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The development of 2-acetylphenol-donepezil hybrids as multifunctional agents for the treatment of Alzheimer's disease

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ABSTRACT

A series of 2-acetylphenol-donepezil hybrids was designed and synthesized based on multi-target-directed ligands strategy. The biological activities were evaluated by AChE/BChE inhibition and MAO-A/MAO-B inhibition. The results revealed that the tertiary amines and methylene chain length significantly affected the eeAChE inhibitory potency, in particular, compound **TM-14** showed the best *ee*AChE inhibitory activity with IC_{50} value of 2.9 μM, in addition, both kinetic analysis of AChE inhibition and docking study displayed that **TM-14** could simultaneously bind to the catalytic active site and peripheral anionic site of AChE. Moreover, compound **TM-14** was a selective metal chelator and could form 1:1 **TM-14**-Cu^{2+} complex. The structure-active-relationship also indicated that the *O*-alkylamine fragment remarkably decreased hMAO-B inhibitory activity, compound **TM-2** exhibited potent hMAO-B inhibitory activity (IC_{50} = 6.8 μM), which was supported by the molecular docking study. More interestingly, compounds **TM-14** and **TM-2** could cross the blood-brain barrier *in vitro*. Therefore, the structure-active-relationship of 2-acetylphenol-donepezil hybrids could encourage the development of multifunction agents with selective AChE inhibition or selective MAO-B inhibition for the treatment of Alzheimer's disease.

Alzheimer's disease (AD) is a devastating neurodegenerative disease and the most common cause of dementia, affecting nearly 36 million people today and the number will be approximately triple to 131 million by 2050.[1] Up to now, the etiology of AD is not fully understood, but several factor including cholinergic dysfunction, amyloid-β (Aβ) deposits, tau protein aggregation, and metal ion disorder are considered to be important roles in the pathophysiology of AD.[2] Therefore, there are several acetylcholinesterase inhibitors (AChEIs), named donepezil, rivastigmine and galantamine, have been approved by Food and Drug Administration (FDA) for the treatment of AD by increasing acetylcholine (ACh) level. However, these single target drugs could temporarily delay the progression of cognitive decline in AD, their effects are modest. Moreover, AChE inhibition for long time lead to undesirable side effects, such as gastrointestinal disturbances, bradycardia, and excessive salivation, which are associated with peripheral cholinergic receptors.[3–5]

Due to the multi-factors pathogenesis of AD, the multi-target-directed ligands (MTDLs), one single molecule could hit different targets involved in the cascade of AD pathological events, have been considered as an appropriate strategy to achieve better therapeutic efficacy for AD. Further, the MTDLs strategy has been applied and developed by many researchers.[6–8] It is gratified that several MTDLs candidate drugs with disease modifying potential are now in the pipeline and have reached testing stage in clinical trials, such as Latrepirdine hydrochloride (Phase III), Eltoprazine hydrochloride (Phase II), Bexarotene (Phase II), Bryostatin 1 (Phase II), Ladostigil tartrate (Phase II), Indole-3-propionic acid (Phase I), Telmisartan (Phase I) and so on.[9]

In our previous work, novel 2-acetylphenol-*O*-alkylamine derivatives were designed and synthesized as multi-function agents for the treatment of AD by combining 2-acetylphenol analogs **DDDT-2d** with

Abbreviations: AD, Alzheimer's disease; A*β*, *β*-amyloid; AChEIs, acetylcholinesterase inhibitors; FDA, Food and Drug Administration; ACh, acetylcholine; MTDLs, multi-target-directed ligands; BChE, butyrylcholinesterase; *Ee*AChE, *Electrophorus electricus* AChE; *eq*BuChE, *equine serum* BuChE; CAS, catalytic active site; PAS, peripheral anionic site; MAO-B, monoamine oxidase B; MAO-A, monoamine oxidase A; PAMPA-BBB, parallel artificial membrane assay for the blood-brain barrier

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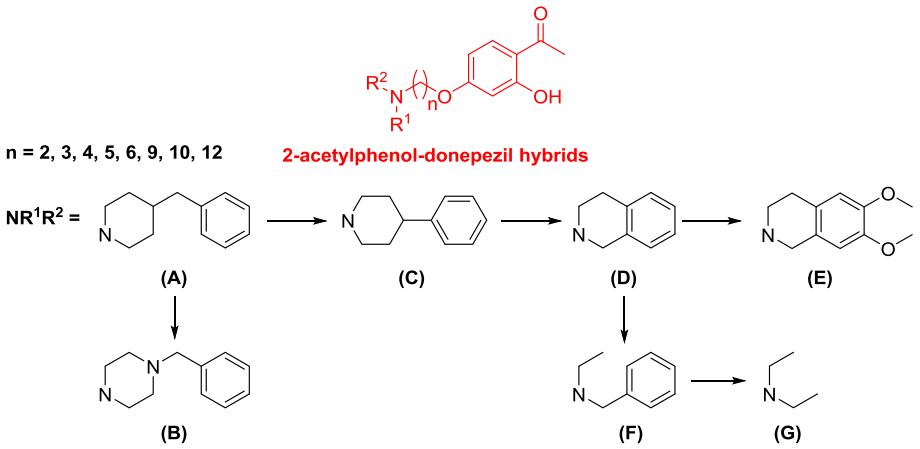


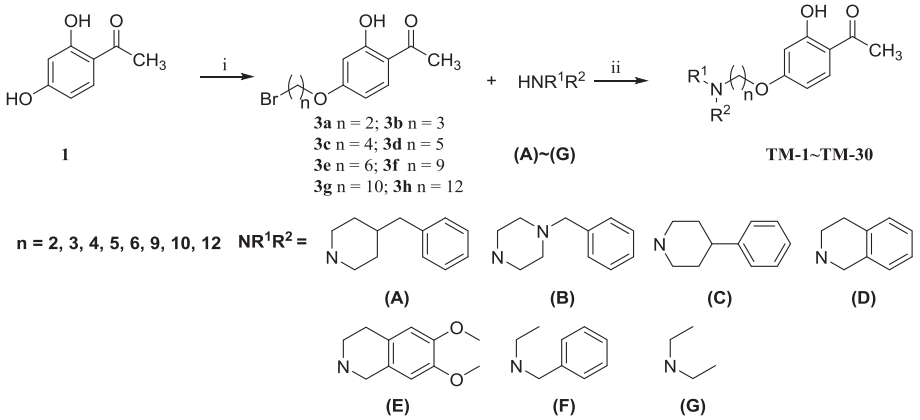
Fig. 1. Design strategy for the 2-acetylphenol-donepezil hybrids.

scutellarein-*O*-alkylamine derivatives **EJMC-16d**, the lead compound obtained provided significant data for the development of anti-AD drugs.[10] As we all known, donepezil is a selective AChE inhibitor approved by FDA to treat mild-to-moderate AD, and the benzylpiperidine fragment is the key pharmacophore.[11] In addition, the methylene chain length is also an important factor on AChE and butyrylcholinesterase (BChE) inhibitory activity based on our preliminary work.[12–16] Therefore, a series of novel 2-acetylphenol-donepezil hybrids was designed and synthesized (Fig. 1), the tertiary amine groups focused on benzylpiperidine fragment, as well as its modified derivatives, such as benzylpiperazine, 4-phenylpiperidine, 1,2,3,4-tetrahydroisoquinoline, 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline, *N*-ethylbenzylamine and diethylamine, and the methylene chain length including n = 2, 3, 4, 5, 6, 9, 10 and 12. Furthermore, the biological activities were evaluated by AChE/BuChE inhibition and MAO-A/MAO-B inhibition. Hoping the structure-active-relationship would give important clues for developing multi-function agents to treat AD.

The synthetic route of target compounds **TM-1** ~ **TM-30** was described in Scheme 1. Briefly, 2,4-dihydroxyacetophenone **1** as raw material was treated with excessive amounts of 1,2-dibromoethane (**2a**), 1,3-dibromopropane (**2b**), 1,4-dibromobutane (**2c**), 1,5-dibromopentane (**2d**), 1,6-dibromohexane (**2e**), 1,9-dibromononane (**2f**), 1,10-dibromodecane (**2 g**) or 1,12-dibromododecane (**2 h**) in the presence of K_2CO_3 in CH_3CN at 65 °C to get the bromine alkoxy intermediates **3a** ~ **3 h**. Finally, the intermediates **3a** ~ **3 h** were treated with secondary amines (**A** ~ **G**) in the presence of K_2CO_3 in anhydrous CH_3CN at 65 °C to obtain the desired products **TM-1** ~ **TM-30**.

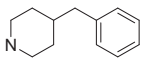
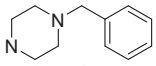
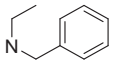
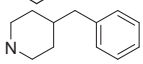
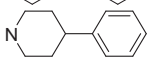
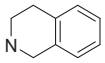
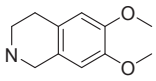
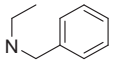
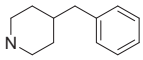
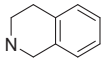
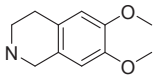
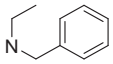
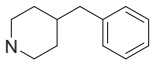
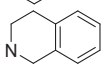
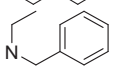
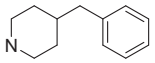
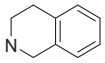
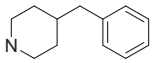
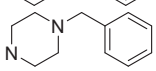
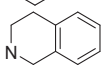
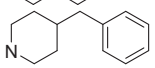
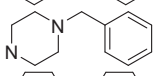
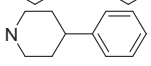
The cholinesterase inhibitory activities of 30 2-acetylphenol-donepezil hybrids were evaluated using the modified Ellman method.[17] The *ee*AChE was from *electric eel* and *eq*BuChE was from *equine serum*. The positive compound donepezil and compound **1** were also tested as control purpose. As shown in Table 1, the target compounds **TM-1** ~ **TM-30** displayed significant *ee*AChE inhibitory activity with IC_{50} values ranging from 35.2 μM to 6.6 μM, implying that introduction of *O*-alkylamine fragment could increase AChE inhibitory activity, which was consist with our design strategy. In addition, these target compounds **TM-1** ~ **TM-30** indicated weak *eq*BuChE inhibitory potency, so the target compounds were selective AChE inhibitors, as similar with donepezil.

According to the screening data in Table 1, the tertiary amine fragment NR^1R^2 and methylene chain length were focused on to explore the structure-active-relationship of AChE inhibitory activity. Firstly, the tertiary amine fragment remarkably affected AChE inhibitory potency, the general tendency was *N*-ethylbenzylamine > 1,2,3,4-tetrahydroisoquinoline > benzylpiperazine > benzylpiperidine > 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline > 4-phenylpiperidine > diethylamine. For example, when the methylene chain length was three, compound **TM-5** with benzylpiperidine fragment showed potent AChE inhibitory activity with IC_{50} value of 11.7 μM, replacing benzylpiperidine of **TM-5** with 4-phenylpiperidine to get compound **TM-6**, the AChE inhibitory activity slightly decreased to 13.4 μM, and then exchanging 4-phenylpiperidine of **TM-6** with 1,2,3,4-tetrahydroisoquinoline to obtain **TM-7**, which showed better AChE inhibitory activity (IC_{50} = 8.9 μM) than compounds **TM-5** and **TM-6**. And further, adding methoxy groups into the 1,2,3,4-tetrahydroisoquinoline of **TM-7** to get compound **TM-8**, the AChE inhibitory potency decreased



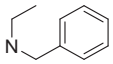
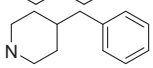
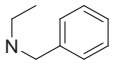
Scheme 1. Synthesis of target compounds **TM-1** ~ **TM-30**. *Reagents and conditions:* (i) $Br(CH_2)_nBr$ (**2a** ~ **2 h**), K_2CO_3, CH_3CN, 65 °C, 6–8 h; (ii) K_2CO_3, NR^1R^2 (**A** ~ **G**) anhydrous CH_3CN, 65 °C, 6–10 h.

Table 1
*ee*AChE and *eq*BuChE inhibitory activities, MAO-A and MAO-B inhibitory potencies of target compounds and control compounds.

Comp.	n	NR^1R^2	IC_{50} (μM)[a]		SI[f]	IC_{50} (μM)[a]	
			*ee*AChE[b]	*eq*BuChE[c]		MAO-A[d]	MAO-B[e]
TM-1	2		16.5 ± 0.82	n.a.[h]	–	n.a.[j]	22.8 ± 0.05
TM-2	2		6.6 ± 0.19	11.2 ± 0.03%[g]	–	26.1 ± 0.07	10.2 ± 0.44
TM-3	2		18.9 ± 0.22	n.a.[h]	–	n.a.[j]	13.8 ± 0.31
TM-4	2		35.2 ± 0.39	n.a.[h]	–	n.a.[j]	11.7 ± 0.20
TM-5	3		11.7 ± 0.18	63.1 ± 0.44	5.4	n.a.[j]	18.7 ± 0.42
TM-6	3		13.4 ± 0.21	29.4 ± 0.18	2.2	24.7 ± 0.09	19.8 ± 0.13
TM-7	3		8.9 ± 0.19	n.a.[h]	–	n.a.[j]	16.7 ± 0.28
TM-8	3		10.1 ± 0.27	57.9 ± 0.99	5.7	8.1 ± 0.01%[j]	13.8 ± 0.16
TM-9	3		7.2 ± 0.13	18.7 ± 0.18	2.6	n.a.[j]	14.9 ± 0.57
TM-10	3		26.7 ± 0.56	n.a.[h]	–	n.a.[j]	16.1 ± 0.19
TM-11	4		8.9 ± 0.17	n.a.[h]	–	n.a.[j]	13.5 ± 0.26
TM-12	4		5.9 ± 0.19	47.5 ± 0.81	8.1	6.3 ± 0.02%[j]	12.8 ± 0.05
TM-13	4		15.9 ± 0.89	56.4 ± 0.91	3.5	n.a.[j]	15.7 ± 0.86
TM-14	4		2.9 ± 0.03	34.6 ± 0.11	11.9	n.a.[j]	16.6 ± 0.43
TM-15	4		20.9 ± 0.19	n.a.[h]	–	n.a.[j]	22.2 ± 0.29
TM-16	5		8.2 ± 0.29	n.a.[h]	–	n.a.[j]	20.8 ± 0.22
TM-17	5		7.5 ± 0.17	n.a.[h]	–	n.a.[j]	15.2 ± 0.36
TM-18	5		5.2 ± 0.12	37.2 ± 0.64	7.1	n.a.[j]	29.8 ± 0.33
TM-19	6		7.1 ± 0.34	66.9 ± 0.49	9.4	22.1 ± 0.17	15.8 ± 0.24
TM-20	6		9.2 ± 0.15	32.6 ± 0.76	3.5	21.2 ± 0.04	26.9 ± 0.36
TM-21	6		16.3 ± 0.11	n.a.[h]	–	n.a.[j]	22.1 ± 0.15
TM-22	9		8.5 ± 0.71	n.a.[h]	–	n.a.[j]	27.9 ± 0.31
TM-23	9		6.7 ± 0.15	19.8 ± 0.24	2.9	19.7 ± 0.17	21.6 ± 0.22
TM-24	9		11.6 ± 0.15	n.a.[h]	–	n.a.[j]	19.4 ± 0.31
TM-25	10		9.2 ± 0.17	13.8 ± 0.06%[g]	–	n.a.[j]	21.6 ± 0.28
TM-26	10		6.9 ± 0.49	15.1 ± 0.02%[g]	–	19.8 ± 0.04	17.3 ± 0.16
TM-27	10		18.4 ± 0.24	0.8 ± 0.01%[g]	–	n.a.[j]	14.2 ± 0.11

(*continued on next page*)

Table 1 (*continued*)

Comp.	n	NR^1R^2	IC_{50} (μM)[a]		SI[f]	IC_{50} (μM)[a]	
			*ee*AChE[b]	*eq*BuChE[c]		MAO-A[d]	MAO-B[e]
TM-28	10		6.5 ± 0.18	50.7 ± 0.31	7.8	n.a.[j]	23.4 ± 0.41
TM-29	12		9.5 ± 0.42	n.a.[h]	–	n.a.[j]	25.8 ± 0.39
TM-30	12		7.7 ± 0.59	38.9 ± 0.67	5.0	n.a.[j]	33.1 ± 0.64
1			n.a.[h]	n.a.[h]		n.t.[i]	n.t.[i]
donepezil			0.019 ± 0.0003	4.76 ± 0.02	251	n.t.[i]	n.t.[i]
rasagiline			n.t.[i]	n.t.[i]	–	0.63 ± 0.01	0.036 ± 0.002
iproniazid			n.t.[i]	n.t.[i]	–	6.18 ± 0.07	1.78 ± 0.01

[a]IC_{50} values represent the concentration of inhibitor required to decrease enzyme activity by 50% and are the mean of three independent experiments, each performed in triplicate (SD = standard deviation). [b]From *electrophorus electricus*. [c]From *equine serum*. [d]From recombinant human MAO-A. [e]From recombinant human MAO-B. [f]SI (selectivity index) = IC_{50}(*eq*BuChE)/IC_{50} (*Ee*AChE). [g]The inhibition percent ratio of compounds for *eq*BuChE at a concentration of 50 μM in the assay conditions. [h]n.a. = no active. Compounds defined "no active" means percent inhibition less than 5.0% at a concentration of 50 μM in the assay conditions. [i]n.t. = no test. [j]n.a. = no active. Compounds defined "no active" means percent inhibition less than 10.0% at a concentration of 10 μM in the assay conditions.

to 10.1 μM. Moreover, ring opening of 1,2,3,4-tetrahydroisoquinoline of **TM-7** to get compound **TM-9** with *N*-ethylbenzylamine, the AChE inhibitory activity increased to 7.2 μM. And further removing benzene ring of *N*-ethylbenzylamine in **TM-9** to gain compound **TM-10** with diethylamine, the AChE inhibitory activity sharply decreased to 26.7 μM. Besides, the compounds **TM-2**, **TM-23** and **TM-26** with benzylpiperazine fragment showed significant AChE inhibitory activity, while exhibited lower inhibitory activity than compound **TM-28** with *N*-ethylbenzylamine. The similar phenomenon were also observed, such as **TM-14** < **TM-12** < **TM-11** < **TM-13** < **TM-15**; **TM-18** < **TM-17** < **TM-16**; **TM-19** < **TM-20** < **TM-21**; **TM-23** < **TM-22** < **TM-24**; **TM-28** < **TM-26** < **TM-25** < **TM-27**; **TM-30** < **TM-29**. Particularly, compound **TM-14** with *N*-ethylbenzylamine fragment exhibited the best AChE inhibitory activity with IC_{50} value of 2.9 μM. Secondly, the methylene chain length also influenced AChE inhibitory activity, in general, the AChE inhibitory activity enhanced as methylene chain increased, but the inflection point was presented in appropriate methylene chain. For example, when the tertiary amine was benzylpiperidine, the optimal methylene chain was 6, **TM-1** (n = 2) > **TM-5** (n = 3) > **TM-11** **(n = 4)** > **TM-16** (n = 5) > **TM-19** (n = 6) < **TM-22** (n = 9) < **TM-25** (n = 10) < **TM-29** (n = 12); when the tertiary amine was *N*-ethylbenzylamine and 1,2,3,4-tetrahydroisoquinoline, the optimal methylene chain was 4, **TM-3** (n = 2) > **TM-9** (n = 3) > **TM-14** (n = 4) < **TM-18** (n = 5) < **TM-28** (n = 10) < **TM-30** (n = 12), **TM-3** (n = 3) > **TM-4** (n = 4) < **TM-17** (n = 5) < **TM-20** (n = 6) < **TM-24** (n = 9). However, when the tertiary amine was diethylamine, the AChE inhibitory potency gradually strengthened as methylene increased, **TM-4** (n = 2) > **TM-10** (n = 3) > **TM-15** (n = 4) > **TM-21** (n = 6), and the optimal methylene was six. Therefore, compound **TM-14** was a potent selective AChE inhibitor (IC_{50} = 2.9 μM), and the selective index was 11.9.

The further kinetic study of compound **TM-14** was performed to explore the inhibition mechanism of AChE.[13] As shown in Fig. 2, the Lineweaver–Burk plots displayed that both inhibitions had rising slopes and increasing intercepts at higher concentration, indicating a mixed-type inhibition.

The docking study was performed to explore possible mechanism of AChE (PDB code: 1EVE) (x: 2.023, y: 63.295, z: 67.062) with compound **TM-14** using AutoDock 4.2 package with Discovery Studio 2.5.[13,16] As shown in Fig. 3, **TM-14** occupied the entire enzymatic catalytic site (CAS), the mid-gorge sites and the peripheral site (PAS), and could simultaneously bind to both the CAS and the PAS. The 2-hydroxy and carbonyl group at the 2,4-dihydroxyacetophenone nucleus formed one

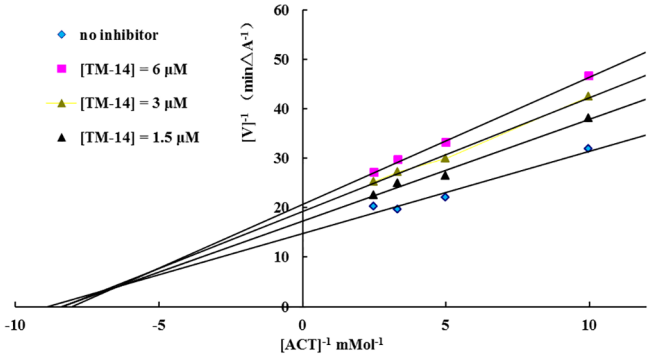


Fig. 2. Steady state inhibition by compound **TM-14** of the AChE hydrolysis of ACh. The plots show mixed-type AChE inhibition for compound **TM-14**.

intramolecular hydrogen bonding, the carbonyl group interacted with Arg289 *via* one intermolecular hydrogen bonding, and the hydroxyl group interacted with Phe288 and Arg289 *via* one intermolecular hydrogen bonding, respectively. In addition, the N atom of *N*-ethylbenzylamine interacted with Tyr121 *via* one intermolecular hydrogen bonding. Moreover, the benzene ring of *N*-ethylbenzylamine could interact with Asp70 via σ-π interaction. Meanwhile, the potential hydrophobic interactions could be observed between the **TM-14** and residues Tyr121, Tyr334, Phe288, Phe331, Arg289, Asp72, Tyr70 and Phe330. Therefore, the molecular docking of **TM-14** provided reasonably explanation for its potent AChE inhibitory activity.

The metal chelation ability of **TM-14** was tested by UV–visual spectrometry using Cu^{2+}, Fe^{2+}, Zn^{2+} and Al^{3+}.[18] As shown in Fig. 4, the electronic spectra of **TM-14** presented a red shift (the peak at 315 nm shifted to 359 nm) after adding $CuCl_2$. However, the electronic spectra of **TM-14** displayed no obvious change after adding $FeSO_4$, $ZnCl_2$ and $AlCl_3$. Therefore, **TM-14** would be a selective metal chelator.

The molar ratio method was performed to test the stoichiometry of the **TM-14**-Cu^{2+} complex by compound **TM-14** with ascending amounts of $CuCl_2$. As shown in Fig. 5, the absorbance linearly increased initially and then plateaued. The two straight lines intersected at a mole fraction of 0.97, meaning a 1:1 stoichiometry for the complex **TM-14**-Cu^{2+}.

Moreover, the inhibitory activities against huMAOs for target compounds **TM-1** ~ **TM-30** were tested by fluorimetric assay.[19] Rasagiline and iproniazid were also tested as control drugs. The results were shown in Table 1, all the target compounds showed moderate hMAO-B inhibitory activity with IC_{50} values ranging from 10.2 μM to 33.1 μM,

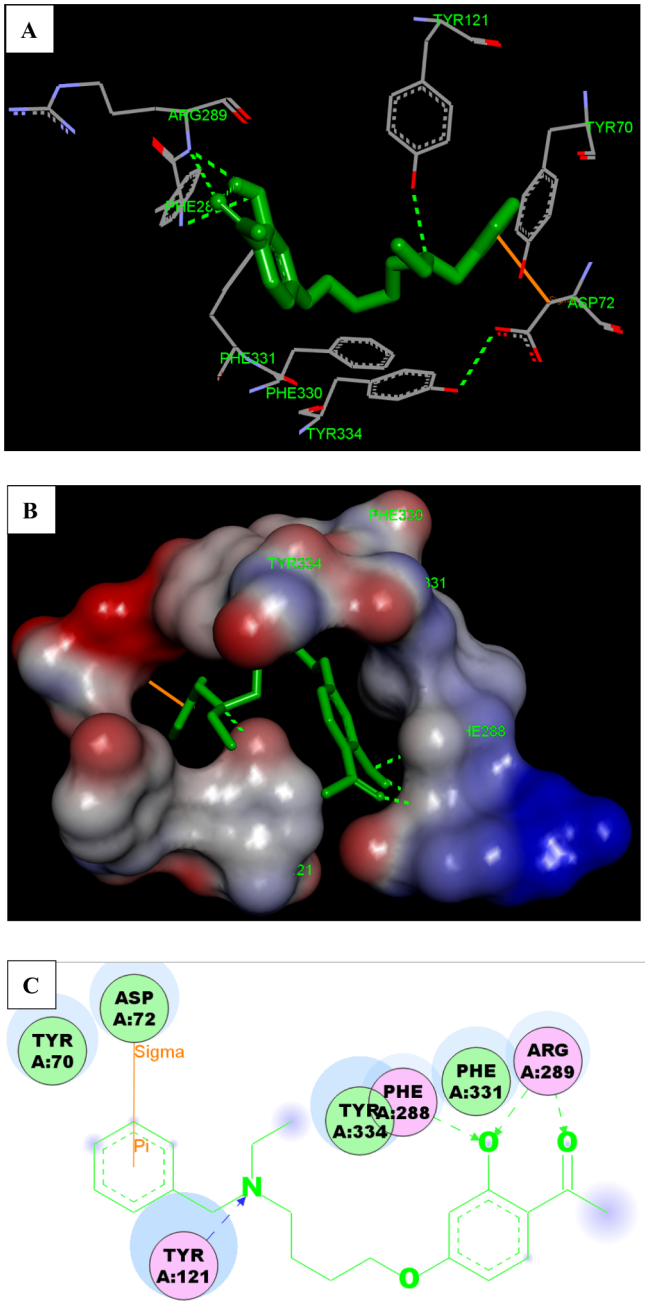


Fig. 3. (A) Representation of compound **TM-14** (green stick) interacted with residues in the binding site of *Tc*AChE (PDB code: 1EVE). (B) 3D docking model of compound **TM-14** with *Tc*AChE. (C) 2D schematic diagram of docking model of compound **TM-14** with *Tc*AChE.

and indicated lower hMAO-B inhibitory potency than the referenced compounds. It meant that the *O*-alkylamine fragment sharply decreased hMAO-B inhibitory activity. In addition, the tertiary amine groups and methylene chain length led to slightly influence on hMAO-B inhibitory potency. In general, when the methylene chain was 2, the tertiary amine inhibition tendency was benzylpiperazine > diethylamine > *N*-ethylbenzylamine > benzylpiperidine, such as **TM-2** < **TM-4** < **TM-3** < **TM-1**; when the methylene chain was 4, 1,2,3,4-tetrahydroisoquinoline > benzylpiperidine > 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline > *N*-ethylbenzylamine > diethylamine. Meanwhile, it appeared that the compounds with n = 2 showed better hMAO-B inhibitory activity than the other compounds (n = 3, 4, 5, 6, 9, 10, 12), indicating that the extended methylene chain produced

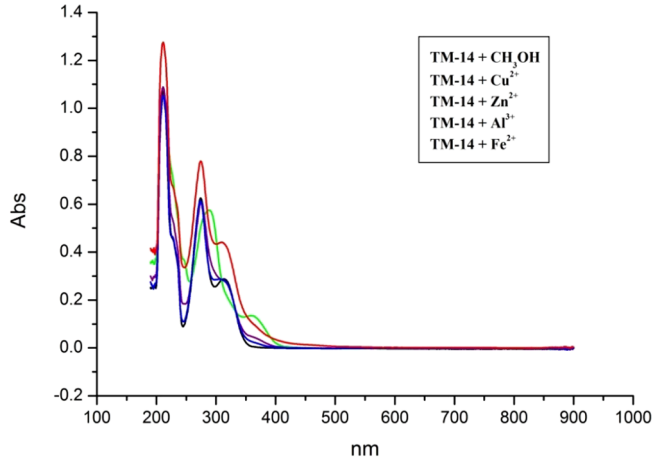


Fig. 4. The UV spectrum of compound **TM-14** (37.5 μM, in methanol) alone or in the presence of $CuCl_2$, $FeSO_4$, $ZnCl_2$ and $AlCl_3$ (37.5 μM, in methanol).

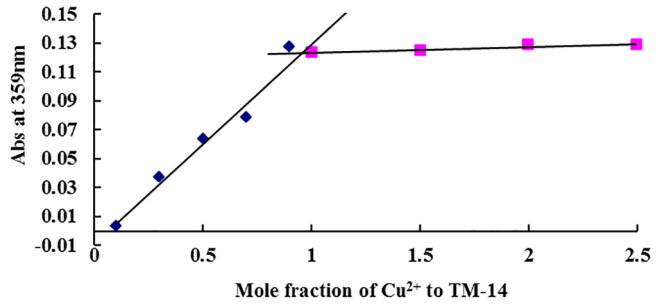


Fig. 5. Determination of the stoichiometry of complex-Cu^{2+} was used molar ratio method by titrating the methanol solution of compound **TM-14** with ascending amounts of $CuCl_2$. The final concentration of tested compound was 37.5 μM, and the final concentration of Cu^{2+} ranged from 3.75 to 93.75 μM.

negative effect on hMAO-B inhibitory activity. Especially, the compound **TM-2** with benzylpiperazine and n = 2 exhibited the best hMAO-B inhibitory activity with IC_{50} value of 10.2 μM. Furthermore, all the target compounds displayed weak hMAO-A inhibitory activity, implying that the target compounds were selective hMAO-B inhibitors, which was beneficial for the treatment of AD.

In order to explore mechanism of **TM-2** with MAO-B, docking study was performed based on the X-ray crystal structures of human MAO-B (PDB code: 2V60) (x: 14.846, y: 128.673, z: 24.971).[20] As shown in Fig. 6, the 2-hydroxyacetophenone nucleus was located in the *hu*MAO-B binding pocket, 2-hydroxy and carbonyl group formed one intramolecular hydrogen bonding. In addition, the carbonyl group could interact with the enzymatic cofactor FAD *via* intermolecular hydrogen bonding. Moreover, the benzene of 2-hydroxyacetophenone nucleus could interact with Tyr398 *via* one π-π interaction. Meanwhile, some hydrophobic interactions were observed between **TM-2** and Trp119, Ile316, Ile199, Phe103, Ile198, Leu171, Ieu167, Phe343, Gln206 and Tyr398. Therefore, the above interactions observed could explain the potent MAO-B inhibitory activity for **TM-2**.

To test the brain penetration of compounds **TM-14** and **TM-2**, a parallel artificial membrane assay for the blood- brain barrier (PAMPA-BBB) was performed.[21,22] The assay was validated by comparing the permeability of 11 commercial drugs with reported values (Table 2). A good linear correlation, P_e (exp) = 0.9163P_e (bibl.) − 0.2247 (r^2 = 0.9558) was produced (Fig. 7). Based on this equation, and considering the limit conditions established by Di et al., we determined that compound with permeability above 3.44×10^{-6} cm/s could cross the blood–brain barrier (Table 3). According to the measured permeability (Table 4), **TM-14** and **TM-2** could cross the BBB *in vitro*.

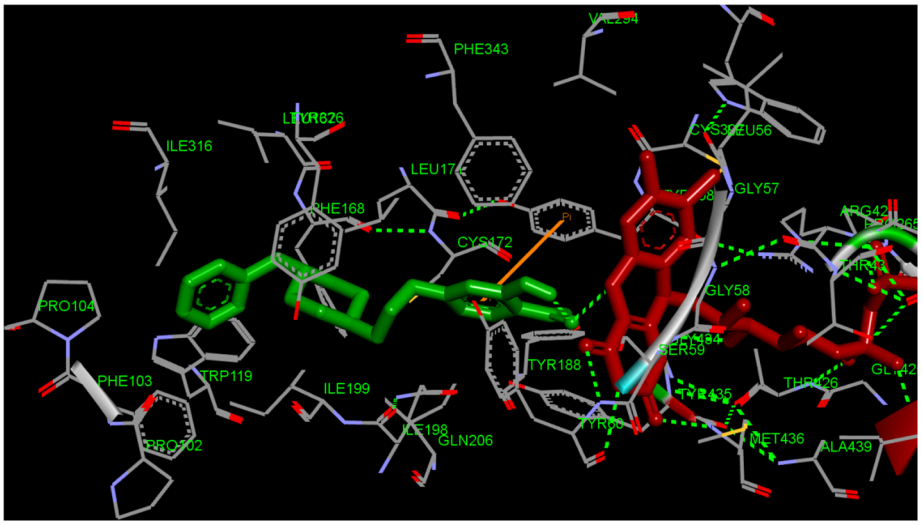


Fig. 6. Compound **TM-2** (green stick) interacting with residues in the binding site of MAO-B (PDB code: 2V60), highlighting the protein residues that participate in the main interactions with the inhibitor.

Table 2
Permeability ($P_e \times 10^{-6}$ cm/s) in the PAMPA-BBB assay for 11 commercial drugs used in the experiment validation.

Commercial drugs	Bibl[a]	PBS:EtOH(70:30) [b]
Verapamil	16	16.90
Oxazepam	10	9.60
Diazepam	16	11.86
Clonidine	5.3	5.10
Imipramine	13	10.10
Testosterone	17	16.30
Caffeine	1.3	1.28
Enoxacine	0.9	0.47
Piroxicam	2.5	0.72
Norfloxacin	0.1	0.42
Theophylline	0.12	0.10

[a] Taken from Ref. 21.
[b] Data are the mean ± SD of three independent experiments.

Table 3
Ranges of permeability of PAMPA-BBB assays ($P_e \times 10^{-6}$ cm/s).

Compounds of high BBB permeation (CNS +)	$P_e > 3.44$
Compounds of uncertain BBB permeation (CNS +/−)	$3.44 > P_e > 1.61$
Compounds of low BBB permeation (CNS −)	$P_e < 1.61$

Table 4
Permeability P_e ($\times 10^{-6}$ cm/s) in the PAMPA-BBB assay of the selected compounds **TM-2** and **TM-14** and the predictive penetration in the CNS.

Compound[a]	P_e ($\times 10^{-6}$ cm/s)[b]	Prediction
TM-14	15.13 ± 0.29	CNS +
TM-2	17.37 ± 0.83	CNS +

[a] Compounds **TM-14** and **TM-2** was dissolved in DMSO at 5 mg/mL and diluted with PBS/EtOH (70:30). The final concentration of the compound was 100 μg/mL.
[b] Values are expressed as the mean ± SD of three independent experiments.

Briefly, a series of 2-acetylphenol-donepezil hybrids was developed as multi-function agents. The structure-active-relationship showed that both tertiary amines groups and methylene chain length remarkably affected AChE inhibitory activity, in particular, compound **TM-14** showed the best eeAChE inhibitory potency with IC_{50} value of 2.9 μM, in addition, both the kinetic analysis and docking study exhibited that **TM-14** was a mixed-type inhibitor, and could simultaneously bind to both CAS and PAS of AChE. And compound **TM-14** served as a selective metal chelator. Moreover, all the target compounds displayed moderate hMAO-B inhibitory activity, the extended methylene chain decreased hMAO-B inhibitory potency, and the tertiary amine did not produce positive effect, compound **TM-2** showed good hMAO-B inhibitory activity with IC_{50} value of 10.2 μM. Furthermore, compounds **TM-2** and

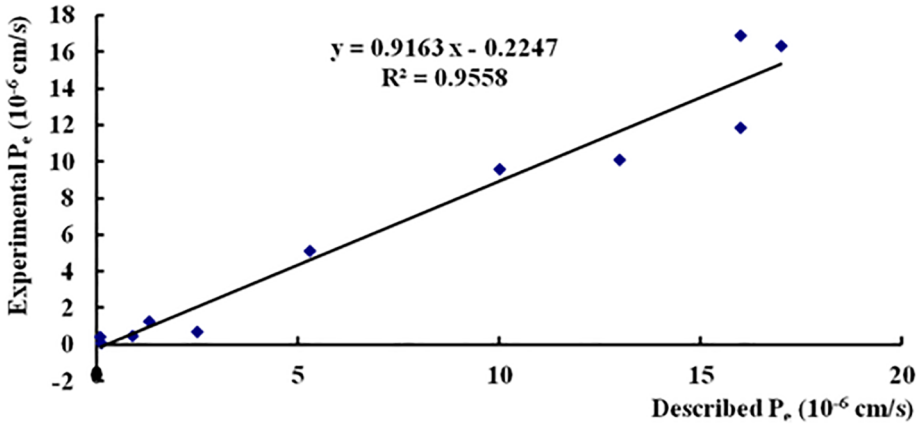


Fig. 7. Linear correlation between experimental and reported permeability of commercial drugs using the PAMPA-BBB assay. P_e(exp) = 0.9163, P_e(bibl.) −0.2247 (r^2 = 0.9558).

TM-14 could cross BBB *in vitro*. Therefore, the structure-active-relationship of 2-acetylphenol-donepezil hybrids encouraged the discovery of selective AChE inhibitors or selective hMAO-B inhibitors, and improved the development of multifunction agents for the treatment of AD.

Declaration of Competing Interest

The authors declare no competing financial interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2019.126625.

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