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Inhibition of cholinesterase activity and amyloid aggregation by berberine-phenyl-benzoheterocyclic and tacrine-phenyl-benzoheterocyclic hybrids

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ABSTRACT

A series of berberine-phenyl-benzoheterocyclic (**26–29**) and tacrine-phenyl-benzoheterocyclic hybrids (**44–46**) were synthesised and evaluated as multifunctional anti-Alzheimer's disease agents. Compound **44b**, tacrine linked with phenyl-benzothiazole by 3-carbon spacers, was the most potent AChE inhibitor with an IC_{50} value of 0.017 μM. This compound demonstrated similar Aβ aggregation inhibitory activity with cucurmin (51.8% vs 52.1% at 20 μM, respectively), indicating that this hybrid is an excellent multi-functional drug candidate for AD.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that, as of 2010, has affected approximately 36 million people.[1] The etiology of AD is still elusive, and multiple factors, such as amyloid β (Aβ) deposits, τ-protein aggregation, oxidative stress, and low levels of acetylcholine (ACh), have been suggested to contribute to the development of AD.[2] In the past decade, treatment strategies for AD have mainly been aimed at improving cholinergic neurotransmission in brain, which was mostly based on the 'cholinergic hypothesis'.[3] Many approaches have been investigated based on this hypothesis, but cholinesterase inhibitors (ChEIs) were the first and, to date, the only species that showed some promise in the treatment of AD.[4] The following four cholinesterase inhibitors have been approved by the US Food and Drug Administration: tacrine, donepezil, rivastigmine and galantamine. These inhibitors have beneficial effects on cognitive, functional and behavioural symptoms of AD. The amyloid hypothesis, which is another hypothesis regarding the etiology of AD, states that the accumulation of Aβ in the brain is the primary influence driving AD pathogenesis.[5] According to this hypothesis, the development of anti-AD drugs to reduce the concentration of Aβ oligomers by inhibiting aggregation could possibly be another therapeutic strategy.[6] Recently, a number of Aβ fibril formation inhibitors have been reported, including curcumin and its analogs (**1**; Fig. 1) as well as bisphenol A derivatives (**2**; Fig. 1).[7]

Because AD is a complex neurodegenerative disorder resulting from multiple factors, molecules that modulate the activity of a single protein target are unable to significantly alter the progression of the disease. Recently, a strategy of developing drugs that simultaneously affect multiple targets,[8] which is also called the 'multitarget-directed ligands' (MTDLs) design strategy, has been proposed instead of the 'one protein, one target, one drug' strategy. Following the MTDLs design strategy, we have developed a novel series of benzenediol-berberine hybrids (**3**; Fig. 1), which simultaneously target three molecular abnormalities of AD.[9] Herein, we describe the design, synthesis, and evaluation of berberine-phenyl-benzoheterocyclic and tacrine-phenyl-benzoheterocyclic hybrids as multifunctional anti-AD agents that target cholinesterase activity and amyloid aggregation.

2. Results and discussion

2.1. Design and chemistry

Howlett and coworkers described a series of benzofuran analogues (**4**; Fig. 2) as inhibitors of Aβ fibril formation via a process involving the binding of benzofuran to a peptide.[10] Recently, a new series of hybrid molecules based on benzofuran analogues and *N*-methyl-*N*-benzylamine as inhibitors of acetylcholinesterase (AChE)/butylcholinesterase (BuChE) and Aβ aggregation were reported.[11] The results of this study suggest that MTDLs could be

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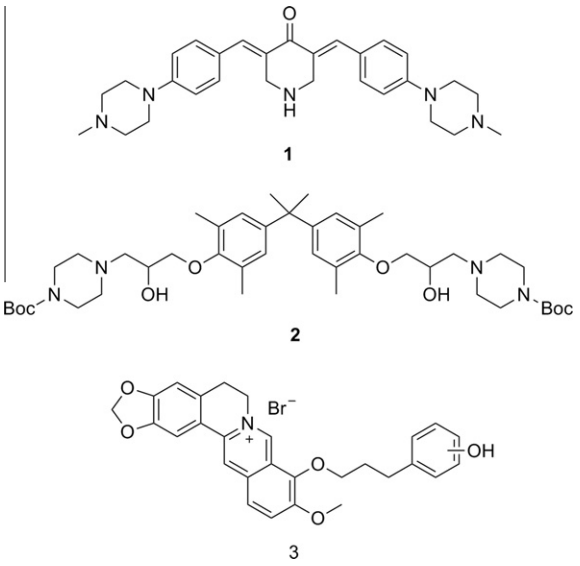


Figure 1. Structures of a curcumin analog (**1**), a bisphenol A derivative (**2**) and benzenediol-berberine hybrids (**3**).

more therapeutically effective if their structural elements or pharmacophores were combined rationally to a single molecule. In previous work, we determined that berberine (**5**; Fig. 2) could inhibit AChE in the sub-micromolar range (IC_{50} = 0.374 μM), which suggested that berberine was an appropriate leading compound for development of anti-AD drugs.[12] With this new paradigm, we combined the phenyl-benzofuran moiety (anti-Aβ aggregation activity) with berberine and tacrine (**6**; Fig. 2) (anti-cholinesterase moiety) using carbon spacers of varying lengths (**26a–b**, **46b–c**; Fig. 2) to investigate their activity when simultaneously affecting multiple targets. To evaluate the effect on AChE on the phenyl-benzoheterocyclic moiety, we substituted the benzofuran moiety with indole (**27a–b**; Fig. 2), benzothiazole (**28a–b**, **44b–e**; Fig. 2) and benzoxazole (**29a–b**, **45b–c**; Fig. 2) for comparison.

The synthetic route of berberine-phenyl-benzoheterocyclic hybrids is shown in Scheme 1. Firstly, 4-methoxy-phenyl-benzofuran (**10**) was synthesised from the commercially available salicylic aldehyde (**7**) as previously reported.[13] Then, the *O*-methyl group of **10** was removed via a reaction with BBr_3 to yield intermediate **11**. Compound **14,** 4-hydroxybenzoindole, was synthesised by reacting aniline and 2-bromoacetophenone (**13**) under microwave irradiation without any catalysts. Compound **17**, 4-hydroxy-phenyl-benzothiazole, was prepared from 2-aminobenzenethiol and *p*-hydroxybenzaldehyde with good yields (80%). Compound **20**, 4-hydroxy-phenyl-benzooxazole, was synthesised via the condensation of 2-aminophenol (**18**) with 4-hydroxybenzoic acid (**19**) in the presence of *p*-toluenesulfonic acid. Alkylations of intermediates **11**, **14**, **17** and **20** with 1,2-dibromoethane or 1,3-dibromopropane in acetone afforded compounds **21–24**, respectively. Target molecules **26**–**29** were obtained via reactions of **21–24** with berberrubine (**25**) in 31–69% yields.

The preparation of hybrids **34–35** is outlined in Scheme 2. The reaction of commercially available indole-2-carboxylic acid (**30**) and benzofuran-2-carboxylic acid (**31**) with 3-bromopropylmine produced amides **32–33**, which reacted with berberrubine to yield target compounds **34** and **35**.

Tacrine-phenyl-benzoheterocyclic hybrids were synthesised with phthalimide **36** as the starting material (Scheme 3). Firstly, the reaction of phthalimide with α,ω-dibromoalkanes in the presence of K_2CO_3 provided intermediates **37a–d**, which were

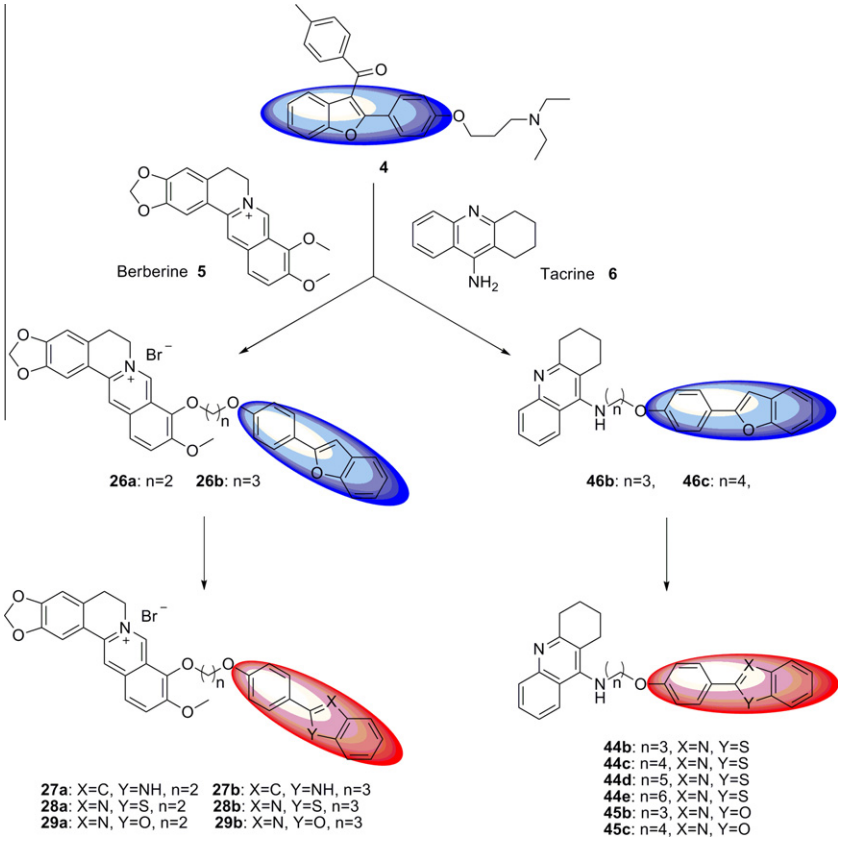
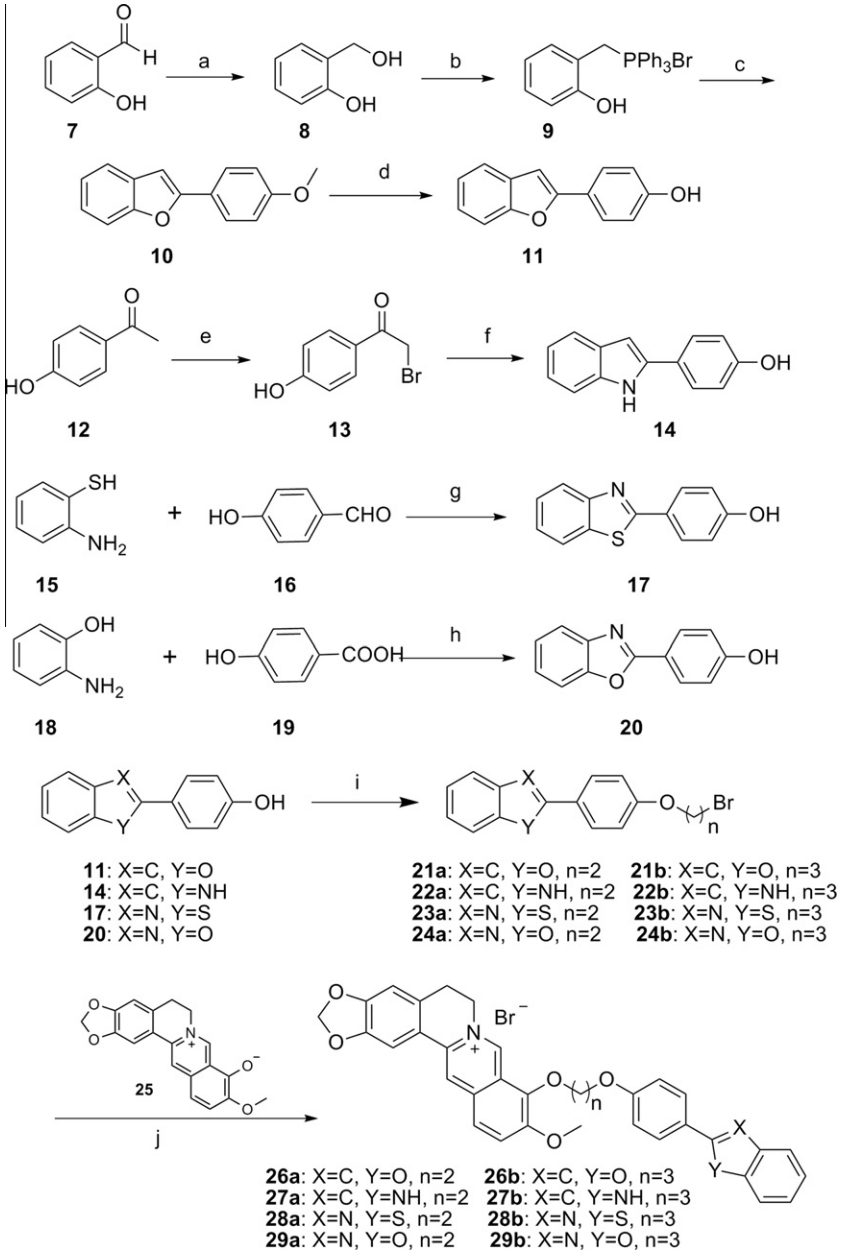
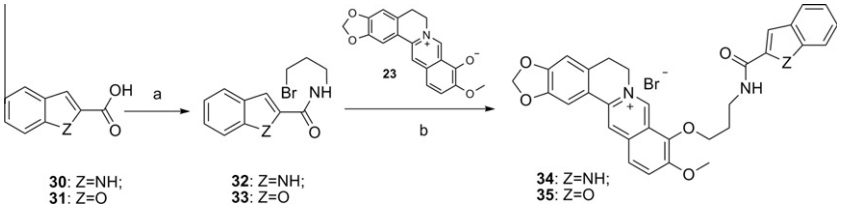


Figure 2. Design strategy for compounds **26–29, 44–46**.



Scheme 1. Reagents and conditions: (a) EtOH, $NaBH_4$; (b) acetonitrile, PPh_3 HBr; (c) toluene, 4-methoxybenzoyl chloride, NEt_3; (d) CH_2Cl_2, BBr_3 (e) $CuBr_2$, EtOH; (f) aniline, $C_2H_5OC_2H_4OH$, microwave; (g) toluene, reflux, overnight; (h) p-TsOH, xylene, reflux, 12 h; (i) $Br(CH_2)_nBr$, K_2CO_3, actone, reflux; (j) K_2CO_3, DMF, 80 °C.

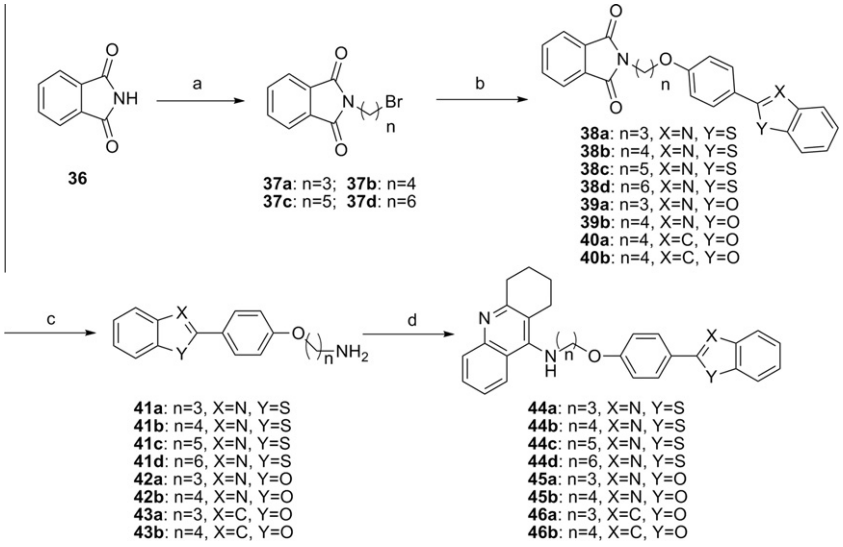


Scheme 2. Reagents and conditions: (a) 3-bromopropylamine, EDCI, THF/DMF; (b) K_2CO_3, DMF, 80 °C, 24 h.

subsequently reacted with compounds **11**, **17** and **20** to give compounds **38–40**, respectively. Reduced with hydrazine, compounds **38–40** were converted to amines **41–42** with almost quantitative yields. Finally, the reaction of 9-chloro-1,2,3,4-tetrahydroacridine with amines **41–42** provided hybrids **44–46** in good yields.

2.2. In vitro inhibition studies of AChE and BuChE

The AChE and BuChE inhibitory effects of the hybrids were determined by the spectroscopic method described by Ellman et al. using tacrine as the standard.[14] AChE (E.C.3.1.1.7) and BuChE



Scheme 3. Reagents and conditions: (a) $Br(CH_2)_nBr$, K_2CO_3, TEBAC, acetone, 24 h; (b) **11**, **17**, **20**, K_2CO_3, DMF; (c) hydrazine hydrate, EtOH, reflux, 4 h; (d) 9-chloro-1,2,3,4-tetrahydroacridine, pentanol, reflux, 24 h.

(E.C. 3.1.1.8) were obtained from electric eel and equine serum, respectively.

The IC_{50} values for AChE and BuChE inhibition are summarised in Table 1. Most of the hybrids demonstrated potent inhibitory activity against AChE and BuChE with IC_{50} values in the micromolar to sub-micromolar range. In particular, compound **44b**, tacrine and phenyl-benzofuran linked with a 3-carbon spacer, exhibited the most potent inhibition of AChE (IC_{50} = 0.017 μM), which is 18-fold more potent than tacrine. A simple structure–activity relationship analysis showed that the AChE inhibitory potency was closely related to the length of the alkylene chain. From the IC_{50} values of the tested compounds, it appears that a 3-carbon spacer seems to be the proper length for a linker between the two pharmacophore groups in both series. For example, compounds **26b, 27b, 28b, 29b** and **44b,** in which berberine or tacrine was linked with phenyl-benzofuran via a 3-carbon spacer, exhibited greater inhibition (IC_{50} = 1.92, 0.774, 1.48, 2.12, and 0.017 μM, respectively) than their relatives, **26a, 27a, 28a, 29a** and **44c–e,** which contained linkers that were either 2-, 4-, or 5-carbon spacers (IC_{50} = 3.59, 1.07, 1.64, 3.30, 0.105, 0.040, and 0.093 μM, respectively). The replacement of the alkylene chain with an acyl alkyl chain resulted in only minor changes in AChE inhibitory activity (**27b** vs **34,** with IC_{50} values of 0.774 and 0.976 μM; **26b** vs **35,** with IC_{50} values 1.92 and 1.94 μM, respectively). Most tacrine-phenyl-benzoheterocyclic hybrids were more potent inhibitors of AChE than the berberine-phenyl-benzoheterocyclic hybrids, indicating that the stereospecificity of the functional groups is very important for inhibitory activity. The results also indicated that the structure of the benzoheterocyclic side chain also affects the inhibitory activity of these compounds (Table 1). The order of activity for the four benzoheterocyclic groups is as follows: indole > benzothiazole > benzofuran > benzoxazole. However, the same trend was not observed in BuChE inhibition. Almost all the compounds showed potent activity against BuChE. Additionally, some compounds demonstrated higher selectivity (i.e., **26–29, 45c**) for BuChE than AChE. Compound **44e**, the most potent for BuChE inhibition (IC_{50} = 0.082 μM), exhibited almost equal inhibitory activity with AChE. Based on these results, these hybrids should be good dual AChE/BChE inhibitors to treat AD.

2.3. Kinetics of AChE inhibition

The inhibition type of AChE was investigated by graphical analysis of steady state inhibition data (Fig. 3) using compound **44b** as a typical example. Reciprocal plots (Lineweaver–Burk plots) describing **44b** inhibition showed both increasing slopes and increasing intercepts with higher inhibitor concentration, indicating a mixed-type inhibition. These results revealed that compound **44b** was able to bind to both the active site and PAS of AChE, which is also in agreement with the results of our molecular modeling studies.

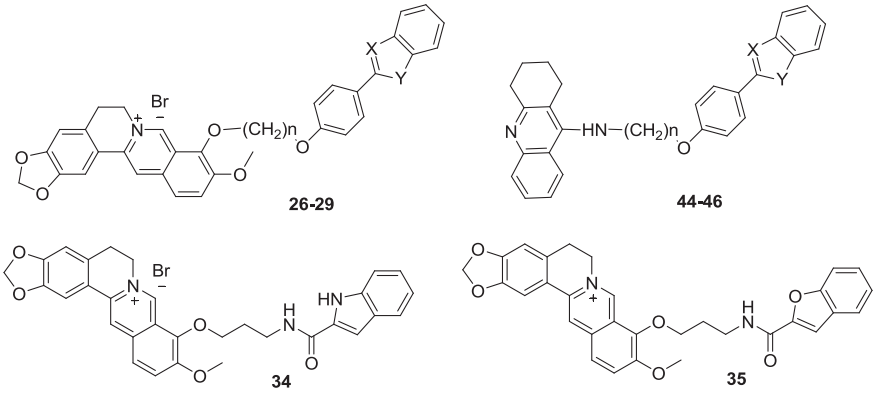
2.4. Molecular modeling studies

To explain the interaction modes of the hybrids to AChE, molecular docking simulations for derivative **44b** to TcAChE were performed using the CDOCKER program in the Discovery studio 2.1 software based on the X-ray crystal structure of the *Tc*AChE-bis-(7)-tacine complex (PDB entry 2CMF). The docking results showed that hybrid **44b** could simultaneously bind to both the central pocket and peripheral sites (Fig. 4a, b). The tacrine moiety of the hybrid was observed to enter the AChE gorge, adopting parallel π–π interactions with Trp84 (3.67 Å) and Phe330 (4.32 Å) in a 'sandwich' form. As shown in Figure 4b, the benzothiazole moiety stacks with Trp279 of PAS with a distance of 4.65 Å of ring-to-ring. In addition, the benzyl moiety, linked at the rim of the gorge, has a face-to-edge π–π interaction with Phe331 (4.71 Å), which could also fold in a proper conformation into the gorge to interact with Tyr70, Asp72, Trp271, Phe330 and Tyr334 through hydrophobic interactions.

2.5. Inhibition of $A\beta_{1-42}$ aggregation

$A\beta_{1-40}$ and $A\beta_{1-42}$ are the main forms of Aβ peptides found in the amyloid plaques, which are released from an amyloid precursor protein through sequential cleavages by β- and γ-secretases. Although $A\beta_{1-40}$ is the predominant product of this proteolytic pathway, $A\beta_{1-42}$ is far more fibrillogenic.[15] Therefore, we chose $A\beta_{1-42}$ to study the inhibition of the hybrid compounds. The ability of novel hybrids to inhibit $A\beta_{1-42}$ aggregation was assessed by the

Table 1
In vitro inhibition of AChE and BuChE and Aβ self-aggregation of berberine, cucurmin, and compounds **27–29, 34–35,** and **44–46**



Compound	X	Y	*n*	IC_{50} [a](μM)		Selectivity for AChE[d]	Aβ self-aggregation inhibitory[e] (IC_{50}, μM)
				AChE/ACh[b]	**BuChE/BuCh**[c]		
Berberine	—	—	—	0.374 ± 0.024	18.2 ± 0.683	48.6	36.3%[f]
26a	C	O	2	3.59 ± 0.270	0.688 ± 0.062	0.2	5.78 ± 0.37
26b	C	O	3	1.92 ± 0.058	0.990 ± 0.028	0.5	84.6% [f]
27a	C	NH	2	1.07 ± 0.005	0.421 ± 0.001	0.4	4.03 ± 0.14
27b	C	NH	3	0.774 ± 0.013	0.711 ± 0.048	0.9	4.69 ± 0.50
28a	N	S	2	1.64 ± 0.015	0.175 ± 0.013	0.1	6.45 ± 0.44
28b	N	S	3	1.48 ± 0.091	0.587 ± 0.031	0.4	79.2% [f]
29a	N	O	2	3.30 ± 0.018	0.346 ± 0.007	0.1	3.61 ± 0.09
29b	N	O	3	2.12 ± 0.021	0.639 ± 0.011	0.3	3.75 ± 0.10
34	—	—	—	0.976 ± 0.060	2.19 ± 0.127	1.1	73.5% [f]
35	—	—	—	1.94 ± 0.094	1.54 ± 0.137	1.7	69.1% [f]
44b	N	S	3	0.017 ± 0.002	0.122 ± 0.006	7.2	51.8% [f]
44c	N	S	4	0.105 ± 0.014	0.186 ± 0.010	1.8	37.8% [f]
44d	N	S	5	0.040 ± 0.004	0.171 ± 0.015	4.2	40.7% [f]
44e	N	S	6	0.093 ± 0.006	0.082 ± 0.007	0.9	41.9% [f]
45b	N	O	3	0.475 ± 0.002	0.352 ± 0.010	0.7	57.6% [f]
45c	N	O	4	0.947 ± 0.023	0.207 ± 0.018	0.2	33.2% [f]
46b	C	O	3	0.058 ± 0.001	0.156 ± 0.002	2.7	62.8% [f]
46c	C	O	4	0.201 ± 0.014	0.238 ± 0.008	1.2	46.3% [f]
Tacrine	—	—	—	0.311 ± 0.009	0.041 ± 0.003	0.13	n.t.[g]
Curcumin	—	—	—	n.t.	n.t.	—	18.7 ± 0.9952.1%[f]

[a] Mean ± SD of at least three independent measurements.
[b] Acetylcholine substrate for evaluation of antiacetylcholinesterase activity.
[c] Butyrylcholine substrate for evaluation of antibutyrylcholinesterase activity.
[d] Selectivity for AChE = IC_{50} (BuChE)/IC_{50} (AChE).
[e] The thioflavin-T fluorescence method was used. Value represents as the mean ± SD from at least two independent measurements.
[f] All values were measured at the inhibitors' concentration of 20 μM.
[g] n.t. = not tested.

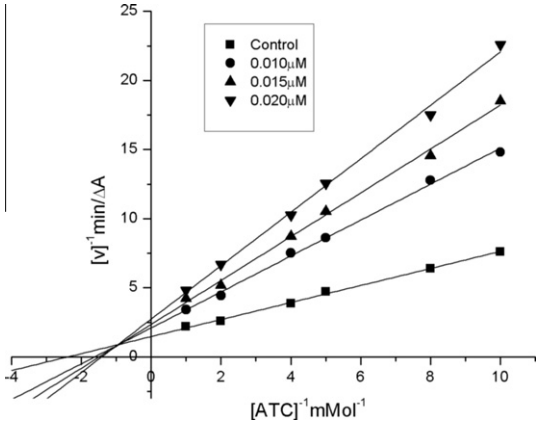


Figure 3. Steady state inhibition by **44b** of AChE hydrolysis of Ach. The plots show mixed-type inhibition for **44b** on AChE.

thioflavin T fluorescence assay using curcumin as a standard (Table 1). Significantly, all the berberine-phenyl-benzoheterocyclic hybrids (IC_{50} = 3.61–6.45 μM, 69.1–84.6% at 20 μM) exhibited greater inhibitory activities than curcumin (IC_{50} = 18.7 μM, 52.1% at 20 μM) and berberine (36.3% at 20 μM). Among these, compound **29a**, the berberine-phenyl-benzoxazole hybrid, was the most potent inhibitor of Aβ aggregation (IC_{50} = 3.61 μM), which is 5.2-fold more potent than curcumin. Conversely, most of the berberine-phenyl-benzoheterocyclic hybrids exhibited higher or similar Aβ aggregation inhibitory activity with cucurmin. Among them, compound **46b** exhibits the highest inhibitory activity of 62.8% at 20 μM. In particular, compound **44b**, the most potent AChE inhibitor, demonstrated similar Aβ aggregation inhibitory activity with cucurmin (51.8% vs 52.1% at 20 μM, respectively), which indicated that compound **44b** is a good multifunctional candidate drug for AD.

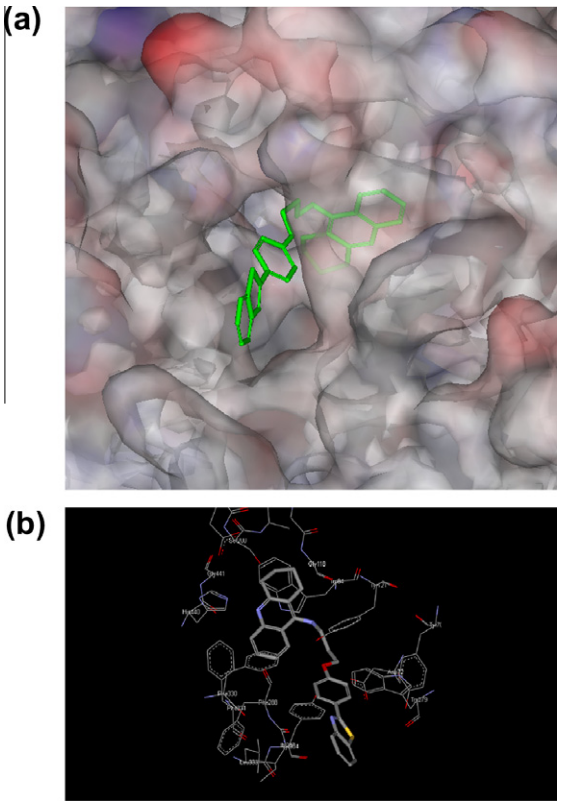


Figure 4. Docking models of the compound–enzyme complex. (a) Stereoviews looking down the gorge of TcAChE binding with **44b.** (b) Representation of compound **44b** docked into the binding site of AChE, highlighting the protein residues that form the main interactions with the inhibitor.

3. Conclusion

In conclusion, we have synthesised and evaluated a series of berberine-phenyl-benzoheterocyclic (**26–29**) and tacrine-phenyl-benzoheterocyclic hybrids (**44–46**) as multifunctional anti-AD agents. Our results demonstrate that these compounds possess potent AChE and Aβ aggregation inhibitory activities. Compound **44b**, tacrine linked with phenyl-benzothiazole by 3-carbon spacers, was the most potent AChE inhibitor with an IC_{50} of 0.017 μM. Additionally, compound **44b** demonstrated similar Aβ aggregation inhibitory activity with cucurmin (51.8% vs 52.1% at 20 μM, respectively), indicating that this compound is an excellent multifunctional drug candidate for AD. Kinetic analyses and molecular docking stimulations revealed that the inhibitors could simultaneously interact with the central pocket, gorge, and peripheral pocket of AChE. Finally, our results indicate that these new compounds represent useful templates for the development of new multifunctional anti-AD agents.

4. Experimental section

4.1. Chemistry

The ^{1}H NMR and ^{13}C NMR spectra were recorded using TMS as the internal standard on a Bruker BioSpin GmbH spectrometer at 400.132 MHz and 100.614 MHz, respectively. Coupling constants are given in Hz. High-resolution mass spectra were obtained using a Shimadzu LCMS-IT-TOF mass spectrometer. Flash column chromatography was performed using Silica gel (200–300 mesh) purchased from Qingdao Haiyang Chemical Co. Ltd or alumina from Sinopharm Chemical Reagent Co. Ltd. All the reactions were monitored by thin layer chromatography using silica gel.

4.1.1. 2-(Hydroxymethyl)phenol (8)

Sodium borohydride (6 mmol) was added to a stirring solution of 2-hydroxybenzaldehyde (12 mmol) in ethanol (20 mL) at 0 °C. The reaction mixture was stirred at room temperature for 1 h. After the solvent was removed, 40 mL of 1 N HCl solution was added to the residue and extracted with diethyl ether (40 mL). The organic phase was dried over Na_2SO_4 and filtered. The filtrate was concentrated to give compound **8** (90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 (s, 1H), 7.24 (d, J = 6.9 Hz, 1H), 7.22–7.12 (m, 1H), 7.02 (d, J = 6.9 Hz, 1H), 6.92–6.78 (m, 2H), 4.78 (s, 2H), 2.91 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.80, 129.48, 128.04, 124.87, 120.19, 116.40, 64.27.

4.1.2. 2-Hydroxybenzyltriphenylphosphonium bromide (9)

A solution of **8** (10 mmol) and triphenylphosphine hydrobromide (10 mmol) in acetonitrile (40 mL) was stirred under reflux for 1 h. The solid that formed was filtered and washed with acetonitrile to give compound **9** as white solid. (85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.00 (s, 1H), 7.79–7.68 (m, 3H), 7.63–7.44 (m, 12H), 7.27 (m, 1H), 7.02–6.95 (m, 1H), 6.95–6.90 (m, 1H), 6.59 (t, J = 7.4 Hz, 1H), 4.55 (d, J = 13.5 Hz, 2H).

4.1.3. 2-(4-Methoxyphenyl)benzofuran (10)

A mixture of **9** (5 mmol) and 4-methoxybenzoyl chloride (5 mmol) in a mixed solvent (toluene 20 mL and triethylamine 0.5 mL) was stirred under reflux for 2 h. The precipitate was removed by filtration. The filtrate was concentrated, and the residue was recrystalized with ethyl acetate to give **10** (30%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76–7.62 (m, 2H), 7.43 (ddd, J = 26.7, 9.3, 4.8 Hz, 2H), 7.19–7.07 (m, 2H), 6.91–6.82 (m, 2H), 6.77 (d, J = 0.7 Hz, 1H), 3.74 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.94, 155.02, 153.66, 128.46, 125.37, 122.69, 122.30, 121.79, 119.53, 113.21, 109.94, 98.64, 54.29.

4.1.4. 4-(Benzofuran-2-yl)phenol (11)

BBr_3 (12 mL,1 M solution in CH_2Cl_2) was added to a solution of **10** (2 mmol) in CH_2Cl_2 (30 mL) dropwise in an ice bath. The mixture was allowed to warm to room temperature and stirred for 30 min. Water (40 mL) was added while the reaction mixture was cooled in an ice bath. The mixture was extracted with ethyl acetate, and the organic phase was dried over Na_2SO_4 and filtered. The filtrate was concentrated, and the residue was purified by chromatography on a silica gel column with EtOAc/petroleum ether as eluent to give **11** (63%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.35 (s, 1H), 7.61 (d, J = 8.2 Hz, 2H), 7.45 (d, J = 6.9 Hz, 1H), 7.39 (d, J = 7.5 Hz, 1H), 7.18–7.07 (m, 2H), 6.87–6.77 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.10, 161.18, 159.15, 134.26, 131.18, 128.33, 127.63, 126.30, 125.26, 120.74, 115.58, 103.72.

4.1.5. 2-Bromo-1-(4-hydroxyphenyl)ethanone (13)

$CuBr_2$ (17.92 g, 80 mmol) was added to a stirring solution of **12** (5.44 g, 40 mmol) in ethanol (120 mL) in room temperature. The mixture was stirred under reflux for 6 h. The precipitate was removed by filtration. The filtrate was concentrated, and the residue was dissolved in ethyl acetate. The solution was washed with water, and the organic phase was dried over Na_2SO_4 and filtered. The filtrate was concentrated, and the residue was purified by recrystalization with ethanol to give 6.25 g of **13** (75.8%). ^{1}H NMR (400 MHz, DMSO) δ 10.44 (d, J = 64.4 Hz, 1H), 7.89 (d, J = 6.6 Hz, 2H), 6.87 (d, J = 8.0 Hz, 2H), 4.99–4.56 (m, 2H); ^{13}C NMR (101 MHz, DMSO) δ 189.81, 162.61, 131.40, 130.01, 125.35, 115.38, 40.45, 33.43.

4.1.6. 4-(1*H*-Indol-2-yl)phenol (14)

A solution of **13** (0.56 g, 2.5 mmol) and aniline (0.7 g, 7.5 mmol) in 2-ethoxyethanol (5 mL) was placed in a microwave tube. The

mixture was heated in a microwave reactor at 140 °C for 10–12 min. The mixture was filtered and then evaporated under vacuum .The residue was purified by recrystalizaiton with ethanol and water,and then was purified again by chromatography on a silica gel column to give a yellow solid 0.2 g of **14** (38.3%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.68 (s, 1H), 9.08 (s, 1H), 7.54 (d, J = 8.3 Hz, 2H), 7.42 (d, J = 7.7 Hz, 1H), 7.30 (d, J = 7.9 Hz, 1H), 6.97 (dd, J = 14.7, 6.8 Hz, 1H), 6.91 (t, J = 7.1 Hz, 1H), 6.80 (d, J = 8.2 Hz, 2H), 6.52 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.99, 137.61, 135.80, 128.43, 125.69, 123.22, 120.38, 119.03, 118.76, 115.04, 109.88, 96.86.

4.1.7. 4-(Benzothiazole-2-yl)phenol (17)

A mixture of **15** (1.88 g, 15 mmol) and **16** (1.83 g, 15 mmol) in toluene (50 mL) was heated to reflux for overnight. After cooling to room temperature, the precipitate was removed by filtration. The filtrate was concentrated, and the residue was purified by recrystalization with toluene to give 2.1 g of **13**(61.7%). ^{1}H NMR (400 MHz, DMSO) δ 10.20 (s, 1H), 8.07 (d, J = 7.9 Hz, 1H), 7.97 (d, J = 7.9 Hz, 1H), 7.95–7.90 (m, 2H), 7.53–7.46 (m, 1H), 7.42–7.36 (m, 1H), 6.96–6.89 (m, 2H).

4.1.8. 4-(Benzoxazole-2-yl)phenol (20)

A mixture of **18** (1.09 g, 10 mmol) and **19**(1.66 g, 12 mmol) and *p*-toluenesulfonic(9.51 g, 50 mmol) in xylene (40 mL) was heated to reflux for 12 h. Ethyl acetate(120 ml) was added to the mixture, and then washed with saturated sodium bicarbonate (20 × 50 ml) and brine (50 ml).The organic phase was dried over Na_2SO_4 and filtered. The filtrate was concentrated to get the crude product 1.26 g, which was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.67 (s, 1H), 12.74 (d, J = 8.1 Hz, 2H), 12.35 (d, J = 5.9 Hz, 1H), 12.29–12.23 (m, 1H), 12.01 (d, J = 2.2 Hz, 2H), 11.64 (d, J = 8.1 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.08, 165.94, 155.26, 147.03, 134.19, 129.33, 129.24, 124.09, 122.55.

4.2. General procedures for the preparation of 21a–b, 22a–b, 23a–b, 24a–b

To a stirred suspension of selective compounds (**11**, **14**, **17**, **20**) (5 mmol) and K_2CO_3 (15 mmol) in acetone (35 mL), dibromoalkanes (20 mmol) were added. The mixture was heated to reflux for 12–24 h, and then was filtered. The filtrate evaporated under vacuum to afford the crude product, which was purified by chromatography on an silica gel column with EtOAc/petroleum ether as eluent to give the target products.

4.2.1. 2-(4-(2-Bromoethoxy)phenyl)benzofuran (21a)

Compound **10** was treated with 1,2-dibromethane according to general procedure to give the desired product **21a** (47% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (dd, J = 12.6, 5.7 Hz, 2H), 7.55 (dd, J = 7.8, 6.6 Hz, 1H), 7.50 (d, J = 8.0 Hz, 1H), 7.30–7.27 (m, 1H), 7.25–7.18 (m, 1H), 6.99 (d, J = 8.9 Hz, 2H), 6.93–6.88 (m, 1H), 4.35 (t, J = 6.3 Hz, 2H), 3.67 (t, J = 6.3 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.41, 154.76, 153.70, 128.39, 125.48, 123.07, 122.85, 121.85, 119.62, 114.02, 109.99, 98.96, 66.89, 27.90.

4.2.2. 2-(4-(3-Bromopropoxy)phenyl)benzofuran (21b)

Compound **10** was treated with 1,3-dibrompropane according to general procedure to give the desired product **21b** (62% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (d, J = 8.8 Hz, 2H), 7.48 (d, J = 7.2 Hz, 1H), 7.42 (d, J = 7.7 Hz, 1H), 7.20 (d, J = 1.2 Hz, 1H), 7.14 (dd, J = 12.5, 4.6 Hz, 1H), 6.90 (d, J = 8.8 Hz, 2H), 6.82 (s, 1H), 4.13–4.01 (m, 2H), 3.59–3.49 (m, 2H), 2.34–2.21 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.05, 154.93, 153.68, 128.44, 125.42, 122.76, 122.57, 121.82, 119.57, 113.80, 109.97, 98.75, 64.40, 31.29, 28.88.

4.2.3. 2-(4-(3-Bromoethoxy)phenyl)-1*H*-indole (22a)

Compound **14** was treated with 1,2-dibromethane according to general procedure to give the desired product **22a** (38% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (s, 1H), 7.59 (t, J = 8.7 Hz, 3H), 7.37 (d, J = 8.0 Hz, 1H), 7.17 (dd, J = 11.1, 4.0 Hz, 1H), 7.11 (t, J = 7.4 Hz, 1H), 6.97 (d, J = 8.7 Hz, 2H), 6.72 (d, J = 1.3 Hz, 1H), 4.32 (t, J = 6.2 Hz, 2H), 3.65 (t, J = 6.2 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.48, 137.87, 136.68, 129.43, 126.51, 125.49, 121.96, 120.38, 120.20, 115.10, 110.72, 98.94, 65.48, 32.34, 29.90.

4.2.4. 2-(4-(3-Bromopropoxy)phenyl)-1*H*-indole (22b)

Compound **14** was treated with 1,3-dibrompropane according to general procedure to give the desired product **22b** (54% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (s, 1H), 7.59 (t, J = 8.3 Hz, 3H), 7.38 (d, J = 7.9 Hz, 1H), 7.17 (t, J = 7.4 Hz, 1H), 7.11 (t, J = 7.3 Hz, 1H), 6.98 (d, J = 8.6 Hz, 2H), 6.71 (s, 1H), 4.15 (t, J = 5.8 Hz, 2H), 3.62 (t, J = 6.4 Hz, 2H), 2.42–2.25 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.48, 137.87, 136.68, 129.43, 126.51, 125.49, 121.96, 120.38, 120.20, 115.10, 110.72, 98.94, 76.41, 65.48, 32.34, 29.90.

4.2.5. 2-(4-(3-Bromoethoxy)phenyl)benzo[*d*]thiazole (23a)

Compound **17** was treated with 1,2-dibromethane according to general procedure to give the desired product **23a** (22% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (dd, J = 9.2, 2.4 Hz, 3H), 7.87 (d, J = 7.9 Hz, 1H), 7.50–7.44 (m, 1H), 7.39–7.32 (m, 1H), 7.03–6.97 (m, 2H), 4.34 (t, J = 6.2 Hz, 2H), 3.66 (t, J = 6.2 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.55, 159.32, 153.16, 133.85, 128.16, 126.08, 125.23, 123.87, 121.86, 120.51, 114.01, 66.87, 27.72.

4.2.6. 2-(4-(3-Bromopropoxy)phenyl)benzo[*d*]thiazole (23b)

Compound **17** was treated with 1,3-dibrompropane according to general procedure to give the desired product **23b** (35% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (t, J = 7.3 Hz, 3H), 7.77 (d, J = 7.9 Hz, 1H), 7.38 (t, J = 7.5 Hz, 1H), 7.26 (t, J = 7.5 Hz, 1H), 6.89 (d, J = 8.7 Hz, 2H), 4.09 (dt, J = 11.5, 5.9 Hz, 2H), 3.52 (t, J = 6.4 Hz, 2H), 2.31–2.18 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.79, 159.96, 153.10, 133.77, 128.09, 125.52, 125.20, 123.80, 121.76, 120.48, 113.82, 64.43, 31.15, 28.79.

4.2.7. 2-(4-(3-Bromoethoxy)phenyl)benzo[*d*]oxazole (24a)

Compound **20** was treated with 1,2-dibromethane according to general procedure to give the desired product **24a** (40% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (d, J = 8.7 Hz, 2H), 7.67 (dd, J = 8.4, 4.4 Hz, 1H), 7.48 (dd, J = 8.4, 4.4 Hz, 1H), 7.29–7.23 (m, 2H), 6.97 (d, J = 8.7 Hz, 2H), 4.30 (t, J = 6.2 Hz, 2H), 3.60 (t, J = 6.2 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.92, 159.75, 149.67, 141.20, 128.46, 123.71, 123.47, 119.40, 118.67, 113.99, 109.40, 66.87, 27.66, 21.11.

4.2.8. 2-(4-(3-Bromopropoxy)phenyl)benzo[*d*]oxazole (24b)

Compound **20** was treated with 1,3-dibrompropane according to general procedure to give the desired product **24b** (32% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (d, J = 8.7 Hz, 2H), 7.67 (dd, J = 7.8, 5.1 Hz, 1H), 7.51–7.44 (m, 1H), 7.26 (p, J = 7.0 Hz, 2H), 6.96 (d, J = 8.8 Hz, 2H), 4.21–4.06 (m, 2H), 3.56 (t, J = 6.4 Hz, 2H), 2.35–2.22 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.07, 160.39, 149.65, 141.22, 128.40, 123.62, 123.42, 118.90, 118.61, 113.83, 109.37, 64.47, 31.17, 28.73.

4.3. General procedures for the preparation of 26a–b, 27a–b, 28a–b, 29a–b

Compounds **21a**–**b, 22a**–**b, 23a**–**b, 24a**–**b** (2 mmol) was added to a magnetically stirred suspension of **25** (2 mmol), K_2CO_3 (6 mmol) in DMF(15 mL) under an argon atmosphere. The mixture was allowed to heat in reflux for 24 h, and monitored by TLC. When

the mixture was cooled to room temperature, filtered, and then evaporated under vacuum. The crude product was chromatographed on an Al_2O_3 column, eluted with $CHCl_3$/MeOH as eluent to afford the proposed compound.

4.3.1. 9-*O*-2-(4-(2-Ethoxy)phenyl)benzofuran-berberine bromide (26a)

Compound **21a** was treated with berberrubine **25** according to general procedure to give the desired product **26a** as a yellow solid (39% yield). Mp 216.3–218.3 °C; ^{1}H NMR (400 MHz, DMSO) δ 9.79 (s, 1H), 8.93 (s, 1H), 8.23 (d, J = 9.1 Hz, 1H), 8.03 (d, J = 9.1 Hz, 1H), 7.80 (d, J = 8.7 Hz, 2H), 7.76 (s, 1H), 7.61 (d, J = 7.1 Hz, 1H), 7.57 (d, J = 7.8 Hz, 1H), 7.31–7.22 (m, 3H), 7.02 (s, 1H), 6.98 (d, J = 8.7 Hz, 2H), 6.14 (s, 2H), 4.83 (s, 2H), 4.71 (s, 2H), 4.53 (s, 2H), 4.08 (s, 3H), 3.13 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ 154.17, 150.94, 148.91, 148.05, 144.33, 141.99, 139.38, 138.14, 135.22, 133.07, 131.27, 128.46, 127.02, 126.47, 125.59, 125.98, 123.02, 121.94, 120.36, 120.11, 119.23, 119.09, 118.20, 113.54, 110.71, 109.13, 106.47, 100.13, 96.41, 74.29, 63.12, 57.88, 52.07. HRMS m/z [M-Br]$^+$ Calcd for $C_{35}H_{27}NO_6$ 558.1917, found 558.1929.

4.3.2. 9-*O*-2-(4-(3-Propoxy)phenyl)benzofuran-berberine bromide (26b)

Compound **21b** was treated with berberrubine **25** according to general procedure to give the desired product **26b** as a yellow solid (42% yield). Mp 210.3–213.0 °C; ^{1}H NMR (400 MHz, DMSO) 9.82 (s, 1H), 8.57 (s, 1H), 8.30 (d, J = 9.1 Hz, 1H), 8.01 (d, J = 9.1 Hz, 1H), 7.80–7.67 (m, 3H), 7.50 (d, J = 7.9 Hz, 1H), 7.35 (d, J = 8.0 Hz, 1H), 7.17–7.01 (m, 4H), 6.90–6.81 (m, 1H), 6.75 (s, 1H), 6.16 (s, 2H), 4.91 (s, 2H), 4.49 (s, 2H), 4.37 (s, 2H), 4.07 (s, 3H), 3.22 (s, 2H), 2.41 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ 159.41, 153.66, 151.71, 149.37, 146.13, 144.50, 139.21, 138.86, 138.78, 134.10, 133.75, 126.79, 126.38, 125.21, 124.89, 124.10, 122.41, 121.57, 120.87, 120.01, 119.94, 118.32, 116.35, 110.59, 107.48, 105.29, 103.90, 96.09, 72.14, 65.37, 57.71, 56.91, 54.05, 28.62. HRMS m/z [M-Br]$^+$ Calcd for $C_{36}H_{29}NO_6$ 572.2073, found 572.2094.

4.3.3. 9-*O*-2-(4-(3-Ethoxy)phenyl)-1*H*-indole-berberine bromide (27a)

Compound **22a** was treated with berberrubine **25** according to general procedure to give the desired product **27a** as a yellow solid (30% yield). Mp 211.4–213.7 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.36 (s, 1H), 9.79 (s, 1H), 8.93 (s, 1H), 8.23 (d, J = 9.2 Hz, 1H), 8.03 (d, J = 9.1 Hz, 1H), 7.76 (d, J = 3.5 Hz, 2H), 7.74 (s, 1H), 7.48 (d, J = 7.7 Hz, 1H), 7.36 (d, J = 7.9 Hz, 1H), 7.10–7.02 (m, 2H), 6.97 (t, J = 8.0 Hz, 3H), 6.72 (d, J = 1.5 Hz, 1H), 6.07 (d, J = 59.8 Hz, 2H), 4.92–4.75 (m, 2H), 4.70 (dd, J = 5.3, 2.7 Hz, 2H), 4.60–4.44 (m, 2H), 4.19–3.96 (m, 3H), 3.17–3.06 (m, 2H); ^{13}C NMR (101 MHz, DMSO) δ 157.47, 150.29, 149.79, 147.65, 145.21, 142.48, 137.48, 137.44, 136.87, 132.97, 130.47, 128.72, 126.62, 126.29, 125.15, 124.31, 123.61, 121.76, 121.06, 120.31, 120.23, 119.61, 119.20, 114.83, 111.01, 108.32, 105.37, 102.03, 97.42, 72.21, 67.12, 57.02, 54.85. HRMS m/z [M-Br]$^+$ Calcd for $C_{35}H_{28}N_2O_5$ 557.2100, found 557.2076.

4.3.4. 9-*O*-2-(4-(3-Propoxy)phenyl)-1*H*-indole-berberine bromide (27b)

Compound **22b** was treated with berberrubine **25** according to general procedure to give the desired product **27b** as a yellow solid (38% yield). Mp 192.3–194.9 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.44 (s, 1H), 9.81 (s, 1H), 8.94 (s, 1H), 8.20 (d, J = 9.1 Hz, 1H), 8.00 (d, J = 9.1 Hz, 1H), 7.80 (d, J = 9.0 Hz, 3H), 7.49 (d, J = 7.7 Hz, 1H), 7.37 (d, J = 8.0 Hz, 1H), 7.04 (dd, J = 19.3, 14.0 Hz, 4H), 6.97 (t, J = 7.4 Hz, 1H), 6.75 (s, 1H), 6.16 (s, 2H), 4.90 (s, 2H), 4.51 (s, 2H), 4.33 (s, 2H), 4.03 (s, 3H), 3.19 (s, 2H), 2.37 (s, 2H); ^{13}C NMR (101 MHz, DMSO) δ 158.01, 150.28, 149.80, 147.65, 145.23, 142.69, 137.63, 137.47, 136.88, 133.00, 130.62, 128.75, 126.68, 126.34, 124.98, 123.40, 121.55, 121.00, 120.39, 120.21, 119.60, 119.18, 114.88, 111.04, 108.36, 105.39, 102.04, 97.30, 71.24, 64.44, 59.71, 57.01, 55.22, 29.43. HRMS m/z [M-Br]$^+$ Calcd for $C_{36}H_{30}N_2O_5$ 571.2233, found 571.2249.

4.3.5. 9-*O*-2-(4-(3-Ethoxy)phenyl)benzo[d]thiazole-berberine bromide (28a)

Compound **23a** was treated with berberrubine **25** according to general procedure to give the desired product **28a** as a yellow solid (35% yield). Mp 219.3–220.8 °C; ^{1}H NMR (400 MHz, DMSO) δ 9.79 (s, 1H), 8.92 (s, 1H), 8.23 (d, J = 9.2 Hz, 1H), 8.10 (d, J = 7.8 Hz, 1H), 8.04–7.94 (m, 4H), 7.74 (s, 1H), 7.52 (dd, J = 11.2, 4.1 Hz, 1H), 7.45–7.41 (m, 1H), 7.09–6.98 (m, 3H), 6.13 (s, 2H), 4.89–4.80 (m, 2H), 4.73 (s, 2H), 4.57 (s, 2H), 4.11 (d, J = 21.9 Hz, 3H), 3.16–3.10 (m, 2H); ^{13}C NMR (101 MHz, DMSO) δ 166.75, 160.52, 153.55, 150.21, 149.75, 147.60, 145.16, 142.40, 137.42, 134.17, 132.99, 130.44, 128.79, 126.62, 126.49, 125.65, 125.12, 123.62, 122.42, 122.12, 121.71, 120.27, 120.21, 115.23, 108.28, 105.32, 102.00, 72.08, 67.52, 57.02, 55.26, 30.63. HRMS m/z [M-Br]$^+$ Calcd for $C_{34}H_{26}N_2O_5S$ 575.1641, found 575.1663.

4.3.6. 9-*O*-2-(4-(3-Opropoxy)phenyl)benzo[*d*]thiazole-berberine bromide (28b)

Compound **23b** was treated with berberrubine **25** according to general procedure to give the desired product **28b** as a yellow solid (25% yield). Mp 204.3–206.6 °C; ^{1}H NMR (400 MHz, DMSO) δ 9.82 (s, 1H), 8.93 (s, 1H), 8.19 (d, J = 9.1 Hz, 1H), 8.13–8.06 (m, 1H), 8.01 (dd, J = 15.0, 8.9 Hz, 4H), 7.77 (s, 1H), 7.52 (t, J = 7.3 Hz, 1H), 7.43 (t, J = 7.2 Hz, 1H), 7.14 (d, J = 8.7 Hz, 2H), 7.06 (s, 1H), 6.15 (s, 2H), 4.92 (s, 2H), 4.52 (t, J = 6.0 Hz, 2H), 4.39 (t, J = 6.0 Hz, 2H), 4.02 (s, 3H), 3.20 (s, 2H), 2.42–2.36 (m, 2H); ^{13}C NMR (101 MHz, DMSO) δ 166.88, 161.04, 153.63, 150.23, 149.83, 147.67, 145.18, 142.73, 137.48, 134.19, 133.05, 130.54, 128.83, 126.71, 126.43, 125.59, 125.05, 123.38, 122.40, 122.08, 121.54, 120.35, 120.22, 115.21, 108.33, 105.38, 102.02, 71.14, 64.79, 57.02, 55.24, 29.36, 26.33. HRMS m/z [M-Br]$^+$ Calcd for $C_{35}H_{28}N_2O_5S$ 589.1797, found 589.1826.

4.3.7. 9-*O*-2-(4-(3-Ethoxy)phenyl)benzo[*d*]oxazole-berberine bromide (29a)

Compound **24a** was treated with berberrubine **25** according to general procedure to give the desired product **29a** as a yellow solid (33% yield). Mp 221.4–224.1 °C; ^{1}H NMR (400 MHz, DMSO) δ 9.80 (s, 1H), 8.92 (s, 1H), 8.23 (d, J = 9.2 Hz, 1H), 8.09 (d, J = 8.8 Hz, 2H), 8.03 (d, J = 9.1 Hz, 1H), 7.74 (td, J = 5.2, 1.7 Hz, 3H), 7.42–7.38 (m, 2H), 7.08 (d, J = 8.8 Hz, 2H), 7.02 (s, 1H), 6.13 (s, 2H), 4.88–4.79 (m, 2H), 4.73 (s, 2H), 4.58 (s, 2H), 4.07 (s, 3H), 3.17–3.19 (m, 2H); ^{13}C NMR (101 MHz, DMSO) δ 163.45, 163.01, 151.43, 151.09, 150.37, 146.21, 144.79, 141.97, 141.29, 138.62, 135.34, 132.77, 130.25, 129.07, 127.04, 125.33, 124.86, 122.42, 121.55, 121.10, 120.17, 117.46, 116.36, 111.11, 106.24, 104.98, 100.86, 78.88, 70.57, 67.75, 55.67, 54.12. HRMS m/z [M-Br]$^+$ Calcd for $C_{34}H_{26}N_2O_6$ 559.1869, found 559.1883.

4.3.8. 9-*O*-2-(4-(3-Propoxy)phenyl)benzo[*d*]oxazole-berberine bromide (29b)

Compound **24b** was treated with berberrubine **25** according to general procedure to give the desired product **29b** as a yellow solid (42% yield). Mp 210.5–212.4 °C; ^{1}H NMR (400 MHz, DMSO) δ 9.81 (s, 1H), 8.92 (s, 1H), 8.19 (d, J = 9.2 Hz, 1H), 8.15 (d, J = 8.9 Hz, 2H), 8.00 (d, J = 9.1 Hz, 1H), 7.78–7.73 (m, 3H), 7.42–7.38 (m, 2H), 7.19 (d, J = 8.9 Hz, 2H), 7.07 (s, 1H), 6.16 (s, 2H), 4.94–4.88 (m, 2H), 4.51 (t, J = 6.2 Hz, 2H), 4.40 (t, J = 6.1 Hz, 2H), 4.02 (s, 3H), 3.22–3.17 (m, 2H), 2.42–2.36 (m, 2H); ^{13}C NMR (101 MHz, DMSO) δ 162.28, 161.44, 150.23, 150.06, 149.78, 147.64, 145.20, 142.67, 141.60,

137.45, 132.99, 130.60, 129.13, 126.66, 125.00, 124.71, 123.41, 121.52, 120.36, 120.19, 119.38, 118.76, 115.25, 110.66, 108.36, 105.35, 102.03, 79.12, 71.11, 64.80, 56.99, 55.21, 30.64, 28.27. HRMS *m*/*z* [M-Br]$^+$ Calcd for $C_{35}H_{28}N_2O_6$ 573.2026.1869, found 573.2048.

4.4. General procedures for the preparation of 32, 33

To **30, 31** (10 mmol) solution in THF/DMF (50 ml), 3-bromopropan-1-amine (11 mmol) was slowly added at 0 °C and then EDCI (11 mmol). After stirred for 1 h at room temperature, the reaction mixture was washed with $NaHCO_3$ solution (2×50 ml), water (2×50 ml), brine (1×50 ml), and then dried with Na_2SO_4, concentrated in vacuo to afford the resulting compound (**32, 33**) which was used for the next step without purification.

4.5. General procedures for the preparation of 34, 35

Compounds **32, 33** (2 mmol) was added to a magnetically stirred suspension of berberrubine **25** (2 mmol), K_2CO_3 (6 mmol) in DMF (15 mL) under an argon atmosphere. The mixture was allowed to heated in reflux for 24 h, and monitored by TLC. When the mixture was cooled to room temperature,filtered, and then evaporated under vacuum. The crude product was chromatographed on an Al_2O_3 column, eluted with $CHCl_3$/MeOH as eluent to afford the proposed compound.

4.5.1. 9-*O*-*N*-(3-Bromopropyl)-1*H*-indole-2-carboxamide-berberine bromide (34)

Compound **32** was treated with berberrubine **25** according to general procedure to give the desired product **34** as a yellow solid (34% yield). Mp 173.3–176.1 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.59 (s, 1H), 9.99 (s, 1H), 8.92 (s, 1H), 8.78 (t, *J* = 5.6 Hz, 1H), 8.19 (d, *J* = 9.2 Hz, 1H), 7.98 (d, *J* = 9.1 Hz, 1H), 7.78 (s, 1H), 7.59 (d, *J* = 8.0 Hz, 1H), 7.42 (d, *J* = 8.2 Hz, 1H), 7.22–7.14 (m, 2H), 7.11 (s, 1H), 7.02 (t, *J* = 7.4 Hz, 1H), 6.18 (s, 2H), 5.10–4.96 (m, 2H), 4.39 (t, *J* = 6.0 Hz, 2H), 3.61 (dd, *J* = 12.3, 6.3 Hz, 2H), 3.28–3.17 (m, 2H), 2.21–2.09 (m, 2H); ^{13}C NMR (101 MHz, DMSO) δ 161.32, 150.26, 145.36, 137.42, 136.31, 132.95, 131.61, 130.67, 126.98, 123.35, 121.39, 119.72, 112.22, 108.38, 102.55, 102.02, 71.80, 56.99, 56.00, 29.93, 26.33, 18.44. HRMS *m*/*z* [M-Br]$^+$ Calcd for $C_{31}H_{27}N_3O_5$ 522.2029, found 522.2031.

4.5.2. 9-*O*-*N*-(3-Propyl)benzofuran-2-carboxamide-berberine bromide (35)

Compound **33** was treated with berberrubine **25** according to general procedure to give the desired product **36** as a yellow solid (29% yield). Mp 168.4–169.9 °C; ^{1}H NMR (400 MHz, DMSO) δ 9.77 (s, 1H), 8.89 (s, 1H), 8.69 (t, *J* = 5.7 Hz, 1H), 8.22 (d, *J* = 9.2 Hz, 1H), 8.00 (d, *J* = 9.1 Hz, 1H), 7.74 (s, 1H), 7.60 (d, *J* = 8.1 Hz, 1H), 7.40 (d, *J* = 8.0 Hz, 1H), 7.29–7.20 (m, 2H), 7.06 (s, 1H), 6.94 (t, *J* = 7.3 Hz, 1H), 6.20 (s, 2H), 5.17–5.99 (m, 2H), 4.47 (t, *J* = 6.1 Hz, 2H), 3.67–3.74 (m, 2H), 3.22–3.15 (m, 2H), 2.17–2.05 (m, 2H); ^{13}C NMR (101 MHz, DMSO) δ 163.01, 154.44, 147.91, 138.55, 135.62, 133.85, 131.92, 130.07, 127.68, 125.47, 124.74, 118.45, 110.57, 107.68, 103.27, 103.00, 72.7, 57.18, 56.37, 28.83, 25.47, 20.07. HRMS *m*/*z* [M-Br]$^+$ Calcd for $C_{31}H_{26}N_2O_6$ 523.1869, found 523.2048.

4.6. General procedures for the preparation of 37a–d

Phthalimide (**36**) (40 mmol), K_2CO_3 (120 mmol) and benzyltriethylammonium chloride(4.4 mmol) were suspended in acetone (100 mL). Dibromoalkanes (100 mmol) was added to the mixture and then the reaction mixture was stirred at room temperature for 24 h. The mixture was filtered and then evaporated under vacuum to afford the crude product, which was purified by chromatography on a silica gel column with EtOAc/petroleum ether as eluent to give the products.

4.7. General procedures for the preparation of 38a–d, 39a–b, 40a–b

To a stirred mixture of **11, 17, 20**(12 mmol) and K_2CO_3(20 mmol) in DMF (20 mL), **37a–d** (10 mmol) were added. After stirred at room temperature for 10–12 h, the mixture was quenched with water and extracted with EtOAc for three times.The combined organic phase was dried over Na_2SO_4, filtered and concentrated to give the crude product, which was purified by chromatography on an silica gel column with EtOAc/petroleum ether aseluent to give the products.

4.8. General procedures for the preparation of 41a–d, 42a–b, 43a–b

An solution of **38a–d, 39a–b, 40a–b** (11 mmol) and hydrazine hydrate (80%) (9 mL, 142 mmol) in ethanol (60 mL) was refluxed for 4 h, and then cooled to room temperature. The mixture was filtered and the solid was washed with 95% EtOH. The whole filtrate was concentrated and the solid was dissolved in CH_2Cl_2. The mixture was dried over Na_2SO_4, filtered and concentrated to give the crude product, which was used in the next step without further purification.

4.9. General procedures for the preparation of 44a-d,45a-b,46a-b

To a solution of 9-chloro-1,2,3,4-tetrahydroacridine (0.217 g, 1 mmol) in pentanol, **41a–d, 42a–b, 43a–b** (4–10 mmol) were added. The mixture was stirred in reflux for 10–24 h. After the reaction completed (monitored by TLC), the mixture was concentrated in vacuo and the residual was purified purified by chromatography on an silica gel column with EtOAc/petroleum ether aseluent to give the products.

4.9.1. *N*-(3-(4-(benzo[*d*]thiazol-2-yl)phenoxy)propyl)-1,2,3,4-tetrahydroacridin-9-amine (44a)

Compound **41a** was treated with 9-chloro-1,2,3,4-tetrahydroacridine according to general procedure to give the desired product **44a** as a white solids (39% yield). Mp 152.7–154.0 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09–8.00 (m, 3H), 7.97 (d, *J* = 7.8 Hz, 1H), 7.94–7.85 (m, 2H), 7.56 (ddd, *J* = 8.3, 6.8, 1.3 Hz, 1H), 7.51–7.44 (m, 1H), 7.40–7.30 (m, 2H), 7.05–6.96 (m, 2H), 4.22 (t, *J* = 5.7 Hz, 2H), 3.74 (t, *J* = 6.2 Hz, 2H), 2.76 (t, *J* = 5.5 Hz, 2H), 1.92–1.75 (m, 8H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.68, 160.91, 158.64, 154.21, 150.46, 147.48, 134.99, 129.21, 128.86, 128.37, 126.83, 126.27, 124.90, 123.88, 122.88, 122.60, 121.54, 120.43, 114.82, 66.42, 46.84, 34.05, 30.74, 25.01, 23.06, 22.80. HRMS *m*/*z* [M-Br]$^+$ Calcd for $C_{29}H_{27}N_3OS$ 466.1953, found 466.1959.

4.9.2. *N*-(4-(4-(Benzo[*d*]thiazol-2-yl)phenoxy)butyl)-1,2,3,4-tetrahydroacridin-9-amine (44b)

Compound **41b** was treated with 9-chloro-1,2,3,4-tetrahydroacridine according to general procedure to give the desired product **44b** as a white solids (21% yield). Mp 151.5–152.3 °C;1H NMR (400 MHz, $CDCl_3$) δ 8.06–7.98 (m, 3H), 7.96 (d, *J* = 8.5 Hz, 1H), 7.92 (d, *J* = 7.9 Hz, 1H), 7.87 (d, *J* = 7.5 Hz, 1H), 7.55 (ddd, *J* = 8.3, 6.8, 1.2 Hz, 1H), 7.49–7.42 (m, 1H), 7.38–7.30 (m, 2H), 6.99–6.92 (m, 2H), 4.05 (t, *J* = 5.7 Hz, 2H), 3.57 (t, *J* = 6.6 Hz, 2H), 3.06 (d, *J* = 6.1 Hz, 2H), 2.73 (d, *J* = 5.7 Hz, 2H), 1.96–1.82 (m, 8H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.77, 161.16, 158.57, 154.22, 150.51, 147.50, 134.85, 129.13, 128.83, 128.31, 126.53, 126.22, 124.82,

123.74, 122.74, 121.50, 120.33, 116.23, 114.84, 67.66, 49.07, 34.04, 28.51, 26.65, 24.88, 23.06, 22.78. HRMS *m*/*z* [M-Br]$^+$ Calcd for $C_{30}H_{29}N_3OS$ 480.0210, found 480.2133.

4.9.3. *N*-(5-(4-(Benzo[*d*]thiazol-2-yl)phenoxy)pentyl)-1,2,3,4-tetrahydroacridin-9-amine (44c)

Compound **41c** was treated with 9-chloro-1,2,3,4-tetrahydroacridine according to general procedure to give the desired product **44c** as a glassy solids (27% yield). Mp 145.8–147.8 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07–8.00 (m, 3H), 7.98 (d, *J* = 8.5 Hz, 1H), 7.94 (d, *J* = 8.4 Hz, 1H), 7.91–7.86 (m, 1H), 7.57 (ddd, *J* = 8.3, 6.9, 1.2 Hz, 1H), 7.51–7.45 (m, 1H), 7.40–7.33 (m, 2H), 7.01- 6.94 (m, 2H), 4.04 (t, *J* = 6.2 Hz, 2H), 3.55 (t, *J* = 7.1 Hz, 2H), 3.08 (s, 2H), 2.73 (s, 2H), 1.93 (t, *J* = 3.2 Hz, 4H), 1.89–1.83 (m, 2H), 1.80–1.74 (m, 2H), 1.65–1.60 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.85, 161.31, 158.38, 154.21, 150.74, 147.30, 134.83, 129.31, 129.11, 128.53, 126.30, 124.81, 123.74, 122.80, 120.86, 116.02, 114.81, 67.76, 49.33, 33.93, 31.47, 28.91, 24.81, 23.54, 23.04, 22.75. HRMS *m*/*z* [M-Br]$^+$ Calcd for $C_{31}H_{31}N_3OS$ 494.2266, found 494.2257.

4.9.4. *N*-(6-(4-(Benzo[*d*]thiazol-2-yl)phenoxy)hexyl)-1,2,3,4-tetrahydroacridin-9-amine (44d)

Compound **41d** was treated with 9-chloro-1,2,3,4-tetrahydroacridine according to general procedure to give the desired product **44d** as a glassy solids (24% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04–8.00 (m, 3H), 7.96 (d, *J* = 7.8 Hz, 1H), 7.92 (d, *J* = 8.2 Hz, 1H), 7.87 (dd, *J* = 7.9, 0.5 Hz, 1H), 7.59–7.51 (m, 1H), 7.49–7.44 (m, 1H), 7.39–7.30 (m, 2H), 7.02–6.93 (m, 2H), 4.02 (t, *J* = 6.4 Hz, 2H), 3.51 (t, *J* = 7.2 Hz, 2H), 3.07 (s, 2H), 2.71 (s, 2H), 1.92 (dd, *J* = 6.5, 3.3 Hz, 4H), 1.81 (dd, *J* = 14.0, 6.5 Hz, 2H), 1.70 (dd, *J* = 14.2, 7.0 Hz, 2H), 1.50 (dt, *J* = 11.9, 6.3 Hz, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.88, 161.40, 158.37, 154.21, 150.84, 134.84, 129.10, 128.65, 128.38, 126.26-, 124.79, 123.68, 122.80, 121.51, 120.20, 117.66, 115.92, 114.83, 67.92, 49.41, 33.95, 31.72, 29.06, 26.72, 25.87, 24.80, 23.04, 22.76. HRMS *m*/*z* [M-Br]$^+$ Calcd for $C_{32}H_{33}N_3OS$ 508.2423, found 508.2427.

4.9.5. *N*-(3-(4-(Benzo[*d*]oxazol-2-yl)phenoxy)propyl)-1,2,3,4-tetrahydroacridin-9-amine (45a)

Compound **42a** was treated with 9-chloro-1,2,3,4-tetrahydroacridine according to general procedure to give the desired product **45a** as a white solids (26% yield). Mp 148.9–150.1 °C; ^{1}H NMR (400 MHz, DMSO) δ 8.13 (t, *J* = 9.7 Hz, 3H), 7.84–7.67 (m, 3H), 7.52 (t, *J* = 7.5 Hz, 1H), 7.43–7.36 (m, 2H), 7.33 (t, *J* = 7.6 Hz, 1H), 7.16–7.05 (m, 2H), 4.17 (d, *J* = 5.9 Hz, 2H), 3.60 (d, *J* = 6.3 Hz, 2H), 2.89 (d, *J* = 5.9 Hz, 2H), 2.74 (s, 2H), 2.12–2.03 (m, 2H), 1.79 (m, 4H). ^{13}C NMR (101 MHz, DMSO) δ 159.00, 155.37, 153.98, 129.07, 128.19, 127.89, 126.21, 123.96, 123.30, 123.02 (d, *J* = 9.7 Hz), 122.38, 120.72, 120.31, 114.94, 110.86, 65.46, 52.02, 33.40, 30.03, 25.06, 22.67. HRMS *m*/*z* [M-Br]$^+$ Calcd for $C_{29}H_{27}N_3O_2$ 450.2281, found 450.2293.

4.9.6. *N*-(4-(4-(Benzo[*d*]oxazol-2-yl)phenoxy)butyl)-1,2,3,4-tetrahydroacridin-9-amine (45b)

Compound **42b** was treated with 9-chloro-1,2,3,4-tetrahydroacridine according to general procedure to give the desired product **45b** as a white solids (30% yield). Mp 145.8–147.8 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (d, *J* = 8.9 Hz, 2H), 7.97 (d, *J* = 8.5 Hz, 1H), 7.92 (d, *J* = 8.5 Hz, 1H), 7.76–7.70 (m, 1H), 7.59–7.52 (m, 2H), 7.37–7.30 (m, 3H), 7.00 (d, *J* = 8.9 Hz, 2H), 4.08 (t, *J* = 5.7 Hz, 2H), 3.59 (t, *J* = 6.5 Hz, 2H), 3.07 (s, 2H), 2.74 (s, 2H), 1.96–1.87 (m, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.56, 158.59, 150.51, 142.25, 129.41, 128.86, 128.33, 124.55 (d, *J* = 19.5 Hz), 123.75, 122.68, 120.32, 119.63, 116.25, 114.80, 110.39, 67.65, 49.10, 34.07, 28.53, 26.64, 24.89, 23.06, 22.79. HRMS *m*/*z* [M-Br]$^+$ Calcd for $C_{30}H_{29}N_3O_2$ 464.2338, found 464.2349.

4.9.7. *N*-(3-(4-(benzofuran-2-yl)phenoxy)propyl)-1,2,3,4-tetrahydroacridin-9-amine (46a)

Compound **43a** was treated with 9-chloro-1,2,3,4-tetrahydroacridine according to general procedure to give the desired product **46a** as a white solids (38% yield). Mp 147.9–149.3 °C; ^{1}H NMR (400 MHz, DMSO) δ 8.15 (d, *J* = 8.5 Hz, 1H), 7.83 (d, *J* = 8.0 Hz, 2H), 7.71 (d, *J* = 8.4 Hz, 1H), 7.65–7.55 (m, 2H), 7.53 (t, *J* = 7.6 Hz, 1H), 7.32 (dd, *J* = 14.4, 6.6 Hz, 1H), 7.26 (m, 3H), 7.01 (d, *J* = 8.1 Hz, 2H), 4.13 (t, *J* = 5.7 Hz, 2H), 3.66–3.53 (m, 2H), 2.89 (d, *J* = 6.0 Hz, 2H), 2.75 (d, *J* = 5.4 Hz, 2H), 2.13–2.00 (m, 2H), 1.80 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.20, 158.51, 155.97, 154.70, 150.59, 147.43, 129.46, 128.77, 128.35, 126.44, 123.76, 123.45, 122.79, 120.59, 120.27, 116.14, 114.77, 110.98, 99.74, 67.53, 49.11, 34.02, 28.55, 26.70, 24.87, 23.05, 22.77. HRMS *m*/*z* [M-Br]$^+$ Calcd for $C_{30}H_{28}N_2O_2$ 449.3129, found 449.3140.

4.9.8. *N*-(4-(4-(Benzofuran-2-yl)phenoxy)butyl)-1,2,3,4-tetrahydroacridin-9-amine (46b)

Compound **43b** was treated with 9-chloro-1,2,3,4-tetrahydroacridine according to general procedure to give the desired product **46b** as a glassy solids (41% yield). Mp 146.6–148.4 °C; ^{1}H NMR (400 MHz, DMSO) δ 8.15 (d, *J* = 8.5 Hz, 1H), 7.83 (d, *J* = 8.0 Hz, 2H), 7.71 (d, *J* = 8.4 Hz, 1H), 7.65–7.55 (m, 2H), 7.53 (t, *J* = 7.6 Hz, 1H), 7.32 (dd, *J* = 14.4, 6.6 Hz, 1H), 7.26 (m, 3H), 7.01 (d, *J* = 8.1 Hz, 2H), 4.13 (t, *J* = 5.7 Hz, 2H), 3.66–3.53 (m, 2H), 2.89 (d, *J* = 6.0 Hz, 2H), 2.75 (d, *J* = 5.4 Hz, 2H), 2.13–2.00 (m, 2H), 1.80 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.20, 158.51, 155.97, 154.70, 150.59, 147.43, 129.46, 128.77, 128.35, 126.44, 123.76, 123.45, 122.79, 120.59, 120.27, 116.14, 114.77, 110.98, 99.74, 67.53, 49.11, 34.02, 28.55, 26.70, 24.87, 23.05, 22.77. HRMS *m*/*z* [M−Br]$^+$ Calcd for $C_{31}H_{30}N_2O_2$ 463.2386, found 463.2392.

4.10. Biological activity

4.10.1. In vitro inhibition studies on AChE and BuChE

Acetylcholinesterase (AChE, E.C. 3.1.1.7, from *electric eel*), butylcholinesterase (BuChE, E.C. 3.1.1.8, from *equine serum*), 5,5′-dithiobis-(2-nitrobenzoic acid) (Ellman's reagent, DTNB), acetylthiocholine chloride (ATC), butylthiocholine chloride (BTC), and tarcine hydrochloride were purchased from Sigma–Aldrich. Berberine derivatives were dissolved in DMSO and diluted in 0.1 M KH_2PO_4/K_2HPO_4 buffer (pH 8.0) to provide a final concentration range. DMSO was diluted to a concentration in excess of 1 in 10000, and no inhibitory action on either AChE or BuChE was detected in separate prior experiments.

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

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

4.10.2. Kinetic characterization of AChE inhibition

Kinetic characterization of AChE was performed using a reported method.[16] Test compound was added into the assay solution and pre-incubated with the enzyme at 37 °C for 15 min, followed by the addition of substrate. Kinetic characterization of the hydrolysis of ATC catalyzed by AChE was done spectrometrically at 412 nm. A

parallel control with no inhibitor in the mixture, allowed adjusting activities to be measured at various times. The plots were assessed by a weighted least squareanalysis that assumed the variance of V to be a constant percentage of V for the entire data set. Slopes of these reciprocal plots were then plotted against the concentration of the inhibitors in a weighted analysis and K_i was determined as the ratio of the replot intercept to the replot slope.

4.10.3. Inhibition of $A\beta_{1-42}$ peptide aggregation[17]

HFIP pretreated $A\beta_{1-42}$ samples (AnaSpec) were resolubilized with a 50 mM phosphate buffer (pH = 7.4) in order to have a stable stock solution ([Aβ] = 200 μM). The peptide was incubated in 50 mM phosphate buffer (pH = 7.4) at 37 °C for 48 h (final Aβ concentration 50 μM) with and without the tested compound at at different concentrations. After incubation, the samples were diluted to a final volume of 200 μL with 50 mM glycine–NaOH buffer (pH 8.0) containing thioflavin T. Then, a 300-s-time scan of fluorescence intensity was performed (λ_{exc} = 450 nm; λ_{em} = 485 nm), and values at plateau were averaged after subtracting the background fluorescence of thioflavin T solution.

4.11. Molecular modeling

The simulation system was built based on the X-ray crystal structure of the bistacrine–AChE complex which was obtained from the Protein Data Bank (PDB entry 2CMF). The original ligand was removed while water molecules present in the PDB file were maintained in their position. 3D structures of the 9-substituted berberine derivatives were generated and optimized by Discovery studio 2.1 package (Accelrys Inc., San Diego, CA). The CDOCKER pro- gramin Discovery studio 2.1 software was used to perform-docking simulations, which allows full flexibility of the ligand.

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