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O-Hydroxyl- or *o*-amino benzylamine-tacrine hybrids: Multifunctional biometals chelators, antioxidants, and inhibitors of cholinesterase activity and amyloid-β aggregation

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ABSTRACT

In an effort to identify novel multifunctional drug candidates for the treatment of Alzheimer's disease (AD), a series of hybrid molecules were synthesised by reacting N-(aminoalkyl)tacrine with salicylic aldehyde or derivatives of 2-aminobenzaldehyde. These compounds were then evaluated as multifunctional anti-Alzheimer's disease agents. All of the hybrids are potential biometal chelators, and in addition, most of them were better antioxidants and inhibitors of cholinesterases and amyloid-β (Aβ) aggregation than the lead compound tacrine. Compound **7c** has the potential to be a candidate for AD therapy: it is a much better inhibitor of acetylcholinesterase (AChE) than tacrine (IC_{50}: 0.55 nM vs 109 nM), has good biometal chelation ability, is able to inhibit Aβ aggregation and has moderate antioxidant activity (1.22 Trolox equivalents).

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1. Introduction

Acetylcholinesterase inhibitors (AChEIs), such as tacrine, donepezil, rivastigmine and galantamine, which increase neurotransmission at cholinergic synapses in the brain and temporarily reduce cognitive deficits, are the current therapeutic options for the treatment of Alzheimer's disease (AD).[1,2] However, many facts strongly suggest that AD is a multifaceted illness. In addition to the hallmark low levels of acetylcholine, the following are important in the aetiology of AD: β-amyloid (Aβ) deposits, τ-protein aggregation, oxidative stress and the dyshomeostasis of biometals. Recent studies have indicated that biometals, such as Cu(II), Zn(II), and Fe(II, III), play a very important role in many critical aspects of AD. Raman spectra demonstrated that Zn(II) and Cu(II) are coordinated to histidine residues in senile plaque (SP) cores. Treatment of SPs with the chelator EDTA reverses Cu(II) binding to SP histidines and leads to a broadening of the amide features, indicating a 'loosening' of the β-structure.[3] Furthermore, Cu(II) and Fe(II, III) have been linked to the production of reactive oxygen species (ROS) and oxidative stress (OS).[4,5] Thus, the modulation of these biometals in the brain has been proposed as a potential therapeutic strategy for the treatment of AD.[6] In view of this proposal, the biometal chelators desferrioxamine (DFO, Fig. 1), EDTA, clioquinol (Fig. 1), and 5-[*N*-methyl-*N*-propargylaminomethyl]-8-hydroxyquinoline (M30, Fig. 1) have been used for the treatment of AD.[7] Recently, the small molecules *N*-(pyridin-2-ylmethyl) aniline (**1**, Fig. 1) and N^1,N^1-dimethyl-N^4-(pyridin-2-ylmethyl)benzene-1,4-diamine (**2**, Fig. 1) were designed to regulate metal-induced Aβ aggregation and neurotoxicity.[8] Many studies have been devoted to the search for multifunctional agents that simultaneously inhibit cholinesterase, decrease Aβ levels and chelate redox-active metals because of the potential uses of these agents for the treatment of AD.[9]

Tacrine, the first ChE inhibitor approved by the FDA for the treatment of AD, suffers from therapy-limiting liver toxicity. Despite this limitation, tacrine has been widely used as scaffold for the development of new multifunctional agents with additional biological properties other than AChE inhibition.[10–13] For example, Pang et al. developed bis(7)tacrine, which is composed of two tacrine units linked by a seven-carbon linker. This compound exhibited a 1000-fold greater AChE inhibition potency than tacrine.[14,15] Rodríguez-Franco reported that tacrine-8-hydroxyquinoline hybrids are potential multifunctional drugs for the treatment of AD because these compounds exhibited more potent AChE inhibitory activities than tacrine and had good copper-complexing properties.[16] Other compounds, such as compounds containing both mercapto groups and tacrine,[17] hybrids of tacrine and 4-fluorobenzoic acid,[18] heterobivalent tacrine derivatives and tacrine-multialkoxybenzene hybrids,[19,20] were also developed recently.

In our previous work, we designed and synthesised a series of berberine derivatives as multifunctional anti-AD agents.[21] Herein, we describe the design, synthesis, and evaluation a series of *o*-hydroxyl and *o*-amino benzylamine-tacrine hybrids **6a**–**6g**, **7a**–**7e** and

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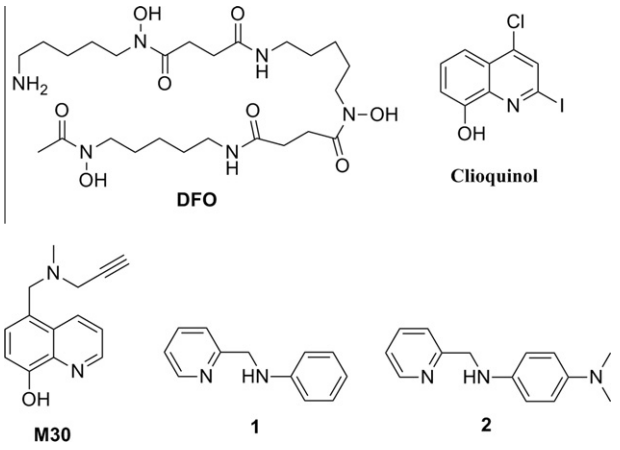


Figure 1. Chemical structure of metal-chelating agents.

o-hydroxyl benzylamine-(7-chloro tacrine) hybrids **6h**-**6n** (their leading compound exhibited better inhibitive activity than tacrine[22]) as multifunctional anti-AD agents with metal-chelating ability, antioxidative activity, and cholinesterase and Aβ aggregation inhibition activities.

2. Results and discussion

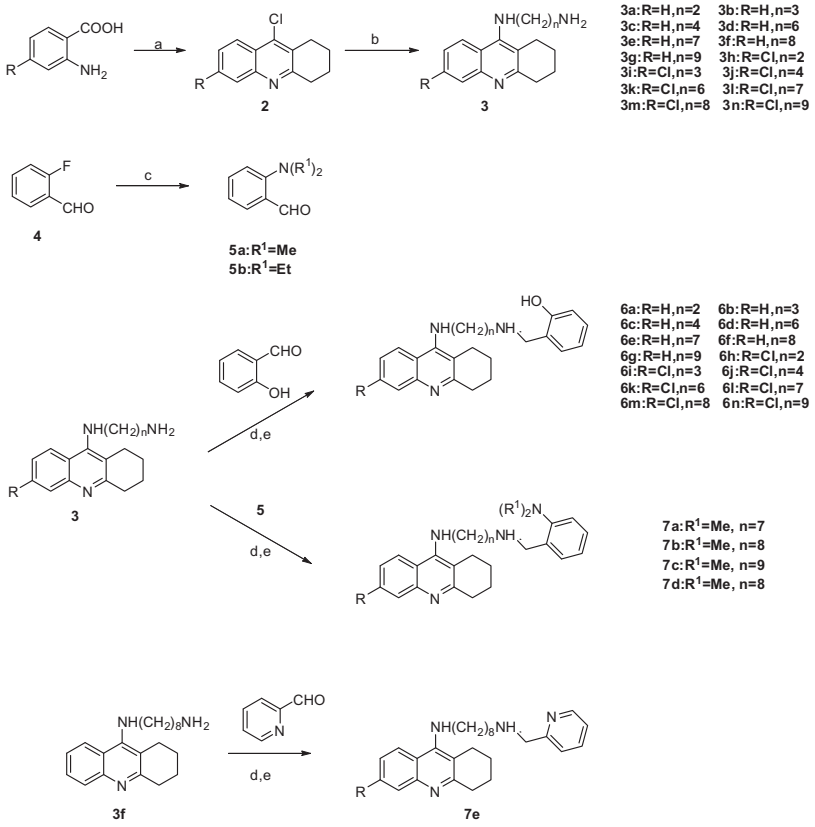
2.1. Chemistry

The synthetic pathway of the *o*-hydroxyl and *o*-amino benzylamine-tacrine hybrids is shown in Scheme 1. First, the reaction of 2-amino benzoic acid or 2-amino-4-chlorobenzoic acid with cyclohexanone and phosphorus oxychloride at a temperature of 0 °C to reflux provided the intermediate **2**.[22] Compound **2** was then reacted with diamine in the presence of a catalytic amount of potassium iodide to give **3**. *o*-Fluorobenzaldehyde was reacted with dimethylamine or diethylamine in the presence of K_2CO_3 to provide the substituted *o*-amino benzaldehyde **5**. Finally, compound **3** was reacted with salicylaldehyde or **5** to give the corresponding Schiff base, which was then reduced by sodium borohydride to afford the target product **6** or **7** in good chemical yields. Compound **7e**, the hybrid consisting of tacrine and pyridine-2-yl methanamine, was obtained by reacting compound **3** with pyridine-2-carboxaldehyde under the same reaction conditions.

2.2. Biological activities

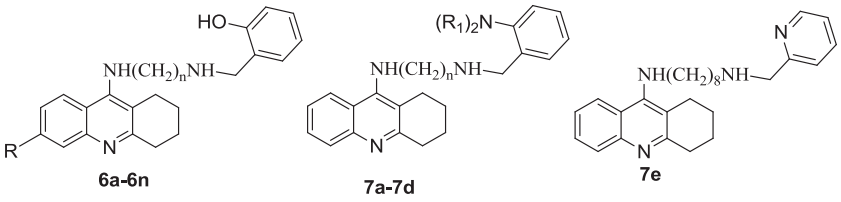
2.2.1. In vitro inhibition studies of AChE and BuChE

The AChE and BuChE inhibitory activities of all the *o*-hydroxyl and *o*-amino benzylamine-tacrine hybrids were examined by the method of Ellman et al.[23] using AChE from the electric eel and BuChE from equine serum. Tacrine was used as the reference standard. The IC_{50} values are summarised in Table 1. Relative to tacrine, which had an IC_{50} value of 109 nM, fifteen of the nineteen hybrids were more potent AChEIs, with IC_{50} values ranging from 0.55 to 43.3 nM. A structure-activity relationship analysis showed that the AChE inhibitory potency was closely related to the length of the alkylene chain. Hybrids **6a**, **6b**, and **6c**, with two, three and four carbon spacers between tacrine and an *o*-hydroxyl unit had weak inhibitory activities than tacrine (**6a**, two methylene groups, with an IC_{50} value of 146.19 nM; **6b**, three methylene groups, 131.13 nM; **6c**, four methylene groups, 252.5 nM). Generally, the



Scheme 1. Synthesis of *o*-hydroxyl- or *o*-amino benzylamine tacrine. Reagents and conditions: (a) cyclohexanone, $POCl_3$, 0 °C→reflux; (b) diamine, KI, 1-pentanol, 160 °C; (c) $NHMe_2$ or $NHEt_2$, K_2CO_3, DMF, 70 °C; (d) ethanol, rt; (e) $NaBH_4$, ethanol, rt.

Table 1
In vitro inhibition and selectivity of AChE and BuChE and Oxygen Radical Absorbance Capacity (ORAC, Trolox Equivalents) by tacrine and tacrine derivatives **6a-6n** and **7a-7e**



Compound	R	R_1	*n*	IC_{50}[a](nM)		Selectivity for AChE[d]	Trolox equiv[e]
				AChE[b]	BuChE[c]		
6a	H	—	2	146.19 ± 4.53	10.00 ± 0.76	0.07	11.86
6b	H	—	3	131.13 ± 28.6	46.13 ± 1.11	0.35	13.81
6c	H	—	4	252.5 ± 3.54	20.25 ± 1.64	0.08	11.76
6d	H	—	6	25.15 ± 3.49	9.65 ± 0.46	0.38	11.14
6e	H	—	7	9.55 ± 1.15	5.84 ± 0.16	0.61	7.91
6f	H	—	8	6.17 ± 1.19	6.25 ± 0.29	1.01	5.45
6g	H	—	9	7.52 ± 0.41	8.20 ± 0.45	1.09	4.41
6h	Cl	—	2	22.49 ± 2.64	9.97 ± 0.6	0.44	8.76
6i	Cl	—	3	43.32 ± 3.05	76.51 ± 8.11	1.77	11.32
6j	Cl	—	4	125.0 ± 2.83	28.09 ± 0.34	0.22	8.04
6k	Cl	—	6	43 ± 2.55	11.1 ± 0.35	0.26	7.87
6l	Cl	—	7	10.48 ± 0.23	17.79 ± 1.72	1.7	5.83
6m	Cl	—	8	6.77 ± 0.66	18.48 ± 0.68	2.73	4.79
6n	Cl	—	9	7.78 ± 0.14	22.98 ± 2.2	2.95	4.54
7a	—	Me	7	3.50 ± 0.19	2.92 ± 0.36	0.83	2.16
7b	—	Me	8	1.55 ± 0.067	2.11 ± 0.11	1.36	1.97
7c	—	Me	9	0.55 ± 0.034	2.65 ± 0.17	4.78	1.22
7d	—	Et	8	1.36 ± 0.046	2.82 ± 0.30	2.07	0.88
7e	—	—	—	2.95 ± 0.02	2.99 ± 0.1	1.01	n.t.[f]
Tacrine	—	—	—	109 ± 10.3	15.8 ± 1.32	0.14	n.t. [f]

[a] Mean ± SD of at least three independent measurements.
[b] AChE from the electric eel.
[c] BuChE from equine serum.
[d] Selectivity for AChE = IC_{50} (BuChE)/ IC_{50} (AChE).
[e] Data are expressed as (μmol trolox)/(μmol tested compound).
[f] n.t. = not tested.

AChE inhibitory activity intensifies as the number of methylene groups increases. Compound **6f**, which has an eight-carbon linker, had the best IC_{50} value (6.17 nM). The same trend was also observed for the hybrids derived from salicylaldehyde and 7-chloro tacrine, among which compound **6m**, possessing an eight-carbon linker, also gave the best result in this series. Substituted amino groups seem to have a favourable effect on the AChE inhibitory activity. For example, hybrids **7a-d**, which were derived from tacrine and substituted *o*-amino benzaldehyde, gave better results than the corresponding hybrids of salicylaldehyde and tacrine. Among **7a-d**, compound **7c**, in which tacrine was linked with *o*-(*N*,*N*-dimethylamino) benzylamine by a 9-carbon spacer, exhibited the greatest inhibitory potency towards AChE (IC_{50} value: 0.55 nM).

A selection of the compounds (**7a-7c**) was evaluated as inhibitors of human AChE and the results were listed in table 2. All tested derivatives gave IC_{50} values in the nano- and subnanomolar range (5.18, 4.04, 3.52 nM), which showed the same tendency as that of electric eel (Table 1, 3.50, 1.55, 0.55 nM). The different activities to the enzymes should be attributed to a slightly better fit of the compound to the active sites of electric eel.

Most of the hybrids also exhibited very good BuChE inhibitory activities. Compounds **6f** and **7c**, which had the best AChE inhibitory activities in the two series, also had very good BuChE inhibitory activities (IC_{50} values: 6.25 and 2.63 nM, respectively). However, the trend for the structure-activity relationship found for the AChE inhibitory activity was not found for the BuChE inhibitory activity.

Table 2
Inhibition of human AChE by selected tacrine derivatives **7a-7c**

Compound	R_1	*n*	IC_{50}[a](nM) hAChE[b]
7a	Me	7	5.18 ± 0.24
7b	Me	8	4.04 ± 0.028
7c	Me	9	3.52 ± 0.057

[a] Mean ± SD of at least three independent measurements.
[b] AChE from human recombinant.

2.3. Kinetics of AChE inhibition

To study the inhibitory mechanism for this class of tacrine-derived hybrids, the most potent AChE inhibitor among these compounds, compound **7c**, was chosen for further kinetic studies. The graphical presentation of the steady-state inhibition data of compound **7c** for AChE is shown in Figure 2. The results showed that there was an increasing slope and an increasing intercept at higher inhibitor concentrations, indicating a mixed-type inhibitory behaviour for compound **7c** as the result of binding to both the catalytic active site (CAS) and peripheral anionic site(PAS) of AChE.

2.4. Molecular modelling studies

To explore the possible interaction mode of the tacrine hybrids with TcAChE, molecular docking simulations for the derivative **7c** onto TcAChE were performed using the CDOCKER program in Discovery Studio 2.1. These simulations were based on the X-ray crystal structure of the TcAChE-bis-(7)-tacrine complex (PDB entry 2CMF), and the result is shown in Figure 3a and b. These

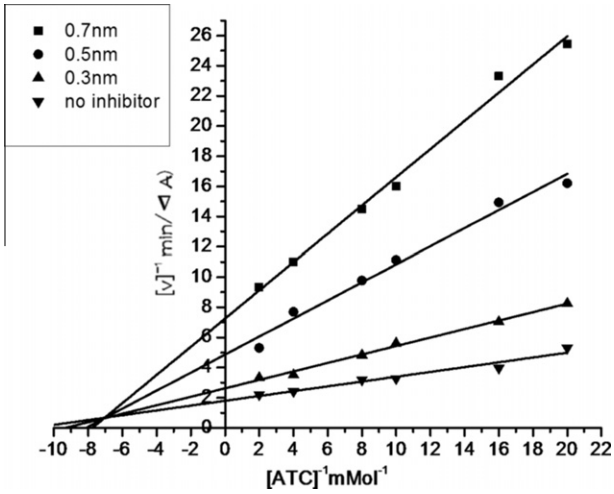


Figure 2. Steady state inhibition by compound **7c** of the AChE hydrolysis of Ach. The plots show mixed-type AChE inhibition for compound **7c**.

simulations indicated that compound **7c** occupies the entire enzymatic CAS, the mid-gorge sites and the PAS and could simultaneously bind to both the central pocket and peripheral sites. The tacrine moiety of the hybrid was observed to bind to the CAS via a parallel π–π interaction between Trp84 (4.195 Å) and Phe330 (4.048 Å) in a 'sandwich' form. The protonated nitrogen atom of benzylamine interacted with Trp279 of the PAS with an atom-to-ring distance of 3.935 Å. In addition, the methylene moiety of the long chain, bound to the gorge, could fold in a conformation in the gorge that allows the linker to interact with Tyr334, Gly335, Phe330, Tyr121, Asp72 and Tyr70 through hydrophobic interactions. The benzyl moiety, bound to the rim of the gorge, also could interact with Leu282 and Ile287 through hydrophobic interactions.

2.5. Antioxidant activity

The antioxidant activities of all of the hybrids were determined by measuring the oxygen radical absorbance capacity of fluorescein (ORAC-FL), and the results are presented in Table 1.[24,25] A vitamin E analogue, Trolox was used as a standard (the activities are expressed as Trolox equivalents, μmol of Trolox equivalents/μmol of tested compound). It could be observed from the results that most of the hybrids had very potent peroxyl radical absorbance capacities, ranging from 1.22- to 13.81-fold the value of Trolox. The exception was compound **7d**, *o*-(*N*,*N*-diethylamino) benzylamine linked with tacrine by an eight-carbon spacer, which had an activity of 0.88 Trolox equivalents. In contrast to the AChE inhibitory activity, the antioxidant activities of the hybrids decreased as the length of the carbon spacer increased. Compounds **6a**-**d**, which have 2- to 6-carbon spacers linking tacrine to *o*-hydroxyl benzylamine, had activities of more than 10 Trolox equivalents, whereas compound **6g**, a hybrid with a similar structure apart from its nine-carbon spacer, had an activity of only 4.41 Trolox equivalents. Substituted amino groups seem to have an unfavourable effect on the antioxidant activity, and compounds **7a**-**d** all had lower activities than their relatives **6e**-**g**.

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

On the basis of the results of the in vitro inhibition studies for AChE and BuChE, the ability of tacrine-derived hybrids to reduce $A\beta_{1-42}$ self-aggregation was studied through a thioflavin T-based fluorimetric assay with curcumin (Cur) as the standard compound. The results in Figure 4 indicate that these hybrids showed moderate to good potencies (25.8–40.5% at 20 μM) relative to that of curcumin (52.1% at 20 μM). Among the hybrids, compound **7c**, which exhibited the most potent AChE inhibition activity, had good Aβ aggregation inhibition properties (39.4% at 20 μM). In contrast, compound **6m**, which was the most potent AChE inhibitor in the series of *o*-hydroxyl benzylamine-tacrine hybrids, exhibited 25.8% inhibition at 20 μM, indicating that substituted amino groups have a favourable effect on $A\beta_{1-42}$ peptide aggregation inhibition.

2.7. Metal-chelating properties of compounds 6f, 7c and 7e

The complexation abilities of compounds **6f**, **7c** and **7e** for biometals such as Cu^{2+}, Fe^{2+} and Zn^{2+} in ethanol were studied by UV–vis spectrometry,[26,27] and the results are shown in Figures 5, 6 and 7. All the electronic spectra of **6f**, **7c** and **7e** exhibited a red shift (**6f**: the peak at 323 nm shifted to 337 nm; **7c**: the peak at 325 nm shifted to 336 nm; **7e**: the peak at 323 nm shifted to 337 nm). After the addition of Cu^{2+}. These results indicated that the two hybrids could interact with the Cu^{2+} ion effectively. Two other biometals, Fe^{2+} and Zn^{2+}, also expressed similar results; the peak in the electronic spectra of **6f** at 323 nm shifted to 335 nm when Fe^{2+} was added, and the peak changed from 323 nm to 337 nm when Zn^{2+} was added. The peaks of **7c** changed from 325 nm to 336 nm and from 325 nm to 336 nm, respectively, in response to the addition of the two biometals. The peaks of **7e** changed from 323 nm to 336 nm and from 323 nm to 336 nm, respectively.

3. Conclusion

In conclusion, our study involved the synthesis of a new series of tacrine-derived hybrids incorporating *o*-hydroxyl- or *o*-amino benzylamine and tacrine as synthons. These compounds were

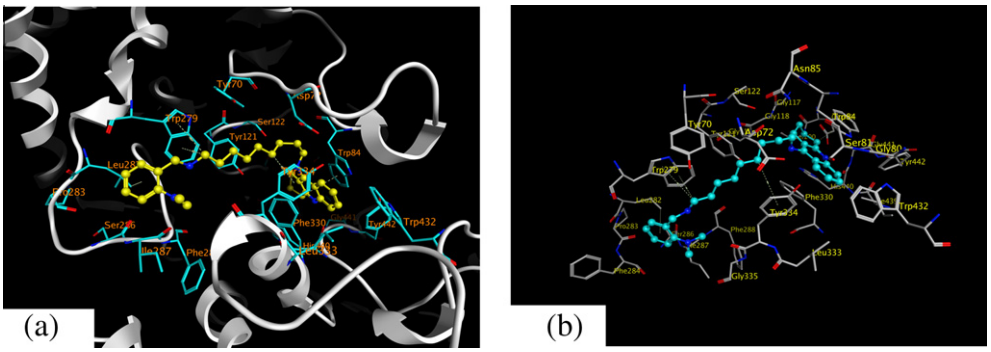


Figure 3. Docking models of the compound–enzyme complex. (a) The binding pattern of compound **7c** (yellow stick) to the TcAChE. (b) Representation of compound **7c** interacting with residues in the binding site of TcAChE, highlighting the protein residues that participate in the main interactions with the inhibitor.

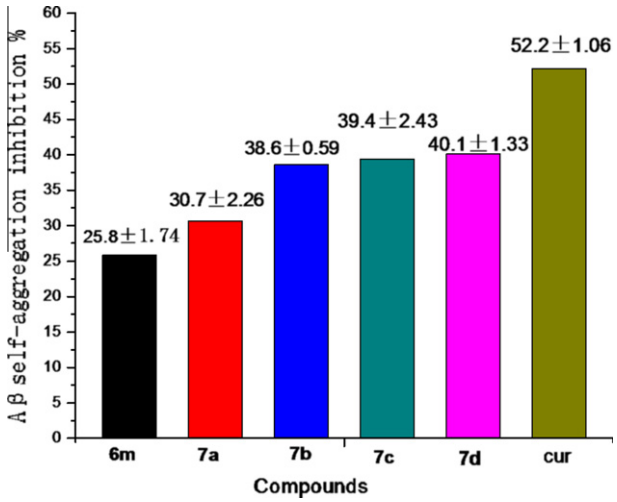


Figure 4. Inhibition of self-mediated $A\beta_{1-42}$ aggregation by compounds **6m**, **7a**, **7b**, **7c**, **7d** and curcumin. The measurements were carried out in the presence of 20 μM test compound. The thioflavin-T fluorescence method was used. The values represent the mean ± SD from at least two independent measurements.

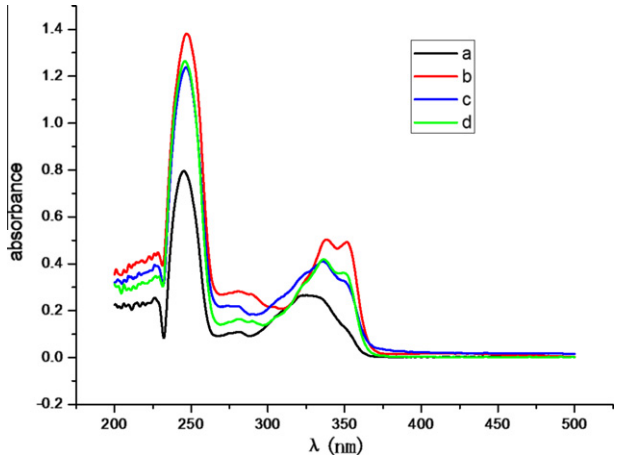


Figure 5. (a) UV spectrum of compound **6f** (40 μM). (b) Spectrum of a mixture of **6f** (40 μM) and $CuSO_4$ (40 μM). (c) Spectrum of a mixture of **6f** (40 μM) and $FeSO_4$ (40 μM). (d) Spectrum of a mixture of **6f** (40 μM) and $ZnCl_2$ (40 μM).

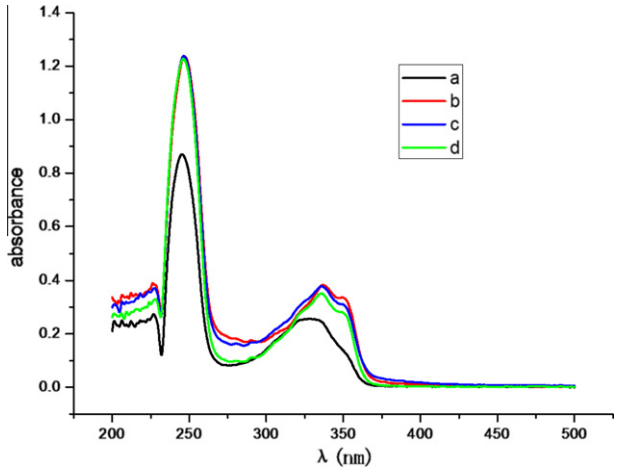


Figure 6. (a) UV spectrum of compound **7c** (40 μM). (b) Spectrum of a mixture of **7c** (40 μM) and $CuSO_4$ (40 μM). (c) Spectrum of a mixture of **7c** (40 μM) and $FeSO_4$ (40 μM). (d) Spectrum of a mixture of **7c** (40 μM) and $ZnCl_2$ (40 μM).

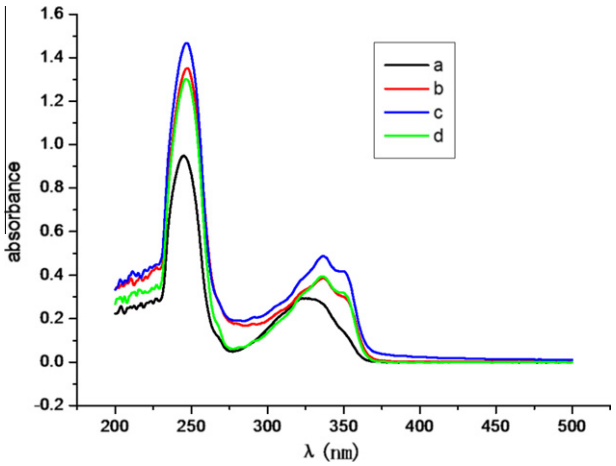


Figure 7. (a) UV spectrum of compound **7e** (40 μM). (b) Spectrum of a mixture of **7e** (40 μM) and $CuSO_4$ (40 μM). (c) Spectrum of a mixture of **7e** (40 μM) and $FeSO_4$ (40 μM). (d) Spectrum of a mixture of **7e** (40 μM) and $ZnCl_2$ (40 μM).

evaluated as potential multi-valent inhibitors of ChE and Aβ aggregation, as antioxidant agents, and as chelators of biometals. Among the synthesised compounds, compound **7c**, in which tacrine was linked with *o*-(*N*,*N*-dimethylamino) benzylamine by a 9-carbon spacer, exhibited the greatest inhibitory potency towards AChE (IC_{50} value: 0.55 nM), good biometal-chelating ability, good inhibition of Aβ aggregation (39.4% at 20 μM) and moderate antioxidant activity (1.22 Trolox equivalents). Further investigations of AD candidates based on these results are in progress.

4. Material and methods

4.1. Chemistry

The ^1H 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. MS spectra were recorded on an Agilent LC–MS 6120 instrument with an ESI mass selective detector. High-resolution mass spectra were obtained using a Shimadzu LCMS-IT-TOF mass spectrometer. Flash column chromatography was performed with 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 on silica gel. The purities of synthesized compounds were confirmed to be higher than 95% by analytical HPLC performed with a dual pump Shimadzu LC-20AB system equipped with a Eclipse Plus XDB-C8 column (4.6 × 150 mm, 5 μm) and eluted with methanol/water (55:45–40:60) containing 0.1% TFA at a flow rate of 0.5 mL/min. Compound **3** and compound **5** was prepared according to the reported procedure.[28–30]

4.2. General procedures for the preparation of compound 6a–6n, 7a–7e

Aromatic aldehydes **5** or salicylic aldehyde or pyridine-2-carboxaldehyde (0.5 mmol) and intermediate **3** (0.5 mmol) were stirred in EtOH for 5 h and then $NaBH_4$ (1 mmol) was added at 0 °C. The solution was stirred for 5 min at 0 °C. After 2 h at room temperature, the solvent was evaporated and the residue was quenched by water (10 mL) followed by extraction with EtOAc (3 × 10 mL). The organic layer was washed with brine (3 × 10 mL), and then dried over $MgSO_4$ and then concentrated, the compounds were purified by flash chromatography with CH_2Cl_2/MeOH/NH_4OH (10:1:1%) elution.

4.2.1. 2-(((2-((1,2,3,4-Tetrahydroacridin-9-yl)amino)ethyl) amino)methyl)phenol (6a)

Salicylic aldehyde was treated with **3a** according to general procedure to give the desired product as light yellow solid (59% yield). m.p. 119.5–121.1 °C; ^{1} (400 MHz, CDCl3) δ 7.93 (t, J = 8.5 Hz, 2H), 7.57 (t, J = 7.4 Hz, 1H), 7.42–7.34 (m, 1H), 7.20 (t, J = 7.7 Hz, 1H), 7.01 (d, J = 7.3 Hz, 1H), 6.87 (d, J = 8.1 Hz, 1H), 6.81 (t, J = 7.4 Hz, 1H), 4.06 (s, 2H), 3.62 (s, 2H), 3.08 (s, 2H), 2.97 (t, J = 5.9 Hz, 2H), 2.73 (s, 2H), 1.92 (s, 4H). ^{13}C NMR (101 MHz, CDCl3) δ 158.01, 157.71, 150.78, 146.55, 128.89, 128.79, 128.72, 127.91, 124.07, 122.81, 122.70, 120.02, 119.25, 116.47, 116.30, 52.18, 49.36, 48.28, 33.40, 24.70, 22.87, 22.53. Purity: 98.0% by HPLC; LC/MS (ESI) m/z:[M+H]$^+$ 348.2. HRMS m/z [M+H]$^+$ Calcd for $C_{22}H_{25}N_3O$ 348.2070, found 348.2075.

4.2.2. 2-(((3-((1,2,3,4-Tetrahydroacridin-9-yl)amino)propyl) amino)methyl)phenol (6b)

Salicylic aldehyde was treated with **3b** according to general procedure to give the desired product as light yellow solid (68% yield). m.p. 144.3-146.2 °C; ^{1} (400 MHz, $CDCl_3$) δ 7.90 (dd, J = 12.1, 8.5 Hz, 2H), 7.55 (t, J = 7.4 Hz, 1H), 7.38–7.30 (m, 1H), 7.18 (t, J = 7.5 Hz, 1H), 6.98 (d, J = 7.2 Hz, 1H), 6.84 (d, J = 8.0 Hz, 1H), 6.78 (t, J = 7.4 Hz, 1H), 4.01 (s, 2H), 3.55 (t, J = 6.7 Hz, 2H), 3.06 (s, 2H), 2.78 (t, J = 6.9 Hz, 2H), 2.68 (s, 2H), 1.98–1.83 (m, 6H). ^{13}C NMR (101 MHz, DMSO) δ 157.77, 157.16, 150.26, 146.79, 128.55, 128.11, 127.82, 127.77, 124.08, 123.14, 123.01, 120.10, 118.38, 115.67, 115.26, 50.26, 46.29, 45.99, 33.41, 30.17, 24.99, 22.68, 22.35. Purity: 99.8% by HPLC; LC/MS (ESI) m/z:[M+H]$^+$ 362.2. HRMS m/z [M+H]$^+$ Calcd for $C_{23}H_{27}N_3O$ 362.2227, found 362.2216.

4.2.3. 2-(((4-((1,2,3,4-Tetrahydroacridin-9-yl)amino)butyl) amino)methyl)phenol (6c)

Salicylic aldehyde was treated with **3c** according to general procedure to give the desired product as light yellow oil (62% yield). ^{1} (400 MHz, $CDCl_3$) δ 7.96 (dd, J = 16.1, 8.4 Hz, 2H), 7.57 (t, J = 7.7 Hz, 1H), 7.36 (t, J = 7.7 Hz, 1H), 7.17 (t, J = 7.8 Hz, 1H), 6.97 (d, J = 7.2 Hz, 1H), 6.82 (d, J = 8.0 Hz, 1H), 6.77 (t, J = 7.4 Hz, 1H), 3.99 (s, 2H), 3.51 (d, J = 7.0 Hz, 2H), 3.10 (s, 2H), 2.80–2.56 (m, 4H), 1.92 (s, 4H), 1.73 (d, J = 7.0 Hz, 2H), 1.66 (d, J = 6.8 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.12, 157.66, 151.15, 146.35, 128.76, 128.69, 128.41, 127.67, 123.89, 122.93, 122.49, 119.80, 119.01, 116.26, 115.67, 52.51, 48.94, 48.19, 33.30, 29.21, 26.94, 24.75, 22.88, 22.50. Purity: 99.7% by HPLC; LC/MS (ESI) m/z:[M+H]$^+$ 376.2. HRMS m/z [M+H]$^+$ Calcd for $C_{24}H_{29}N_3O$ 376.2383, found 376.2388.

4.2.4. 2-(((6-((1,2,3,4-Tetrahydroacridin-9-yl)amino)hexyl) amino)methyl)phenol (6d)

Salicylic aldehyde was treated with **3d** according to general procedure to give the desired product as light yellow oil (76% yield). ^{1} (400 MHz, $CDCl_3$) δ 7.98 (t, J = 8.6 Hz, 2H), 7.56 (t, J = 7.6 Hz, 1H), 7.35 (dd, J = 8.2, 7.0 Hz, 1H), 7.15 (t, J = 7.7 Hz, 1H), 6.97 (d, J = 7.4 Hz, 1H), 6.81 (d, J = 8.1 Hz, 1H), 6.76 (dd, J = 7.9, 6.8 Hz, 1H), 3.98 (s, 2H), 3.54 (t, J = 7.2 Hz, 2H), 3.10 (s, 2H), 2.72–2.61 (m, 4H), 1.91 (s, 4H), 1.74–1.61 (m, 2H), 1.59–1.47 (m, 2H), 1.39 (d, J = 2.1 Hz, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.27, 157.01, 151.64, 145.80, 128.97, 128.57, 128.34, 127.05, 123.82, 123.21, 122.56, 119.39, 118.88, 116.21, 114.92, 52.55, 49.07, 48.41, 32.94, 31.48, 29.34, 26.79, 26.63, 24.65, 22.83, 22.38. Purity: 99.7% by HPLC; LC/MS (ESI) m/z:[M+H]$^+$ 404.2. HRMS m/z [M+H]$^+$ Calcd for $C_{26}H_{33}N_3O$ 404.2696, found 404.2686.

4.2.5. 2-(((7-((1,2,3,4-Tetrahydroacridin-9-yl)amino)heptyl) amino)methyl)phenol (6e)

Salicylic aldehyde was treated with **3e** according to general procedure to give the desired product as light yellow oil (73% yield). ^{1} (400 MHz, $CDCl_3$) δ 7.96 (d, J = 8.5 Hz, 2H), 7.56 (t, J = 7.6 Hz, 1H), 7.35 (t, J = 7.6 Hz, 1H), 7.15 (t, J = 7.7 Hz, 1H), 6.97 (d, J = 7.2 Hz, 1H), 6.82 (d, J = 8.0 Hz, 1H), 6.76 (t, J = 7.4 Hz, 1H), 3.98 (s, 2H), 3.51 (t, J = 7.2 Hz, 2H), 3.09 (s, 2H), 2.67 (dd, J = 15.7, 8.7 Hz, 4H), 1.92 (s, 4H), 1.65 (dd, J = 14.2, 7.1 Hz, 2H), 1.51 (d, J = 6.6 Hz, 2H), 1.43–1.30 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.31, 157.76, 151.19, 146.65, 128.59(2H), 128.26, 127.91, 123.69, 122.96, 122.61, 119.85, 118.88, 116.26, 115.40, 52.64, 49.27, 48.57, 33.45, 31.56, 29.39, 29.05, 26.94, 26.75, 24.71, 22.95, 22.58. Purity: 97.5% by HPLC; LC/MS (ESI) m/z:[M+H]$^+$ 418.2. HRMS m/z [M+H]$^+$ Calcd for $C_{27}H_{35}N_3O$ 418.2853, found 418.2839.

4.2.6. 2-(((8-((1,2,3,4-Tetrahydroacridin-9-yl)amino)octyl) amino)methyl)phenol (6f)

Salicylic aldehyde was treated with **3f** according to general procedure to give the desired product as light yellow oil (70% yield). ^{1} (400 MHz, $CDCl_3$) δ 8.01–7.91 (m, 2H), 7.55 (t, J = 7.6 Hz, 1H), 7.34 (t, J = 7.6 Hz, 1H), 7.15 (t, J = 7.7 Hz, 1H), 6.97 (d, J = 7.3 Hz, 1H), 6.82 (d, J = 8.0 Hz, 1H), 6.76 (t, J = 7.3 Hz, 1H), 3.97 (s, 2H), 3.51 (t, J = 7.9 Hz, 2H), 3.08 (s, 2H), 2.66 (dd, J = 16.0, 8.9 Hz, 4H), 1.91 (s, 4H), 1.72–1.60 (m, 2H), 1.50 (d, J = 6.4 Hz, 2H), 1.41–1.27 (m, 8H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.31, 157.71, 151.24, 146.65, 128.59, 128.54, 128.29, 127.85, 123.65, 123.07, 122.68, 119.79, 118.85, 116.22, 115.30, 52.60, 49.33, 48.62, 33.47, 31.65, 29.47, 29.25, 29.19, 26.97, 26.79, 24.69, 22.94, 22.59. Purity: 99.3% by HPLC; LC/MS (ESI) m/z:[M+H]$^+$ 432.2. HRMS m/z [M+H]$^+$ Calcd for $C_{28}H_{37}N_3O$ 432.3009, found 432.2997.

4.2.7. 2-(((9-((1,2,3,4-Tetrahydroacridin-9-yl)amino)nonyl) amino)methyl)phenol (6g)

Salicylic aldehyde was treated with **3g** according to general procedure to give the desired product as light yellow oil (65% yield). ^{1} (400 MHz, $CDCl_3$) δ 8.00–7.89 (m, 2H), 7.55 (t, J = 7.6 Hz, 1H), 7.34 (t, J = 7.6 Hz, 1H), 7.15 (t, J = 7.7 Hz, 1H), 6.97 (d, J = 7.4 Hz, 1H), 6.81 (d, J = 8.1 Hz, 1H), 6.76 (t, J = 7.4 Hz, 1H), 3.97 (s, 2H), 3.54–3.42 (m, 2H), 3.07 (s, 2H), 2.66 (dd, J = 16.2, 9.0 Hz, 4H), 1.91 (d, J = 2.9 Hz, 4H), 1.64 (dd, J = 13.6, 6.6 Hz, 2H), 1.50 (d, J = 6.5 Hz, 2H), 1.28 (s, 10H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.35, 158.01, 151.05, 146.99, 128.54, 128.43, 128.22(2H), 123.60, 122.94, 122.68, 120.01, 118.85, 116.26, 115.52, 52.67, 49.40, 48.69, 33.68, 31.68, 29.52, 29.34, 29.26, 29.22, 27.05, 26.85, 24.73, 23.00, 22.67. Purity: 99.7% by HPLC; LC/MS (ESI) m/z:[M+H]$^+$ 446.2. HRMS m/z [M+H]$^+$ Calcd for $C_{29}H_{39}N_3O$ 446.3166, found 446.3165.

4.2.8. 2-(((2-((6-chloro-1,2,3,4-Tetrahydroacridin-9-yl) amino)ethyl)amino)methyl)phenol (6h)

Salicylic aldehyde was treated with **3h** according to general procedure to give the desired product as light yellow solid (62% yield). m.p. 151.3–152.6 °C; ^{1} (400 MHz, $CDCl_3$) δ 7.89 (dd, J = 21.5, 5.5 Hz, 2H), 7.29 (dd, J = 9.1, 2.1 Hz, 1H), 7.24–7.16 (m, 1H), 7.02 (d, J = 7.2 Hz, 1H), 6.87 (d, J = 7.9 Hz, 1H), 6.81 (t, J = 7.4 Hz, 1H), 4.06 (s, 2H), 3.63 (s, 2H), 3.04 (s, 2H), 2.98 (t, J = 5.8 Hz, 2H), 2.68 (s, 2H), 1.96–1.85 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.08, 157.59, 150.73, 147.08, 134.50, 129.02, 128.71, 126.78, 124.75, 124.28, 122.40, 119.42, 118.22, 116.48, 116.36, 52.38, 49.36, 48.40, 33.42, 24.54, 22.75, 22.37. Purity: 98.9% by HPLC; LC/MS (ESI) m/z:[M+H]$^+$ 382.3. HRMS m/z [M+H]$^+$ Calcd for $C_{22}H_{24}ClN_3O$ 382.1681, found 382.1680.

4.2.9. 2-(((3-((6-chloro-1,2,3,4-Tetrahydroacridin-9-yl) amino)propyl)amino)methyl)phenol (6i)

Salicylic aldehyde was treated with **3i** according to general procedure to give the desired product as light yellow solid (63% yield). m.p. 153.6–155.2 °C; ^{1} (400 MHz, $CDCl_3$) δ 7.86 (dd, J = 17.4, 5.5 Hz, 2H), 7.20 (dd, J = 9.1, 2.0 Hz, 1H), 7.18–7.13 (m, 1H), 6.98 (d, J = 7.1 Hz, 1H), 6.83 (d, J = 8.0 Hz, 1H), 6.78 (t, J = 7.4 Hz, 1H), 3.99

(s, 2H), 3.61 (t, J = 6.8 Hz, 2H), 2.99 (d, J = 5.7 Hz, 2H), 2.78 (t, J = 6.7 Hz, 2H), 2.58 (d, J = 5.5 Hz, 2H), 1.94–1.76 (m, 6H). ^{13}C NMR (101 MHz, DMSO) δ 159.26, 157.02, 150.36, 147.56, 132.35, 128.64, 127.83, 126.61, 125.41, 123.95, 123.27, 118.45, 118.41, 115.76, 115.23, 50.03, 46.25, 45.92, 33.47, 29.99, 24.92, 22.52, 22.20. Purity: 99.8% by HPLC; LC/MS (ESI) m/z:[M+H]$^+$ 396.3. HRMS m/z [M+H]$^+$ Calcd for $C_{23}H_{26}ClN_3O$ 396.1837, found 396.1831.

4.2.10. 2-(((4-((6-Chloro-1,2,3,4-tetrahydroacridin-9-yl)amino)butyl)amino)methyl)phenol (6j)

Salicylic aldehyde was treated with **3j** according to general procedure to give the desired product as light yellow oil (65% yield). ^{1} (400 MHz, $CDCl_3$) δ 7.87 (dd, J = 13.3, 5.5 Hz, 2H), 7.30–7.23 (m, 1H), 7.16 (dd, J = 11.1, 4.4 Hz, 1H), 6.97 (d, J = 7.0 Hz, 1H), 6.84–6.73 (m, 2H), 3.98 (s, 2H), 3.47 (t, J = 7.0 Hz, 2H), 3.03 (s, 2H), 2.69 (dd, J = 15.7, 9.0 Hz, 4H), 1.97–1.84 (m, 4H), 1.76–1.67 (m, 2H), 1.63 (dd, J = 14.4, 7.7 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.32, 158.08, 150.78, 147.67, 134.03, 128.67, 128.45, 127.07, 124.63, 124.27, 122.53, 119.02, 118.30, 116.20, 115.93, 52.48, 49.08, 48.17, 33.75, 29.26, 26.92, 24.59, 22.83, 22.51. Purity: 99.4% by HPLC; LC/MS (ESI) m/z:[M+H]$^+$ 410.3. HRMS m/z [M+H]$^+$ Calcd for $C_{24}H_{28}ClN_3O$ 410.1994, found 410.1992.

4.2.11. 2-(((6-((6-Chloro-1,2,3,4-tetrahydroacridin-9-yl)amino)hexyl)amino)methyl)phenol (6k)

Salicylic aldehyde was treated with **3k** according to general procedure to give the desired product as light yellow oil (58% yield). ^{1} (400 MHz, $CDCl_3$) δ 7.95–7.84 (m, 2H), 7.28 (d, J = 2.1 Hz, 1H), 7.16 (t, J = 7.1 Hz, 1H), 6.98 (d, J = 7.2 Hz, 1H), 6.86–6.73 (m, 2H), 3.98 (s, 2H), 3.49 (dd, J = 8.4, 6.0 Hz, 2H), 3.04 (s, 2H), 2.67 (t, J = 6.9 Hz, 4H), 1.94–1.88 (m, 4H), 1.69–1.63 (m, 2H), 1.57–1.50 (m, 2H), 1.43–1.36 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.14, 157.79, 151.80, 146.03, 134.96, 128.81, 128.51, 125.56, 124.95, 124.54, 122.13, 119.00, 117.47, 116.34, 114.74, 52.32, 49.06, 48.27, 32.68, 31.42, 29.09, 26.70, 26.55, 24.50, 22.67, 22.15. Purity: 99.9% by HPLC; LC/MS (ESI) m/z:[M+H]$^+$ 438.3. HRMS m/z [M+H]$^+$ Calcd for $C_{26}H_{32}ClN_3O$ 438.2307, found 438.2318.

4.2.12. 2-(((7-((6-Chloro-1,2,3,4-tetrahydroacridin-9-yl)amino)heptyl)amino)methyl)phenol (6l)

Salicylic aldehyde was treated with **3l** according to general procedure to give the desired product as light yellow oil (50% yield). ^{1} (400 MHz, $CDCl_3$) δ 7.99 (s, 1H), 7.93 (d, J = 9.1 Hz, 1H), 7.29 (d, J = 1.8 Hz, 1H), 7.16 (t, J = 7.6 Hz, 1H), 6.98 (d, J = 7.3 Hz, 1H), 6.82 (d, J = 8.1 Hz, 1H), 6.77 (t, J = 7.4 Hz, 1H), 3.99 (s, 2H), 3.55 (t, J = 7.1 Hz, 2H), 3.07 (s, 2H), 2.73–2.61 (m, 5H), 1.91 (s, 4H), 1.67 (dd, J = 14.2, 7.2 Hz, 2H), 1.57–1.48 (m, 2H), 1.37 (d, J = 17.6 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.22, 158.17, 151.58, 146.57, 134.65, 128.71, 128.44, 126.00, 124.96, 124.37, 122.31, 118.94, 117.65, 116.26, 114.90, 52.45, 49.24, 48.44, 33.03, 31.49, 29.23, 28.99, 26.89, 26.70, 24.48, 22.73, 22.28. Purity: 98.1% by HPLC; LC/MS (ESI) m/z:[M+H]$^+$ 452.3. HRMS m/z [M+H]$^+$ Calcd for $C_{27}H_{34}ClN_3O$ 452.2463, found 452.2478.

4.2.13. 2-(((8-((6-Chloro-1,2,3,4-tetrahydroacridin-9-yl)amino)octyl)amino)methyl)phenol (6m)

Salicylic aldehyde was treated with **3m** according to general procedure to give the desired product as light yellow oil (53% yield). ^{1} (400 MHz, $CDCl_3$) δ 7.90 (dd, J = 5.5, 3.3 Hz, 2H), 7.29–7.23 (m, 1H), 7.18–7.12 (m, 1H), 6.97 (d, J = 6.9 Hz, 1H), 6.82 (d, J = 8.1 Hz, 1H), 6.76 (td, J = 7.4, 1.0 Hz, 1H), 3.98 (s, 2H), 3.49 (t, J = 7.2 Hz, 2H), 3.03 (s, 2H), 2.66 (t, J = 7.0 Hz, 4H), 1.90 (t, J = 3.1 Hz, 4H), 1.69–1.60 (m, 2H), 1.56–1.48 (m, 2H), 1.40–1.27 (m, 10H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.57, 158.27, 151.40, 147.02, 134.50, 128.67, 128.33, 126.44, 124.85, 124.33, 122.44, 118.93, 117.84, 116.29, 115.07, 52.60, 49.41, 48.59, 33.29, 31.64, 29.41, 29.22, 29.16, 26.95, 26.75, 24.47, 22.78, 22.38. Purity: 98.6% by HPLC; LC/MS (ESI) m/z:[M+H]$^+$ 466.3. HRMS m/z [M+H]$^+$ Calcd for $C_{28}H_{36}ClN_3O$ 466.2620, found 466.2615.

4.2.14. 2-(((9-((6-Chloro-1,2,3,4-tetrahydroacridin-9-yl)amino)nonyl)amino)methyl)phenol (6n)

Salicylic aldehyde was treated with **3n** according to general procedure to give the desired product as light yellow oil (74% yield). ^{1} (400 MHz, $CDCl_3$) δ 7.90 (dd, J = 5.5, 3.3 Hz, 2H), 7.29–7.23 (m, 1H), 7.18–7.12 (m, 1H), 6.97 (d, J = 6.9 Hz, 1H), 6.82 (d, J = 8.1 Hz, 1H), 6.76 (td, J = 7.4, 1.0 Hz, 1H), 3.98 (s, 2H), 3.49 (t, J = 7.2 Hz, 2H), 3.03 (s, 2H), 2.66 (t, J = 7.0 Hz, 4H), 1.90 (t, J = 3.1 Hz, 4H), 1.69–1.60 (m, 2H), 1.56–1.48 (m, 2H), 1.39–1.26 (m, 11H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.14, 158.28, 151.03, 147.73, 134.06, 128.62, 128.35, 127.05, 124.78, 124.12, 122.51, 118.88, 118.16, 116.26, 115.36, 52.52, 49.49, 48.61, 33.71, 31.71, 29.43, 29.35, 29.27, 29.21, 27.04, 26.82, 24.52, 22.87, 22.55. Purity: 99.8% by HPLC; LC/MS (ESI) m/z:[M+H]$^+$ 480.3. HRMS m/z [M+H]$^+$ Calcd for $C_{29}H_{38}ClN_3O$ 480.2776, found 480.2788.

4.2.15. N^1-(2-(Dimethylamino)benzyl)-N^7-(1,2,3,4-tetrahydroacridin-9-yl)heptane-1,7-diamine (7a)

Aromatic aldehyde **5a** was treated with **3e** according to general procedure to give the desired product as light yellow oil (51% yield). ^{1} (400 MHz, $CDCl_3$) δ 8.02–7.89 (m, 2H), 7.54 (d, J = 6.8 Hz, 1H), 7.43–7.28 (m, 2H), 7.25–7.18 (m, 1H), 7.12 (dd, J = 7.6, 4.0 Hz, 1H), 7.04 (dd, J = 11.1, 7.2 Hz, 1H), 3.88 (s, 2H), 3.49 (d, J = 4.1 Hz, 2H), 3.07 (s, 2H), 2.69 (s, 6H), 2.61 (dt, J = 11.6, 5.9 Hz, 2H), 2.16 (s, 4H), 1.93 (d, J = 3.9 Hz, 4H), 1.66 (d, J = 4.7 Hz, 2H), 1.52–1.51 (m, 2H), 1.34–1.30 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.00, 152.63, 150.98, 147.01, 133.61, 129.84, 128.37, 128.25, 127.90, 123.58, 123.51, 122.89, 120.02, 119.47, 115.56, 50.19, 49.34, 49.01, 44.93(2C), 33.69, 31.61, 29.52, 29.16, 27.13, 26.82, 24.73, 22.99, 22.66. Purity: 97.9% by HPLC; LC/MS (ESI) m/z:[M+H]$^+$ 445.4. HRMS m/z [M+H]$^+$ Calcd for $C_{29}H_{40}N_4$ 445.3253, found 445.3336.

4.2.16. N^1-(2-(Dimethylamino)benzyl)-N^8-(1,2,3,4-tetrahydroacridin-9-yl)octane-1,8-diamine (7b)

Aromatic aldehyde **5a** was treated with **3f** according to general procedure to give the desired product as light yellow oil (57% yield). ^{1} (400 MHz, $CDCl_3$) δ 8.01–7.89 (m, 2H), 7.54 (dd, J = 15.9, 8.0 Hz, 1H), 7.38–7.27 (m, 2H), 7.23 (d, J = 7.2 Hz, 1H), 7.13 (d, J = 7.8 Hz, 1H), 7.05 (t, J = 7.2 Hz, 1H), 3.92 (s, 2H), 3.50 (t, J = 7.1 Hz, 2H), 3.08 (s, 2H), 2.69 (s, 6H), 2.64–2.57 (m, 4H), 1.92 (s, 4H), 1.71–1.63 (m, 2H), 1.53–1.51 (m, 2H), 1.38–1.30 (m 8H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.92, 152.58, 151.00, 146.98, 133.65, 129.80, 128.37, 128.18, 127.85, 123.54, 123.46, 122.97, 119.95, 119.41, 115.46, 50.20, 49.35, 49.12, 44.93(2C), 33.69, 31.65, 29.62, 29.33, 29.24, 27.16, 26.82, 24.71, 22.97, 22.66. Purity: 98.5% by HPLC; LC/MS (ESI) m/z:[M+H]$^+$ 459.4. HRMS m/z [M+H]$^+$ Calcd for $C_{30}H_{42}N_4$ 459.3409, found 459.3493.

4.2.17. N^1-(2-(Dimethylamino)benzyl)-N^9-(1,2,3,4-tetrahydroacridin-9-yl)nonane-1,9-diamine (7c)

Aromatic aldehyde **5a** was treated with **3g** according to general procedure to give the desired product as light yellow oil (53% yield). ^{1} (400 MHz, $CDCl_3$) δ 8.00–7.88 (m, 2H), 7.58–7.48 (m, 1H), 7.38–7.28 (m, 2H), 7.22 (d, J = 7.7 Hz, 1H), 7.12 (d, J = 7.9 Hz, 1H), 7.04 (t, J = 7.2 Hz, 1H), 3.90 (s, 2H), 3.49 (t, J = 7.1 Hz, 2H), 3.07 (s, 2H), 2.69 (s, 6H), 2.61 (t, J = 7.2 Hz, 4H), 1.92 (s, 4H), 1.69–1.60 (m, 2H), 1.531.51 (m, 2H), 1.38–1.28 (m, 10H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.93, 152.63, 151.08, 146.91, 133.17, 129.95, 128.44, 128.18, 128.04, 123.63, 123.61, 122.92, 119.96, 119.55, 115.48, 50.12, 49.40, 48.93, 44.93(2C), 33.64, 31.68, 29.42, 29.37, 29.30, 29.23, 27.18, 26.85, 24.71, 22.99, 22.65. Purity:

98.6% by HPLC; LC/MS (ESI) m/z:$[M+H]^+$ 473.4. HRMS m/z $[M+H]^+$ Calcd for $C_{31}H_{44}N_4$ 473.3566, found 473.3656.

4.2.18. N^1-(2-(Diethylamino)benzyl)-N^8-(1,2,3,4-tetrahydroacridin-9-yl)octane-1,8-diamine (7d)

Aromatic aldehyde **5b** was treated with **3f** according to general procedure to give the desired product as light yellow oil (48% yield). ^{1} (400 MHz, $CDCl_3$) δ 7.96 (d, J = 8.1 Hz, 2H), 7.61–7.51 (m, 1H), 7.29 (s, 2H), 7.23 (d, J = 7.4 Hz, 1H), 7.14 (d, J = 7.8 Hz, 1H), 7.07 (t, J = 7.3 Hz, 1H), 3.91 (s, 2H), 3.55–3.44 (m, 2H), 3.09 (s, 2H), 2.98 (dd, J = 13.9, 6.8 Hz, 4H), 2.70 (s, 2H), 2.58 (t, J = 7.1 Hz, 2H), 1.92 (s, 4H), 1.71–1.62 (m, 2H), 1.54–1.51 (m, 2H), 1.35-1.31 (m, 8H), 1.00 (t, J = 7.1 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.87, 151.11, 149.73, 146.83, 135.73, 130.02, 128.47, 128.10, 127.64, 124.16, 123.62, 122.92, 122.75, 119.92, 115.44, 50.47, 49.37, 48.83, 48.08 (2C), 33.58, 31.65, 29.52, 29.30, 29.21, 27.18, 26.81, 24.70, 22.96, 22.62, 12.60 (2C). Purity: 97.6% by HPLC; LC/MS (ESI) m/z:$[M+H]^+$ 487.4. HRMS m/z $[M+H]^+$ Calcd for $C_{32}H_{46}N_4O$ 487.3795, found 487.3788.

4.2.19. N^1-(Pyridin-2-ylmethyl)-N^8-(1,2,3,4-tetrahydroacridin-9-yl)octane-1,8-diamine (7e)

Pyridine-2-carboxaldehyde was treated with **3f** according to general procedure to give the desired product as light yellow oil (42% yield). ^{1} (400 MHz, $CDCl_3$) δ 8.55 (d, J = 4.7 Hz, 1H), 7.97 (d, J = 8.6 Hz, 2H), 7.63 (td, J = 7.7, 1.7 Hz, 1H), 7.56 (t, J = 7.6 Hz, 1H), 7.35 (t, J = 7.7 Hz, 1H), 7.29 (d, J = 7.8 Hz, 1H), 7.20–7.11 (m, 1H), 3.90 (s, 2H), 3.52 (t, J = 7.1 Hz, 2H), 3.09 (s, 2H), 2.70 (s, 2H), 2.64 (t, J = 7.2 Hz, 2H), 1.92 (d, J = 3.0 Hz, 4H), 1.66 (dd, J = 14.5, 7.4 Hz, 2H), 1.52 (d, J = 6.9 Hz, 2H), 1.41–1.30 (m, 8H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.72, 157.72, 151.19, 149.22, 146.66, 136.44, 128.57, 127.93, 123.64, 122.99, 122.31, 121.91, 119.78, 115.30, 55.24, 49.61, 49.39, 33.48, 31.67, 30.01, 29.37, 29.23, 27.18, 26.82, 24.65, 22.92, 22.57. Purity: 99.7% by HPLC; LC/MS (ESI) m/z:$[M+H]^+$ 431.3. HRMS m/z $[M+H]^+$ Calcd for $C_{27}H_{36}N_4O$ 417.3013, found 417.3013.

4.3. Biological activity

4.3.1. In vitro inhibition of AChE and BuChE

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

All the in vitro AChE assays were carried out in 0.1 M KH_2PO_4/K_2HPO_4 buffer, pH 8.0, using a Shimadzu UV-2450 Spectrophotometer. AChE and BuChE 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. Activity was determined by measuring the increase in absorbance at 412 nm at 1 min intervals at 37 °C. Calculations were performed according to the method of Ellman et al[23] Each concentration was assayed in triplicate.

The in vitro BuChE assay (BuChE or ACh as the enzyme substrate) was performed according to a method similar to that described above.

4.3.2. Kinetic characterization of AChE inhibition

The kinetic characterization of AChE inhibitory activity was performed according to a published protocol.[31] Briefly, test compound was added to the assay solution and incubated with the enzyme at 37 °C for 15 min, then substrate was added. Kinetic characterization of the hydrolysis of ATC catalyzed by AChE was performed spectrophotometrically at 412 nm. A parallel control, with no inhibitor in the mixture, allowed activities to be measured at various times. The plots were analyzed using a weighted least square analysis 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.

4.3.3. The antioxidant activity assay

The antioxidant activity was determined by the oxygen radical absorbance capacity-fluorescein (ORAC-FL) assay.[24,25,32] All the assays were under 75 mM phosphate buffer (pH 7.4) and the final reaction mixture was 200 μL. Antioxidant (20 μL) and fluorescein (120 μL, 300 nM final concentration) were placed in the wells of a black 96-well plate and the mixture was incubated for 10 min at 37 °C. Then, AAPH (Aldrich) solution (60 μL; 12 mM final concentration) was added rapidly. The plate was immediately placed into a Spectrafluor Plus plate reader (Tecan, Crailsheim, Germany) and the fluorescence was measured every 60 s for 4 h with exitation at 485 nm and emission at 535 nm. Trolox was used as standard (1–10 μM, final concentration). A blank (FL + AAPH) using phosphate buffer instead of antioxidant and Trolox calibration were carried out in each assay. The samples were measured at different concentrations (0.5–10 μM). All reaction mixtures were prepared fourfold and at least four independent runs were performed for each sample. Fluorescence measurements were normalized to the curve of the blank (without antioxidant). The ORAC-FL values were calculated as described in the reference[31] and the final results were in μM of Trolox equivalent/μM of pure compound.

4.3.4. Inhibition of $A\beta_{1-42}$ peptide aggregation[33]

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 20 μM. 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-seconds-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.

4.3.5. Metal-chelating properties of compound 6f and 7c

The complexing studies were made in ethanol at 298 K using a UV–vis spectrophotometer (SHIMADZC UV-2450PC) with wavelength ranging from 200 to 500 nm. $CuSO_4$, $FeSO_4$ or $ZnCl_2$ solution were prepared in 200 μM using ethanol using volumetric flask. The tested compounds were dissolved in DMSO and then diluted with ethanol to 400 μM concentration. To a mixture of 100 μL tested compound solution and 700 μL ethanol, 200 μL $CuSO_4$ solution ($FeSO_4$, or $ZnCl_2$) was added. The solution was incubated at 298 K for 30 min and then the absorption spectra were recorded at 298 K in a 1 cm quartz cell. The control was prepared by mixting 100 μL tested compound solution and 900 μL ethanol. The blank was 4 μL DMSO in 996 μL ethanol. All absorption spectra subtracted the blank absorption spectra.

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