,2-diamine).

Compound **3a** (or **3b**, 13.8 mmol), piperazine (5.9 g, 69.0 mmol), NaI (0.2 g, 13.8 mmol) and phenol (2.6 g, 27.6 mmol) were mixed and stirred at 180 °C for 3 h in a flask. Then, the reaction system was cooled to 80 °C and subsequently quenched with NaOH solution (30 mL, 1 mol L^{-1}). The quenched mixture was extracted with dichloromethane ($3 \times 15 \text{ mL}$), and the combined organic phase was washed with saturated salt water ($3 \times 15 \text{ mL}$), dried over anhydrous Na_2SO_4 , filtered, and concentrated under reduced pressure to give the corresponding crude target product, which was purified by flash column chromatography with dichloromethane/methanol to afford compound **4a** (**4b**) as a yellow solid (yellow oil) with a yield of 81% (42%). The same procedure was used to obtain product **4c** with a yield of 82%, except that piperazine was changed to ethane-1,2-diamine.

General procedure for the synthesis of compounds 5a1–3, 5b1 and 5b2. Compound **4a** (**4b**, 3.7 mmol), 3-bromoprop-1-yne (0.49 g, 4.1 mmol), and triethylamine (0.6 g, 5.6 mmol) were stirred at 50 °C for 3 h in dichloromethane solvent. Then, the reaction mixture was cooled to room temperature and concentrated under reduced pressure to obtain the crude product, which was purified by flash column chromatography with dichloromethane/methanol to obtain compound **5a1** (**5b1**) as a yellow solid (yellow solid). Yield: 70% (82%). If 3-bromoprop-1-yne was changed to 5-chloropent-1-yne or 6-chlorohex-1-yne, **5a2**, **5a3** and **5b2** would be obtained with yields of 81%, 69% and 49%, respectively.

General procedure for the synthesis of compounds 5a4, 5b3 and 5b6. Hept-6-ynoic acid (0.5 g, 4.0 mmol), EDCI (1.0

g, 5.0 mmol), and HOBt (0.7 g, 5.0 mmol) were stirred at room temperature for 0.5 h in dichloromethane. Then, compound **4a** (**4b** or **4c**, 3.3 mmol) and triethylamine (0.7 g, 6.6 mmol) were added, and the reaction was continued at room temperature for another 5 h. Once the reaction was finished (monitored by TLC), the reaction mixture was quenched with saturated salt water (3 × 30 mL), dried over anhydrous MgSO₄, filtered, and concentrated under reduced pressure to give the corresponding crude target product, which was purified by flash column chromatography with dichloromethane/methanol to obtain compound **5a4** (**5b3** or **5b6**) as a yellow solid. Yield: 50% (70% or 86%).

General procedure for the synthesis of compounds 5a5 and 5b4–5b6. 4-Chlorobutanoyl chloride (0.8 g, 5.6 mmol) was added dropwise into dichloromethane solvent (25 mL) at 0 °C, which was previously stirred with triethylamine (0.7 g, 7.4 mmol) and compound **4a** (1.0 g, 3.7 mmol). Then, the reaction was continued at 0 °C under magnetic stirring for 0.5 h. Subsequently, stirring was continued for another 2 h at room temperature. Once the reaction was completed (monitored by TLC), the resulting mixture was washed with saturated salt water (3 × 25 mL), dried over anhydrous MgSO₄, filtered, and concentrated under reduced pressure to give the corresponding crude target product, which was purified by flash column chromatography with petroleum ether/ethyl acetate to obtain the precursor compound **5a5** with a yield of 82%. Then, compound **5a5** was reacted with *N*-methylprop-2-yn-1-amine similar to the synthetic procedure for compounds **4a–4c**, and was purified by flash column chromatography. A yellow solid was obtained. Using the same procedure, except that 4-chlorobutanoyl chloride was changed to 2-chloroacetyl chloride or 5-bromopentanoyl chloride, products **5b4** and **5b5** were obtained with yields of 83% and 89%, respectively.

General procedure for the synthesis of compounds 8a1–8a5 and 8b1–8b6. Intermediates **5a1–5a5** (1.0 eq., or **5b1–5b6**), 4-azido-7-chloroquinoline (compound **7**, 1.0 eq.), ascorbic acid sodium (0.6 eq.), CuSO₄·5H₂O (0.3 eq.) and triethylamine (1.2 eq.) were dissolved in a solution of *n*-butyl alcohol/water (v:v = 1:1). The resulting mixture was stirred at room temperature for 5 h. Then, the reaction mixture was extracted with ethyl acetate (3 × 10 mL), and the combined organic phase was washed with saturated salt water (3 × 10 mL), dried over anhydrous MgSO₄, filtered, and concentrated under reduced pressure to give the corresponding crude target product, which was purified by flash column chromatography to afford products **8a1–8a5** (or **8b1–8b6**). Yield: 37–92%.

9-(4-((1-(7-Chloroquinolin-4-yl)-1*H*-1,2,3-triazol-4-yl)-methyl)piperazin-1-yl)-1,2,3,4-tetrahydroacridine (8a1). Yield: 74%; white solid; mp: 105–107 °C. ¹H NMR (400 MHz, CDCl₃, ppm) δ: 9.08 (d, *J* = 4.6 Hz, 1H), 8.26 (d, *J* = 2.0 Hz, 1H), 8.16 (d, *J* = 8.0 Hz, 1H), 8.07–8.05 (m, 2H), 7.97 (d, *J* = 8.3 Hz, 1H), 7.64–7.57 (m, 2H), 7.54 (d, *J* = 4.6 Hz, 1H), 7.42 (t, *J* = 7.2 Hz, 1H), 3.99 (s, 2H), 3.41 (s, 4H), 3.13 (t, *J* = 6.6 Hz, 2H), 2.96–2.87 (m, 6H), 1.97–1.82 (m, 4H); ¹³C NMR (100 MHz, CDCl₃, ppm) δ: 160.3, 152.8, 151.4, 150.3, 147.7, 145.6, 141.1, 137.0,

129.5, 129.1, 128.7, 128.4, 127.7, 125.8, 125.1, 124.7, 124.5, 123.9, 120.6, 115.8, 54.4, 53.7, 50.4, 34.0, 26.9, 23.0, 22.8. ESI-MS: C₂₉H₂₈ClN₇, exact mass: 509.21, *m/z* 511.5 (*M* + 2)⁺.

9-(4-(3-(1-(7-Chloroquinolin-4-yl)-1*H*-1,2,3-triazol-4-yl)-propyl)piperazin-1-yl)-1,2,3,4-tetrahydroacridine (8a2). Yield: 47%; white solid; mp: 98–100 °C. ¹H NMR (400 MHz, CDCl₃, ppm) δ: 9.03 (d, *J* = 4.6 Hz, 1H), 8.22 (d, *J* = 2.0 Hz, 1H), 8.15 (d, *J* = 8.0 Hz, 1H), 8.04 (d, *J* = 9.1 Hz, 1H), 7.95 (d, *J* = 8.3 Hz, 1H), 7.86 (s, 1H), 7.59–7.54 (m, 2H), 7.48 (d, *J* = 4.6 Hz, 1H), 7.40 (t, *J* = 7.6 Hz, 1H), 3.37 (s, 4H), 3.11 (t, *J* = 6.6 Hz, 2H), 3.01–2.92 (m, 4H), 2.72–2.63 (m, 6H), 2.14–2.06 (m, 2H), 1.96–1.80 (m, 4H); ¹³C NMR (100 MHz, CDCl₃, ppm) δ: 160.3, 152.8, 151.4, 150.2, 148.7, 147.8, 141.1, 136.6, 129.2, 128.9, 128.7, 128.3, 127.6, 125.9, 124.9, 124.8, 123.9, 122.6, 120.5, 115.8, 58.2, 54.3, 50.5, 34.1, 26.9, 26.5, 23.5, 23.0, 22.8. ESI-MS: C₃₁H₃₂ClN₇, exact mass: 537.24, *m/z* 539.2 (*M* + 2)⁺.

9-(4-(4-(1-(7-Chloroquinolin-4-yl)-1*H*-1,2,3-triazol-4-yl)-butyl)piperazin-1-yl)-1,2,3,4-tetrahydroacridine (8a3). Yield: 43%; white solid; mp: 112–115 °C. ¹H NMR (400 MHz, CDCl₃, ppm) δ: 9.05 (d, *J* = 4.6 Hz, 1H), 8.24 (d, *J* = 2.0 Hz, 1H), 8.15 (d, *J* = 8.3 Hz, 1H), 8.06–8.00 (m, 2H), 7.83 (s, 1H), 7.61–7.56 (m, 2H), 7.50 (d, *J* = 4.6 Hz, 1H), 7.41 (t, *J* = 7.6 Hz, 1H), 3.38 (s, 4H), 3.15 (t, *J* = 6.6 Hz, 2H), 2.98–2.92 (m, 4H), 2.72 (s, 4H), 2.61–2.57 (m, 2H), 1.97–1.72 (m, 8H); ¹³C NMR (100 MHz, CDCl₃, ppm) δ: 160.3, 153.1, 151.6, 150.3, 149.0, 147.5, 141.2, 136.8, 129.3, 129.0, 128.6, 128.4, 127.6, 125.9, 125.0, 124.8, 124.0, 122.6, 120.7, 115.8, 58.7, 54.4, 50.4, 34.1, 27.3, 26.9, 26.5, 25.5, 23.0, 22.8. ESI-MS: C₃₂H₃₄ClN₇, exact mass: 551.26, *m/z* 553.2 (*M* + 2)⁺.

5-(1-(7-Chloroquinolin-4-yl)-1*H*-1,2,3-triazol-4-yl)-1-(4-(1,2,3,4-tetrahydroacridin-9-yl)piperazin-1-yl)pentan-1-one (8a4). Yield: 64%; white solid; mp: 98–101 °C. ¹H NMR (400 MHz, CDCl₃, ppm) δ: 9.03 (d, *J* = 4.6 Hz, 1H), 8.21 (d, *J* = 2.0 Hz, 1H), 8.09–8.03 (m, 2H), 7.97–7.91 (m, 2H), 7.61–7.55 (m, 2H), 7.49 (d, *J* = 4.6 Hz, 1H), 7.43 (t, *J* = 7.6 Hz, 1H), 4.08–3.71 (m, 4H), 3.33–3.28 (m, 4H), 3.12 (t, *J* = 6.5 Hz, 2H), 2.98–2.89 (m, 4H), 2.52 (t, *J* = 7.0 Hz, 2H), 1.97–1.82 (m, 8H); ¹³C NMR (100 MHz, CDCl₃, ppm) δ: 171.4, 160.6, 152.0, 151.4, 150.2, 148.8, 147.8, 141.1, 136.7, 129.2, 129.0, 128.9, 128.5, 127.9, 125.8, 125.3, 124.8, 123.4, 122.7, 120.6, 115.8, 50.6, 50.2, 46.8, 42.8, 34.1, 32.9, 28.9, 26.8, 25.4, 24.7, 22.9, 22.7. ESI-MS: C₃₃H₃₄ClN₇O, exact mass: 579.25, *m/z* 581.5 (*M* + 2)⁺.

4-(((1-(7-Chloroquinolin-4-yl)-1*H*-1,2,3-triazol-4-yl)methyl)-(methylamino)-1-(4-(1,2,3,4-tetrahydroacridin-9-yl)piperazin-1-yl)butan-1-one (8a5). Yield: 43%; white solid; mp: 110–112 °C. ¹H NMR (400 MHz, CDCl₃, ppm) δ: 9.05 (d, *J* = 4.3 Hz, 1H), 8.23 (d, *J* = 1.7 Hz, 1H), 8.08–8.04 (m, 3H), 7.96 (d, *J* = 8.3 Hz, 1H), 7.61–7.57 (m, 2H), 7.54 (d, *J* = 4.6 Hz, 1H), 7.42 (t, *J* = 7.5 Hz, 1H), 3.91–3.70 (m, 6H), 3.31–3.25 (m, 4H), 3.12 (t, *J* = 6.5 Hz, 2H), 2.89 (t, *J* = 6.2 Hz, 2H), 2.62–2.41 (m, 7H), 2.03–1.83 (m, 6H); ¹³C NMR (100 MHz, CDCl₃, ppm) δ: 171.4, 160.5, 152.0, 151.4, 150.2, 147.9, 145.8, 141.1, 137.0, 129.4, 129.0, 128.5, 127.9, 125.8, 125.3, 124.8, 124.5, 123.4, 120.6, 116.0, 56.3, 52.0, 50.7, 50.2, 46.8, 42.8, 42.6, 34.1, 30.7, 26.8, 22.93, 22.7. ESI-MS: C₃₄H₃₇ClN₈, exact mass: 608.28, *m/z* 609.4 (*M* + 1)⁺.

6-Chloro-9-(4-((1-(7-chloroquinolin-4-yl)-1*H*-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-1,2,3,4-tetrahydroacridine (8b1). Yield: 92%; yellow solid; mp: 115–117 °C. ¹H NMR (400 MHz, CDCl₃, ppm) δ: 9.08 (d, *J* = 4.6 Hz, 1H), 8.26 (d, *J* = 1.9 Hz, 1H), 8.10–8.04 (m, 3H), 7.96 (d, *J* = 1.6 Hz, 1H), 7.63 (dd, *J* = 9.1, 2.0 Hz, 1H), 7.53 (d, *J* = 4.6 Hz, 1H), 7.36 (dd, *J* = 9.0, 2.0 Hz, 1H), 3.99 (s, 2H), 3.39 (s, 4H), 3.11 (t, *J* = 6.6 Hz, 2H), 2.94–2.88 (m, 6H), 2.88 (s, 4H), 1.97–1.82 (m, 4H); ¹³C NMR (100 MHz, CDCl₃, ppm) δ: 161.8, 152.8, 151.4, 150.3, 148.3, 145.6, 141.0, 137.0, 134.1, 129.4, 129.1, 127.9, 127.7, 125.9, 125.4, 124.7, 124.5, 124.3, 120.6, 115.9, 54.3, 53.6, 50.4, 34.1, 26.9, 22.9, 22.7. ESI-MS: C₂₉H₂₇Cl₂N₇, exact mass: 543.17, *m/z* 545.2 (M + 2)⁺.

6-Chloro-9-(4-(4-(1-(7-chloroquinolin-4-yl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-1,2,3,4-tetrahydroacridine (8b2). Yield: 52.6%; white solid; mp: 128–130 °C. ¹H NMR (400 MHz, CDCl₃, ppm) δ: 8.87 (d, *J* = 4.6 Hz, 1H), 8.05 (d, *J* = 1.8 Hz, 1H), 7.95–7.9 (m, 2H), 7.76 (s, 2H), 7.42 (dd, *J* = 9.1, 2.0 Hz, 1H), 7.34 (d, *J* = 4.6 Hz, 1H), 7.17 (dd, *J* = 9.0, 1.9 Hz, 1H), 3.20 (s, 4H), 2.93 (t, *J* = 6.5 Hz, 2H), 2.83 (t, *J* = 7.6 Hz, 2H), 2.77 (t, *J* = 6.2 Hz, 2H), 2.56–2.42 (m, 4H), 1.83–1.59 (m, 8H); ¹³C NMR (100 MHz, CDCl₃, ppm) δ: 161.6, 152.8, 151.3, 150.1, 148.8, 148.0, 141.0, 136.6, 133.8, 129.1, 128.8, 127.8, 127.5, 125.6, 125.5, 124.8, 124.2, 122.5, 120.5, 115.7, 58.6, 54.2, 50.4, 34.0, 27.3, 26.8, 26.5, 25.5, 22.8, 22.6. ESI-MS: C₃₂H₃₃Cl₂N₇, exact mass: 585.22, *m/z* 586.9 (M + 1)⁺.

1-(4-(6-Chloro-1,2,3,4-tetrahydroacridin-9-yl)piperazin-1-yl)-5-(1-(7-chloroquinolin-4-yl)-1*H*-1,2,3-triazol-4-yl)pentan-1-one (8b3). Yield: 80%; yellow solid; mp: 108–110 °C. ¹H NMR (400 MHz, CDCl₃, ppm) δ: 9.06 (d, *J* = 4.6 Hz, 1H), 8.24 (d, *J* = 1.9 Hz, 1H), 8.06–8.0 (m, 3H), 7.87 (s, 1H), 7.60 (dd, *J* = 9.1, 2.0 Hz, 1H), 7.50 (d, *J* = 4.6 Hz, 1H), 7.38 (dd, *J* = 9.0, 2.0 Hz, 1H), 3.72 (s, 4H), 3.32–3.28 (m, 4H), 3.13 (t, *J* = 6.4 Hz, 2H), 2.96 (t, *J* = 7.0 Hz, 2H), 2.89 (t, *J* = 6.3 Hz, 2H), 2.52 (t, *J* = 7.0 Hz, 2H), 1.97–1.84 (m, 8H); ¹³C NMR (100 MHz, CDCl₃, ppm) δ: 171.4, 161.7, 152.3, 151.3, 150.3, 148.6, 148.0, 141.1, 136.8, 134.4, 129.3, 129.0, 128.1, 127.8, 126.3, 125.0, 124.2, 122.7, 115.8, 50.7, 50.3, 46.8, 42.7, 34.0, 32.9, 28.9, 26.8, 25.3, 24.6, 22.8, 22.6. ESI-MS: C₃₃H₃₃Cl₂N₇O, exact mass: 613.21, *m/z* 615.4 (M + 2)⁺.

1-(4-(6-Chloro-1,2,3,4-tetrahydroacridin-9-yl)piperazin-1-yl)-2-(((1-(7-chloroquinolin-4-yl)-1*H*-1,2,3-triazol-4-yl)methyl)-(methyl)amino)ethan-1-one (8b4). Yield: 70%; white solid; mp: 113–116 °C. ¹H NMR (400 MHz, CDCl₃, ppm) δ: 9.06 (d, *J* = 4.6 Hz, 1H), 8.24 (d, *J* = 2.0 Hz, 1H), 8.14 (s, 1H), 8.09–8.02 (m, 2H), 7.94 (d, *J* = 2.0 Hz, 1H), 7.59 (dd, *J* = 9.1, 2.1 Hz, 1H), 7.53 (d, *J* = 4.6 Hz, 1H), 7.35 (dd, *J* = 9.0, 2.1 Hz, 1H), 4.01 (s, 2H), 3.97–3.49 (m, 4H), 3.44 (s, 2H), 3.30 (d, *J* = 29.1 Hz, 4H), 3.09 (t, *J* = 6.6 Hz, 2H), 2.90 (t, *J* = 6.2 Hz, 2H), 2.51 (s, 3H), 2.03–1.77 (m, 4H); ¹³C NMR (100 MHz, CDCl₃, ppm) δ: 168.6, 161.9, 152.1, 151.4, 150.2, 148.3, 144.5, 141.0, 136.9, 134.2, 129.4, 129.0, 128.1, 127.8, 126.2, 125.2, 125.0, 124.62, 124.2, 120.5, 115.9, 59.4, 51.4, 50.8, 50.3, 46.9, 43.0, 34.1, 26.8, 22.8, 22.6. ESI-MS: C₃₂H₃₂Cl₂N₈O, exact mass: 614.21, *m/z* 616.4 (M + 2)⁺.

1-(4-(6-Chloro-1,2,3,4-tetrahydroacridin-9-yl)piperazin-1-yl)-5-(((1-(7-chloroquinolin-4-yl)-1*H*-1,2,3-triazol-4-yl)methyl)-(methyl)amino)pentan-1-one (8b5). Yield: 37%; white solid;

mp: 127–130 °C. ¹H NMR (400 MHz, CDCl₃, ppm) δ: 9.05 (d, *J* = 4.6 Hz, 1H), 8.23 (d, *J* = 2.0 Hz, 1H), 8.09 (s, 1H), 8.05–8.00 (m, 2H), 7.94 (d, *J* = 2.1 Hz, 1H), 7.59 (dd, *J* = 9.1, 2.1 Hz, 1H), 7.53 (d, *J* = 4.6 Hz, 1H), 7.35 (dd, *J* = 9.0, 2.1 Hz, 1H), 4.09–3.64 (m, 6H), 3.09 (t, *J* = 6.6 Hz, 2H), 2.87 (t, *J* = 6.2 Hz, 2H), 2.59 (t, *J* = 7.0 Hz, 2H), 2.48–2.40 (m, 5H), 1.96–1.66 (m, 8H); ¹³C NMR (100 MHz, CDCl₃, ppm) δ: 171.5, 161.9, 152.1, 151.4, 150.2, 148.3, 145.9, 141.0, 136.8, 134.2, 129.4, 129.0, 128.1, 127.8, 126.1, 125.0, 124.7, 124.60, 124.2, 120.5, 115.9, 56.8, 52.2, 50.7, 50.3, 46.8, 42.7, 42.3, 34.1, 33.1, 27.0, 26.8, 23.0, 22.8, 22.6. ESI-MS: C₃₅H₃₈Cl₂N₈O, exact mass: 656.25, *m/z* 657.2 (M + 1)⁺.

N-(2-((6-Chloro-1,2,3,4-tetrahydroacridin-9-yl)amino)ethyl)-5-(1-(7-chloroquinolin-4-yl)-1*H*-1,2,3-triazol-4-yl)pentanamide (8b6). Yield: 65%; white solid; mp: 112–114 °C. ¹H NMR (400 MHz, CDCl₃, ppm) δ: 8.98 (d, *J* = 3.9 Hz, 1H), 8.17 (s, 1H), 7.94–7.73 (m, 4H), 7.51–7.31 (m, 3H), 7.11 (d, *J* = 8.5 Hz, 1H), 5.19 (s, 1H), 3.61 (d, *J* = 23.7 Hz, 4H), 3.92–3.33 (m, 8H), 1.80 (s, 8H); ¹³C NMR (100 MHz, CDCl₃, ppm) δ: 174.7, 159.1, 151.3, 150.9, 150.0, 148.3, 147.4, 140.9, 136.7, 134.0, 129.2, 128.8, 126.6, 124.6, 124.6, 124.0, 122.8, 120.4, 117.8, 115.8, 115.4, 50.2, 40.5, 36.0, 33.8, 28.6, 25.1, 25.0, 22.9, 22.5. ESI-MS: C₃₁H₃₁Cl₂N₇O, exact mass: 587.20, *m/z* 589.0 (M + 2)⁺.

Acetylcholinesterase and butyrylcholinesterase inhibition assay

The inhibition ability of tacrine derivatives **8a1–8a5** and **8b1–8b6** against electric eel acetylcholinesterase (eeAChE; Sigma-Aldrich, USA; Enzyme Information Download Website: https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/Product_Information_Sheet/c3389pis.pdf) and horse serum butyrylcholinesterase (hsBChE; Sigma-Aldrich, USA; Enzyme Information Download Website: <https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/Datasheet/c1057dat.pdf>) was tested using the 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) method with the related assay kit (Keygen, China). DTNB would generate 5-mercapto-2-nitrobenzoic acid – a detectable chromophore at the 405–412 nm range. The final concentrations (100 μM, 20 μM, 4 μM, 0.8 μM, 0.16 μM, and 0.032 μM) of screening compounds were prepared using DMSO. In 96-well plates, 20 μL eeAChE (or hsBChE, diluted to 0.5 U mL⁻¹ using saline) was incubated with the above test compounds of different concentrations at room temperature for 15 min, followed by the addition of relative agents according to the kit instruction. Finally, the chromophore absorbance was examined at the wavelength of 405 nm *via* a microplate reader (Thermo MK3). The percentage of inhibition was calculated by the comparison of compound-treated to various control incubations.

Molecular simulations

Preparation of hAChE/hBChE structures and ligands. The atomic coordinates of hAChE and hBChE were obtained from the Protein Data Bank (PDB ID: hAChE: 1B41 (resolution = 2.76 Å)⁴⁰ and hBChE: 1P0I (resolution = 2.0 Å)).⁴¹ Protein

structures were imported into the Maestro module available in the Schrödinger Suite (Schrödinger, LLC),⁴² and subsequently, both structures were optimized separately using the Protein Preparation Wizard.⁴³ This protein structure optimization includes adding hydrogen atoms, assigning bond orders and building disulfide bonds. The protonation states of the ionisable residues (pH = 7) were predicted using the PROPKA tool provided in the Protein Preparation Wizard.⁴¹ An optimized structure was finally found by energy minimization (*i.e.*, position of the hydrogen atoms) with the OPLS_2005 force field.

The 3D structures of 8a2 and tacrine were built with Maestro and preprocessed using the LigPrep module (v2.5)⁴⁴ of the Schrödinger modeling software. Preprocessing includes energy minimization using the OPLS_2005 force field and determination of ionization states at pH = 7 (± 2).⁴⁵

Molecular docking simulation

The receptor grid generation module of Glide⁴⁶ was used to define both peripheral sites (PASSs) and catalytic sites (CASs) for the docking experiments in hAChE and hBChE. A set of PAS and CAS residues was used as the centroid of the grid box (of size 20 Å).^{40,41} Water molecules at the active site were deleted. Glide uses an in-built docking scoring function, resulting in a Glidescore (standard precision). The docking and scoring function parameters and settings used in this study are described in detail elsewhere.⁴⁷ The best 25 docking solutions for each protein structure were selected for investigation, followed by MM-GBSA calculation.^{48–50} The Prime MM-GBSA method calculates the binding affinity of the ligand (ΔG_{bind}) by energy minimization procedures. The binding energy of the ligand was extracted from an energy-optimized protein–ligand complex and Prime MM-GBSA using the VSGB 2.0 solvation (implicit) model.^{50,51} During binding affinity calculation, the program offers the option to treat the ligand and protein as flexible; in this study, a 5 Å region of the protein around the ligand was treated as flexible.

Ethical statement

All procedures were conducted in strict accordance with Chinese relevant laws and institutional guidelines (School of Pharmaceutical Sciences, Shandong University). This study was performed in strict accordance with the NIH guidelines for the care and use of laboratory animals (NIH Publication No. 85-23 Rev. 1985) and was approved by the Institutional Animal Care and Use Committee of Shandong University (Shandong, China).

Author contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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