Development of Novel 2-Acetylphenol-O-Alkylhydroxyethylamine Derivatives as Multifunctional Agents for Alzheimer's Disease Treatment

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Abstract

Due to the complex pathogenesis of AD, the multi-target-directed ligands (MTDLs) strategy presented the best pharmacological option for AD treatment. Herein, a series of novel 2-acetylphenol-*O*-alkylhydroxyethylamine derivatives was rationally designed and synthesized. Of these derivatives, **5c** was a good multifunctional agent (eeAChE IC_{50} = 7.9 μ M, MAO-B IC_{50} = 9.9 μ M, BACE1 IC_{50} = 8.3 μ M) *in vitro* and displayed a mixed-type AChE inhibition, which could bind to the CAS and PAS of AChE. Compound **5c** also exhibited good antioxidant activity (ORAC = 2.5 eq) and neuroprotective effects. Furthermore, compound **5c** was a selective metal ions chelator. And it could cross blood-brain barrier *in vitro* and complied with drug-like properties rule of 5. Therefore, compound **5c** was a promising multifunctional agent for the treatment of AD.

Introduction

Alzheimer's disease (AD), the utmost common form of dementia, is one of the most complex neurodegenerative disorders, characterized by progressive cognitive decline and behavioral disorders throughout the disease course [1]. Current data exhibits that more than 50 million dementia patients worldwide, and the figure will increase to 152 million by 2050 [2]. The disease course is divided into four stages with a progressive pattern of cognitive decline: preclinical stage, mild cognitive impairment (MCI) stage, moderate stage and severe stage [3]. Up to now, no treatments stop or reverse its progression, through some may temporarily improve symptoms. Furthermore, the failures of the current phase III trials of bapineuzumab and solanezumab offer lessons for the development of $A\beta$ immunotherapies. Anti-amyloid therapies should be given in the early stage of the disease as a preventative measure [4].

Although the cause of AD is poorly understood, scientists accept that low levels of acetylcholine (ACh), accumulation of beta amyloid ($A\beta$), hyperphosphorylated tau protein and loss of neurons serve as crucial role in the progression of AD, and propose several hypotheses, such as cholinergic hypothesis, amyloid cascade hypothesis, metals hypothesis, tau hypothesis, oxidative stress hypothesis, and so on [5].

The cholinergic hypothesis states that ACh is responsible for the learning and memory. In the brain of AD patients, the ACh levels significantly reduces in the hippocampus and the neocortex regions, which directly contributes to cognitive decline. Investigations show that the high levels of acetylcholinesterase (AChE) facilitate the hydrolysis of ACh leading to memory disorder in the early stages of AD [6]. In progressed AD, level of AChE in brain reduces to 55–67% of normal values while butyrylcholinesterase (BuChE), an enzyme closely related to AChE, increases to 120% of normal levels, demonstrating that BuChE takes over the hydrolysis of ACh in the late stage of AD [7]. So, selective inhibiting AChE still be the promising strategy for the early stages of AD.

The amyloid hypothesis states that accumulation of $A\beta$ is the triggering event in the pathogenesis of AD and it further facilitates the form of tau-related neurofibrillary tangles, neuroinflammation, neuronal

degeneration and death [8, 9]. Studies show that the β -site amyloid precursor protein cleaving enzyme 1 (BACE1) initiates the production of $A\beta$ and is a prime target for AD [10]. Several BACE1 inhibitors, such as verubecestat, elenbecestat, lanabecestat and atabecestat have been evaluated in different clinical stages [11, 12]. Thus, inhibition of BACE-1 would be a potent therapeutic strategy for the early stage of AD by reducing the production of $A\beta$.

Metal ion hypothesis states that the dyshomeostasis of metal ions, especially Cu^{2+} , Zn^{2+} , Fe^{2+} , accelerates the formation of metal-induced $A\beta$ aggregates and causes neurofibrillary tangles (NFTs) formation and produces reactive oxygen species (ROS) in the brain [13–15]. Thus, modulation of metal ions in the brain could be useful for treating AD.

In addition, monoamine oxidase-B (MAO-B), one of the flavoproteins present in the outer mitochondrial membranes of neuronal cells, significantly increases up to 3-fold in the parietal and frontal cortex of AD patients [16]. Selective inhibition of MAO-B could improve AD symptoms. Rasagiline, a selective MAO-B inhibitor, is in Phase II clinical trial with mild to moderate AD [17]. Therefore, selective MAO-B inhibitors provide a potential way to treat AD.

The multitarget-directed ligand strategy (MTDLs) has been employed to tackle the multifactorial nature of AD and the encouraging results have convinced researchers that MTDLs display the best pharmacological option for AD treatment [18–20]. Several MTDLs candidate drugs, such as ALZT-OP1, leuco methylthioninium, masitinib mesylate, blarcamesine hydrochloride and troriluzole hydrochloride have reached testing stage in clinical phase II/III trials (Fig. 1) [12].

2-Acetylphenol skeleton is a potent reversible MAO inhibitor, especially, 2-acetyl-5-alkoxyphenol analog **DDDT-15** (Fig. 1) is a potent selective MAO-B inhibitor [21]. Based on this, our previous work has developed a series of 2-acetylphenol-*O*-alkylamine derivatives as multifunctional agents for the treatment of AD, and the lead compound **BMCL-14** displayed selective MAO-B inhibitory activity, metal ions chelation properties and AChE inhibitory potency [22, 23]. Furthermore, hydroxyethylamine, an essential functional group in BACE1 inhibitors, is able to interact through hydrogen bonds and ion pairs with the Asp dyad of BACE enzyme [24]. Thus, a series of novel 2-acetylphenol-*O*-alkylhydroxyethylamine derivatives were designed by introducing hydroxyethylamine fragment into 2-acetylphenol skeleton using MTDLs strategy (Fig. 2). And then the target derivatives were evaluated by inhibition of ChEs (AChE and BuChE) and MAOs (MAO-A and MAO-B), metal chelation and BACE1 inhibitory activity. They were found to show potentially applicable biological activities, including inhibition of AChE/BuChE and MAO-A/MAO-B, antioxidant properties, neuroprotective effects, and metal chelation.

Results And Discussion

Chemistry

Total 14 2-acetylphenol-*O*-alkylhydroxyethylamine derivatives were rationally designed and synthesized as presented in Scheme 1. And the yield of target derivatives were above 78%. Briefly, the key intermediates **3a** and **3b** were obtained by the starting material 2',4'-dihydroxyacetophenone **1** with excessive amounts of (*R*)-(-)-epichlorohydrin **2a** or (*S*)-(+)-epichlorohydrin **2b** in the presence of K₂CO₃ in anhydrous CH₃CN under reflux. After that, the target compounds **5a-g** were got by the reaction of intermediates **3a** with secondary amines NR¹R² (4-benzylpiperidine, **4a**; 1-benzylpiperazine, **4b**; 1-isopropylpiperazine, **4c**; 2-(piperazin-1-yl)pyrimidine, **4d**; piperidine, **4e**; *N*-benzylethanamine, **4f**; diethylamine, **4g**) in the presence of K₂CO₃ in anhydrous CH₃CN under reflux. Similarly, target compounds **6a-g** were obtained by the reaction of **3b** with NR¹R² **4a-g**. The synthetic route for accessing target compounds was outlined in Scheme 1. The target compounds were purified by chromatography, and their structures were confirmed by ¹H NMR, ¹³C NMR and ESI-MS.

Biological activity

AChE and BuChE inhibition studies

The inhibitory potency of 2-acetylphenol-*O*-alkylhydroxyethylamine derivatives against AChE (*rat*AChE, from rat cortex homogenate; *ee*AChE, from electric eel) and BuChE (*rat*BuChE, from rat serum; *eq*BuChE, from equine serum) were measured by Ellman's method [25, 26]. FDA-approved AChE inhibitors donepezil was used as a reference compound. The results were summarized in Table 1. The activity of these derivatives were expressed as IC₅₀. Firstly, the target compounds were evaluated by *rat*AChE and *rat*BuChE. The compounds showed moderate to good *rat*AChE inhibitory activity and moderate *rat*BuChE inhibitory activity. In particular, compound **5c** showed good *rat*AChE inhibitory potency with IC₅₀ value of 10.7 μM. Generally, the compounds with *R* configuration displayed slightly better *rat*AChE inhibitory activity than the compounds with *S* configuration. Moreover, the secondary amine fragment also affected *rat*AChE inhibitory activity. Under the condition with the same configuration, the potency to inhibit *rat*AChE were in the order: 1-isopropylpiperazine (**5c**) > *N*-benzylethanamine (**5f**) > 4-benzylpiperidine (**5a**) > diethylamine (**5g**) > 1-benzyl piperazine (**5b**) > piperidine (**5e**) > 2-(piperazin-1-yl) pyrimidine (**5d**). Further, we re-evaluated the target compounds using *ee*AChE and *eq*BuChE and explored the structure-activity-relationship. All the target compounds showed moderate to good AChE inhibitory activity and weak BuChE inhibitory potency. Particularly, compound **5c** presented the best *ee*AChE inhibitory activity with IC₅₀ value of 7.9 μM and moderate *eq*BuChE inhibitory activity (IC₅₀ = 31.7 μM). In addition, the *R*/*S* configuration and secondary amine fragment significantly affected the AChE inhibitory activity. The target derivatives (**5a** ~ **5g**) with *R* configuration showed better *ee*AChE inhibitory potency than the derivatives (**6a** ~ **6g**) with *S* configuration. Under both the condition of *R* configuration and *S* configuration, the secondary amine fragment of side chain also affected *ee*AChE inhibitory activity. Compound **5a** with 4-benzylpiperidine exhibited good *ee*AChE inhibitory activity with IC₅₀ value of 9.7 μM. Replacing 4-benzylpiperidine of **5a** with 1-benzylpiperazine to obtain compound **5b**, the *ee*AChE inhibitory

activity was 11.2 μM . And then replacing 1-benzylpiperazine of **5b** with 1-isopropylpiperazine to get compound **5c**, the *ee*AChE inhibitory activity was 7.9 μM . While, when 4-benzylpiperidine of **5a** was replaced by 2-(piperazin-1-yl) pyrimidine, piperidine, *N*-benzylethanamine and diethylamine to get compounds **5d**, **5e**, **5f** and **5g**, respectively, all of them showed weak inhibitory activity. Taken together, compound **5c** exhibited the best *ee*AChE inhibitory potency with IC_{50} value of 7.9 μM .

Table 1 The AChE/BuChE inhibitory activity of the target derivatives **5a~5g**, **6a~6g** and donepezil.

Compound	NR ¹ R ²	$\text{IC}_{50} \pm \text{SD} (\mu\text{M})^a$		SI ^d	$\text{IC}_{50} \pm \text{SD} (\mu\text{M})^a$		SI ^d
		<i>Rat</i> AChE ^b	<i>Rat</i> BuChE ^c		<i>Ee</i> AChE ^e	<i>Eq</i> BuChE ^f	
5a		25.7 $\pm$ 1.3	20.9 $\pm$ 0.58	0.8	9.7 $\pm$ 0.31	56.2 $\pm$ 1.7	5.8
5b		39.7 $\pm$ 2.5	37.6 $\pm$ 1.8	0.9	11.2 $\pm$ 0.53	74.6 $\pm$ 2.8	6.7
5c		10.7 $\pm$ 0.67	23.4 $\pm$ 1.5	2.2	7.9 $\pm$ 0.26	31.7 $\pm$ 1.3	4.0
5d		104 $\pm$ 3.1	96 $\pm$ 2.1	0.9	41.4 $\pm$ 1.6	n.a. ^g	---
5e		89 $\pm$ 1.8	n.a. ^g	---	38.2 $\pm$ 0.89	n.a. ^g	---
5f		20.4 $\pm$ 0.81	69.2 $\pm$ 0.88	3.4	15.7 $\pm$ 0.62	59.8 $\pm$ 2.2	3.8
5g		29.8 $\pm$ 0.95	n.a. ^g	---	28.1 $\pm$ 1.2	n.a. ^g	---
6a		32.1 $\pm$ 0.86	36.9 $\pm$ 1.5	1.1	16.8 $\pm$ 0.64	46.2 $\pm$ 1.1	2.8
6b		41.7 $\pm$ 1.9	55.3 $\pm$ 3.3	1.3	26.2 $\pm$ 1.4	59.3 $\pm$ 2.2	2.3
6c		12.8 $\pm$ 0.97	19.7 $\pm$ 0.69	1.5	19.2 $\pm$ 0.89	36.2 $\pm$ 3.1	1.9
6d		n.a. ^g	n.a. ^g	---	n.a. ^g	n.a. ^g	---
6e		n.a. ^g	n.a. ^g	---	37.4 $\pm$ 1.2	66.2 $\pm$ 2.9	1.8
6f		37.6 $\pm$ 1.7	60.8 $\pm$ 2.6	1.6	35.1 $\pm$ 2.6	n.a. ^g	---
6g		59.3 $\pm$ 1.8	63.6 $\pm$ 2.3	1.1	31.8 $\pm$ 2.6	n.a. ^g	---
donepezil		0.014	21.3	1521	0.015	4.7	313

^a IC_{50} values: 50% inhibitory concentration (means $\pm$ SD of three experiments). ^b From 5% rat cortex homogenate. ^c From rat serum. ^d SI = Selectivity Index = IC_{50} (BuChE) / IC_{50} (AChE). ^e From electric eel. ^f From equine serum. ^g n.a. = no active. Compounds defined "no active" means that inhibition is less than 10.0% at a concentration of 50 μM in the assay condition.

Kinetic studies of AChE inhibition.

According to the above results, the representative compound **5c** was chosen to carry out a kinetic study using *ee*AChE [25]. As shown in Fig. 3, the reciprocal Lineweaver-Burk plots displayed that both increasing slopes (decreased V_{max}) and intercepts (higher K_m) at increasing concentration of **5c**, revealing a mixed-type inhibition. Compound **5c** was able to bind to both catalytic active site (CAS) and peripheral anionic site (PAS) of AChE or was able to bind to both free and acylated-AChE [26].

Antioxidant Activity.

The antioxidant potency of all the target compounds were assessed by oxygen radical absorbance capacity fluorescein (ORAC-FL) method using Trolox as a reference compound [27]. As indicated in Table 2, all the target compounds showed good antioxidant activity and ORAC values ranging from 1.9 *eq* to 2.6 *Eq*. According to the screening data, the *R/S* configuration did not obviously influence the antioxidant potency. It was noted that the compounds (**5b**, 2.5 *eq*; **6b**, 2.6 *eq*.) with 1-benzylpiperazine fragment and compounds (**5c**, 2.5 *eq*; **6c**, 2.4 *eq*;) with 1-isopropylpiperazine fragment displayed better antioxidant activity than other compounds.

Table 2
The antioxidant activity, MAO-A/MAO-B inhibitory potency and BACE1 inhibitory activity of target compounds and referenced compounds.

Compound	ORAC ^a	IC ₅₀ ± SD (μM) ^b		BACE1
		huMAO-A	huMAO-B	IC ₅₀ /μM ^d
5a	2.1 ± 0.05	n.a. ^c	16.1 ± 0.32	5.6 ± 0.28
5b	2.5 ± 0.03	n.a. ^c	13.6 ± 0.58	10.7 ± 0.66
5c	2.5 ± 0.04	n.a. ^c	9.9 ± 0.79	8.3 ± 0.71
5d	1.9 ± 0.06	16.1 ± 0.38	14.9 ± 0.41	n.d. ^e
5e	2.1 ± 0.02	n.a. ^c	12.2 ± 0.49	n.d. ^e
5f	2.0 ± 0.07	n.a. ^c	8.9 ± 0.32	13.1 ± 0.49
5g	2.1 ± 0.06	n.a. ^c	23.3 ± 0.49	n.d. ^e
6a	2.0 ± 0.02	n.a. ^c	14.7 ± 0.55	n.d. ^e
6b	2.6 ± 0.05	n.a. ^c	11.7 ± 0.39	n.d. ^e
6c	2.4 ± 0.02	n.a. ^c	9.1 ± 0.28	n.d. ^e
6d	2.0 ± 0.04	n.a. ^c	12.7 ± 0.57	n.d. ^e
6e	1.9 ± 0.03	n.a. ^c	10.1 ± 0.65	n.d. ^e
6f	2.1 ± 0.05	n.a. ^c	10.8 ± 0.44	n.d. ^e
6g	1.9 ± 0.06	n.a. ^c	13.2 ± 0.29	n.d. ^e
rasagiline	n.d. ^e	0.67 ± 0.05	0.035 ± 0.002	n.d. ^e
donepezil	n.d. ^e	n.d. ^e	n.d. ^e	0.21 ± 0.01

^a Results are expressed as μM of Trolox equivalent/μM of tested compound. ^b IC₅₀: 50% inhibitory concentration (means SD of three experiments). ^c n.a. = no active. Compounds defined "no active" means that inhibition is less than 5.0% at a concentration of 10 μM in the assay condition. ^d Values represent the mean values of at least two determinations. ^e n.d = not determined

Monoamine oxidase inhibition studies.

The inhibitory potency of target 2-acetylphenol-*O*-alkylhydroxyethylamine derivatives against human MAO-A and MAO-B was assessed by a fluorimetric method with rasagiline as a positive compound [28].

The results were summarized in Table 2. Most of the target compounds showed good MAO-B inhibitory activity and weak MAO-A inhibitory potency, indicating most of the synthesized compounds were selective MAO-B inhibitors, except compound **5d**, which was a dual MAO-A/MAO-B inhibitor. The data also displayed that the compounds (**6a ~ 6g**) with *S* configuration showed slightly better MAO-B inhibition than the compounds (**5a ~ 5g**) with *R* configuration. In addition, the secondary amine also affected the MAO-B inhibitory activity. Among the target compounds, compound **5f** presented the best MAO-B inhibitory activity with IC₅₀ value of 8.9 μM and the representative compound **5c** displayed good MAO-B inhibitory potency with IC₅₀ value of 9.9 μM.

BACE1 inhibition assay.

According to the results from AChE/BuChE inhibition and MAO-A/MAO-B inhibition, the compounds **5a**, **5b**, **5c** and **5f** were selected to evaluate BACE1 inhibitory effects by fluorometric assay with donepezil (IC₅₀ = 0.21 μM) as positive control [29]. As exhibited in Table 2, compounds **5a**, **5b**, **5c** and **5f** displayed modest BACE1 inhibitory potency with IC₅₀ values of 5.6 μM, 10.7 μM, 8.3 μM and 13.1 μM, respectively.

Metal chelation studies.

The metal ions (Cu²⁺, Zn²⁺, Fe²⁺ and Al³⁺) chelation ability of compound **5c** was tested using UV-visual spectrometry [27, 28]. As presented in Fig. 4A, the characteristic absorption peak of **5c** displayed a red shift from 314 nm to 360 nm after adding CuCl₂. Whereas, the electronic spectra of compound **5c** presented no obvious change after treating with AlCl₃, FeSO₄ and ZnCl₂, respectively. The results revealed that compound **5c** was a selective Cu²⁺ chelator.

The molar ratio method was employed to assess the stoichiometry of the **5c**-Cu²⁺ complex. The UV spectra was treated by numerical subtraction of CuCl₂ and **5c** at different concentrations at 360 nm. As exhibited in Fig. 4B, the two linearly straight lines intersected at a mole fraction of 1.96, revealing a 1:2 stoichiometry for the **5c**-Cu²⁺ complex. The *o*-hydroxyacetophenone structure of **5c** may bind to Cu²⁺ [29], and the hydroxyethylamine structure of **5c** may also bind to Cu²⁺ [30].

Docking studies on AChE and MAO-B.

Compound **5c** was docked into *Tc*AChE (code ID: 1EVE) and *h*MAO-B (code ID: 2V60) to explore the possible binding mode and to explain the reasons for multiple potencies. As shown in Fig. 5, the docking results displayed that compound **5c** occupied the entire enzymatic CAS, the mid-gorge sites and the PAS and could simultaneously bind to both the CAS and PAS. In the *Tc*AChE-**5c** complex, the hydroxyl and carbonyl group at the 2-acetylphenol skeleton formed a intramolecular hydrogen bond. The hydroxyl group of 2-acetylphenol interacted with residue Tyr130 via two intermolecular hydrogen bond interactions. The carbonyl group of 2-acetylphenol fragment interacted with key active residue Gly117 and Tyr130 via one intermolecular hydrogen bond, respectively. The benzene ring of 2-acetylphenol fragment also interacted with key active residue Trp84 via two π-π interactions. In addition, the hydroxyl

group of the hydroxyethylamine group could interact with residue Gln69 via one intermolecular hydrogen bond interaction. Furthermore, compound **5c** could also interact with Trp84, Asp72, Tyr334, and Tyr70 through hydrophobic interaction. In addition, the known AChE inhibitor donepezil was also re-docked with *TcAChE*, the characteristic interaction were also observed in Fig. 5D, such as the benzene ring of 4-benzylpiperidine also interacted with key active residue Trp84 via two π - π interactions, and the benzene ring of indanone interacted with key active residue Phe331 via one π - π interaction.

In **5c**-*hMAO-B* complex (Fig. 6A), the hydroxyl and carbonyl group at the 2-acetylphenol skeleton formed a intramolecular hydrogen bond. The hydroxyl group of 2-acetylphenol interacted with residues Glu84 and Thr314 via intermolecular hydrogen bond interaction, respectively. The carbonyl group of 2-acetylphenol fragment interacted with residues Cys312 and Thr314 via intermolecular hydrogen bond interaction, respectively. The oxygen atom of *O*-alkyl fragment interacted with residue Tyr326 via one intermolecular hydrogen bond interaction. Moreover, the N atom of the 1-isopropylpiperazine fragment interacted with residue Thr201 via one intermolecular hydrogen bond interaction. Furthermore, some hydrophobic interactions were presented between compound **5c** and important active residues Phe99, Gly101, Pro102, Ile199, Leu88, Ser200, Thr202, Glu84, Cys312, Pro104, Thr314, Thr201 and Tyr326. Furthermore, in order to compared with the known selective MAO-B inhibitor 7-(3-chlorobenzyloxy)-4-carboxaldehyde-coumarin, the other docking was performed. The results were displayed in Fig. 6B, the carbonyl group of coumarin fragment interacted with key residues Cys312 and Glu84 via one intermolecular hydrogen bond interaction, respectively. The *O* atom of coumarin interacted with residue Thr314 via one intermolecular hydrogen bond interaction. The benzene ring interacted with important amino acid Ser200 via sigma- π interaction.

Neuroprotective effects against H₂O₂ –induced PC12 cell injury.

Compound **5c** was selected to assess the neuroprotective effect against H₂O₂-induced PC12 cell injury using Vitamine E (VE) as positive control [28]. According to the data in Fig. 7A, compound **5c** did not show obvious cytotoxicity until the concentration increased up to 50 μ M. As displayed in Fig. 7B, when PC12 cells were treated with 100 μ M H₂O₂, the cell viability significantly declined to 48.9% ($p < 0.01$) vs normal group. When adding 100 μ M VE, the cell viability increased to 67.3 % ($p < 0.01$). Moreover, when the PC12 cells were treated with 10 μ M and 50 μ M compound **5c**, respectively, the cell viability was 63.1% ($p < 0.05$) and 75.8% ($p < 0.01$), respectively. The high dose (50 μ M) of **5c** presented better neuroprotective effect than VE, revealing that compound **5c** exhibited potent neuroprotective effect against H₂O₂-induced PC12 cell injury.

In Vitro Blood – Brain Barrier Permeation Assay.

The ability of **5c** to cross blood-brain barrier was assessed by the parallel artificial membrane permeation assay of the blood-brain barrier (PAMPA-BBB) [31, 32]. Compound **BMCL-14** acted as a positive compound. 11 standard drugs were used as the references in our previous work. The following ranges of permeability (P_e 10⁻⁶ cm/s) had been established in our previous work: $P_e > 3.44$ for compounds with

predicted high BBB permeation, $P_e \leq 1.61$ for compounds with predicted low BBB permeation, $3.44 \geq P_e > 1.61$ for compounds with uncertain BBB permeation. As shown in Table 3, compared with **BMCL-14**, compound **5c** presented 5.78×10^{-6} cm/s permeability, revealing that compound **5c** could cross the BBB via passive diffusion.

Table 3
Permeability P_e ($\times 10^{-6}$ cm/s) of compound **5c**
and its predictive penetration in the CNS.

compound ^a	P_e ($\times 10^{-6}$ cm/s) ^b	prediction
BMCL-14	15.79 ± 0.67	CNS+
5c	5.78 ± 0.37	CNS+

^a Compounds **BMCL-14** and **5c** was dissolved in DMSO at 5 mg/mL and diluted with PBS/EtOH (70:30). The final concentration of compounds was 100 μ g/mL.

^b Values are expressed as the mean $\pm$ SD of three independent experiments.

Theoretical evaluation of ADME properties.

The Molinspiration property program was employed to evaluate the druglike properties of **5c**, the items including log P , MW, topological polar surface area (TPSA), the number of hydrogen-bond acceptors, and the number of hydrogen-bond donors [33]. According to the screening data in Table 4, **5c** complied with the Lipinski's rule of 5, which might be a promising candidate compound.

Table 4
Theoretical prediction of the ADME properties of compound **5c**

Comp.	Log P	MW	TPSA ($\text{\AA}^2$)	n-ON	n-OH/NH	nviolations	nrotb	volume ($\text{\AA}^3$)
5c	1.76	336.4	73.24	6	2	0	7	326.92

Conclusion

In conclusion, AD is a complex neurodegenerative disorders with poorly understood pathogenesis. The MTDLs strategy has been considered as the best pharmacological option for AD treatment. Thus, a series of novel 2-acetylphenol-*O*-alkylhydroxyethylamine derivatives was rationally designed based on the MTDLs strategy. The synthesized derivatives were assessed by biological activity evaluation *in vitro* including AChE/BuChE inhibition, antioxidant, MAO-A/MAO-B inhibition, BACE1 inhibition and neuroprotective effects. The results revealed that compound **5c** was a good *ee*AChE inhibitor ($IC_{50} = 7.9$ μ M) and showed a mixed-type AChE inhibition. Compound **5c** also was a good antioxidant agent (ORAC = 2.5 eq) and selective metal ions chelator, as well as a promising neuroprotectant. Furthermore,

compound **5c** was a significant selective MAO-B inhibitor ($IC_{50} = 9.9 \mu M$) and BACE1 inhibitor ($IC_{50} = 8.3 \mu M$). Further, compound **5c** presented good BBB permeability and complied with drug-like properties rule of 5. Therefore, compound **5c** was a promising multifunctional agent for the treatment of AD. The in-depth study and structural modification are in progress.

Experimental

Chemistry

General information

All reagents were obtained from commercial sources and utilized without further purification unless otherwise noted. 1H and ^{13}C NMR spectra were recorded on a Varian INOVA spectrometer and referenced to Tetramethylsilane (TMS), using $CDCl_3$ or $DMSO-d_6$ as solvents at 400 MHz and 100 MHz, respectively. Chemical shifts (d) are given in ppm. Splitting patterns are indicated as follow: s, single; d, doublet; dd, double-doublet; t, triplet; m, multi-plet. HPLC analysis was carried out on a Waters e2695 plus system with the use of a Ultimate CQ-C18 column (4.6 mm $\times$ 250 mm, 5 μm) at a flow ratio of 1 mL/min. Mobile phase: A: 0.1%TFA in H_2O , B: 0.1% TFA in CH_3CN .

General procedure for the preparation of intermediate compounds 3a and 3b. The starting material 2',4'-dihydroxyacetophenone (3 mmol) with excessive amounts of (*R*)-(-)-epichlorohydrin or (*S*)-(+)-epichlorohydrin (3.6 mmol) in the presence of K_2CO_3 (1.2 mmol) in anhydrous CH_3CN (15 mL) under reflux followed by thin layer chromatography (TLC). Upon completion, the solvent was concentrated by vacuum and K_2CO_3 was removed by filtration. The crude products were purified by silica gel chromatography using petroleum ether/acetone (50:1) as eluent to obtain the intermediates **3a** and **3b**.

(*R*)-1-(2-hydroxy-4-(oxiran-2-ylmethoxy)phenyl)ethan-1-one (**3a**). White solid, 60.5% yield. 1H NMR (400 MHz, $CDCl_3$) δ 12.73 (s, 1H, OH), 7.65 (d, $J = 8.8$ Hz, 1H, Ar-H), 6.49 (d, $J = 8.8$ Hz, 1H, Ar-H), 6.43 (s, 1H, Ar-H), 4.30 (d, $J = 9.2$ Hz, 1H, 1/2 OCH_2), 3.96 (d, $J = 6.0$ Hz, 1H, 1/2 OCH_2), 3.38-3.37 (m, 1H, OCH), 2.94 (t, $J = 4.4$ Hz, 1H, 1/2 OCH_2), 2.78-2.77 (m, 1H, 1/2 OCH_2), 2.57 (s, 3H, $COCH_3$).

(*S*)-1-(2-hydroxy-4-(oxiran-2-ylmethoxy)phenyl)ethan-1-one (**3b**). White solid, 45.8% yield. 1H NMR (400 MHz, $CDCl_3$) δ 12.73 (s, 1H, OH), 7.65 (d, $J = 9.2$ Hz, 1H, Ar-H), 6.49 (d, $J = 6.4$ Hz, 1H, Ar-H), 6.42 (d, $J = 2.8$ Hz, 1H, Ar-H), 4.30 (d, $J = 8.4$ Hz, 1H, 1/2 OCH_2), 3.95 (d, $J = 6.0$ Hz, 1H, 1/2 OCH_2), 3.39-3.36 (m, 1H, OCH), 2.94 (t, $J = 4.8$ Hz, 1H, 1/2 OCH_2), 2.77 (d, $J = 2.8$ Hz, 1H, 1/2 OCH_2), 2.57 (s, 3H, $COCH_3$).

General procedure for the preparation of 2-acetylphenol-*O*-alkylhydroxyethylamine derivatives **5a-g** and **6a-g**. The crude intermediate **3a** (1 mmol) was dissolved in anhydrous CH_3CN (10 mL). K_2CO_3 and respective secondary amine (**4a~4g**) (1.5 mmol) was added to the mixture. The reaction mixture was stirred and refluxed. Upon completion, CH_3CN was concentrated under reduced pressure. The residue was extracted with water (40 mL) and dichloromethane (20 mL $\times$ 3). The organic phases were washed with

saturated NaCl aqueous, dried over Na₂SO₄, filtered and evaporated to dryness under reduced pressure. The crude products were purified by silica gel chromatography using petroleum ether/ ethyl acetate (20:1) as eluent to give the target compounds **5a-g**. The target derivatives **6a-g** were also got under the same experiments condition using intermediate **3b** as the starting material.

(R)-1-(4-(3-(4-benzylpiperidin-1-yl)-2-hydroxypropoxy)-2-hydroxyphenyl)ethan-1-one (5a). Light yellow oil, 80.7% yield, 97.8% HPLC purity. ¹H NMR (400 MHz, CDCl₃) δ 7.60 (d, *J* = 8.6 Hz, 1H, Ar-H), 7.27 (d, *J* = 6.4 Hz, 2H, 2 × Ar-H), 7.19 (d, *J* = 7.1 Hz, 1H, Ar-H), 7.13 (d, *J* = 6.7 Hz, 2H, 2 × Ar-H), 6.46 (d, *J* = 8.8 Hz, 1H, Ar-H), 6.41 (s, 1H, Ar-H), 4.17 (s, 1H, OCH), 3.99 (s, 2H, OCH₂), 3.05-3.03 (m, 2H, NCH₂), 2.56 (m, 7H, 2 × NCH₂, COCH₃), 2.33 (m, 1H, 1/2 phCH₂), 2.08 (d, *J* = 11.1 Hz, 1H, 1/2 phCH₂), 1.68 (d, *J* = 12.2 Hz, 2H, CH₂), 1.59 (s, 1H, CH), 1.41 (m, 2H, CH₂). HR-ESI-MS: Calcd. for C₂₃H₂₉NO₄ [M+H]⁺: 384.2130, found: 384.2175. *(R)-1-(4-(3-(4-benzylpiperazin-1-yl)-2-hydroxypropoxy)-2-hydroxyphenyl)ethan-1-one (5b)*. Light yellow oil, 86.1% yield, 97.6% HPLC purity. ¹H NMR (400 MHz, CDCl₃) δ 7.55 (d, *J* = 8.9 Hz, 1H, Ar-H), 7.26 (d, *J* = 4.1 Hz, 4H, 4 × Ar-H), 7.21 (m, 1H, Ar-H), 6.41 (d, *J* = 8.9 Hz, 1H, Ar-H), 6.37 (s, 1H, Ar-H), 4.05 (d, *J* = 8.7 Hz, 1H, OCH), 3.94 (t, *J* = 5.4 Hz, 2H, OCH₂), 3.48 (s, 2H, NCH₂), 2.66 (s, 2H, NCH₂), 2.50 (m, 11H, 4 × NCH₂, COCH₃). ¹³C NMR (100 MHz, CDCl₃) δ 202.6, 165.1, 165.1, 137.6, 132.3, 129.2 (2C), 128.2 (2C), 127.2, 114.0, 107.8, 101.5, 70.6, 65.3, 62.8, 60.2, 52.9 (4C), 26.2. HR-ESI-MS: Calcd. for C₂₂H₂₈N₂O₄ [M+H]⁺: 385.2083, found: 385.2125.

(R)-1-(2-hydroxy-4-(2-hydroxy-3-(4-isopropylpiperazin-1-yl)propoxy)phenyl)ethan-1-one (5c). Light yellow oil, 89.3% yield, 98.1% HPLC purity. ¹H NMR (400 MHz, CDCl₃) δ 7.55 (d, *J* = 8.9 Hz, 1H, Ar-H), 6.40 (d, *J* = 9.0 Hz, 1H, Ar-H), 6.35 (s, 1H, Ar-H), 4.05 (d, *J* = 8.7 Hz, 1H, OCH), 3.94 (d, *J* = 19.9 Hz, 2H, OCH₂), 2.66 (d, *J* = 12.7 Hz, 3H, NCH₂, NCH), 2.48 (m, 11H, 4 × NCH₂, COCH₃), 1.02 (d, *J* = 6.5 Hz, 6H, 2 × CH₃). ¹³C NMR (100 MHz, CDCl₃) δ 202.5, 165.1, 165.0, 132.2, 113.9, 107.7, 101.4, 70.6, 65.3, 60.2, 54.5, 53.2, 48.5 (3C), 26.1, 18.4, 18.3. HR-ESI-MS: Calcd. for C₁₈H₂₈N₂O₄ [M+H]⁺: 337.2083, found: 337.2124.

(R)-1-(2-hydroxy-4-(2-hydroxy-3-(4-(pyrimidin-2-yl)piperazin-1-yl)propoxy)phenyl)ethan-1-one (5d). Light yellow oil, 78.5% yield, 97.5% HPLC purity. ¹H NMR (400 MHz, CDCl₃) δ 12.63 (s, 1H, ph-OH), 8.23 (d, *J* = 4.7 Hz, 2H, 2 × Ar-H), 7.55 (d, *J* = 8.9 Hz, 1H, Ar-H), 6.40 (m, 3H, 3 × Ar-H), 4.12 (m, 1H, OCH), 3.97 (m, 2H, OCH₂), 3.79 (s, 4H, 2 × NCH₂), 3.03 (q, *J* = 7.3 Hz, 2H, NCH₂), 2.66 (m, 2H, NCH₂), 2.50 (m, 2H, NCH₂), 2.46 (s, 3H₂, COCH₃). HR-ESI-MS: Calcd. for C₁₉H₂₄N₄O₄ [M+H]⁺: 373.1831, found: 373.1871.

(R)-1-(2-hydroxy-4-(2-hydroxy-3-(piperidin-1-yl)propoxy)phenyl)ethan-1-one (5e). Light yellow oil, 83.7% yield, 98.8% HPLC purity. ¹H NMR (400 MHz, CDCl₃) δ 7.56 (d, *J* = 8.9 Hz, 1H, Ar-H), 6.41 (d, *J* = 8.9 Hz, 1H, Ar-H), 6.36 (s, 1H, Ar-H), 4.07 (dd, *J* = 8.9, 4.6 Hz, 1H, OCH), 3.94 (d, *J* = 4.6 Hz, 2H, OCH₂), 2.59 (d, *J* = 4.3 Hz, 2H, NCH₂), 2.47 (m, 5H, NCH₂, COCH₃), 2.39 (s, 2H, NCH₂), 1.57 (d, *J* = 10.4 Hz, 4H, 2 × CH₂), 1.41 (m, 2H, CH₂). ¹³C NMR (100 MHz, CDCl₃) δ 202.6, 165.2, 165.0, 132.3, 114.0, 107.8, 101.5, 70.7, 65.1, 61.0 (2C), 54.7, 26.1, 25.8 (2C), 24.0. HR-ESI-MS: Calcd. for C₁₉H₂₄N₄O₄ [M+H]⁺: 294.1661, found: 294.1700.

(R)-1-(4-(3-(benzyl(ethyl)amino)-2-hydroxypropoxy)-2-hydroxyphenyl)ethan-1-one (**5f**). Light yellow oil, 88.6% yield, 98.3% HPLC purity. ^1H NMR (400 MHz, CDCl_3) δ 12.73 (s, 1H, ph-OH), 7.59 (d, J = 8.9 Hz, 1H, Ar-H), 7.31 (d, J = 3.3 Hz, 4H, 4 $\times$ Ar-H), 7.27 (m, 1H, Ar-H), 6.43 (d, J = 8.9 Hz, 1H, Ar-H), 6.38 (s, 1H, Ar-H), 4.04 (d, J = 7.6 Hz, 1H, OCH), 3.96 (d, J = 9.7 Hz, 2H, OCH_2), 3.78 (d, J = 13.5 Hz, 1H, 1/2 NCH_2), 3.57 (d, J = 13.5 Hz, 1H, 1/2 NCH_2), 2.63 (m, 4H, 2 $\times$ NCH_2), 2.51 (s, 3H, COCH_3), 1.09 (t, J = 7.0 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, CDCl_3) δ 202.6, 165.3, 165.1, 138.3, 132.4, 129.1 (2C), 128.5 (2C), 127.4, 114.0, 107.8, 101.6, 70.6, 66.0, 58.3, 55.7, 47.9, 26.2, 11.7. HR-ESI-MS: Calcd. for $\text{C}_{20}\text{H}_{25}\text{N}_4\text{O}_4$ $[\text{M}+\text{H}]^+$: 344.1817, found: 344.1856.

(R)-1-(4-(3-(diethylamino)-2-hydroxypropoxy)-2-hydroxyphenyl)ethan-1-one (**5g**). Light yellow oil, 83.7% yield, 97.9% HPLC purity. ^1H NMR (400 MHz, CDCl_3) δ 7.56 (d, J = 8.9 Hz, 1H, Ar-H), 6.40 (t, J = 10.1 Hz, 1H, Ar-H), 6.33 (d, J = 15.0 Hz, 1H, Ar-H), 4.04 (d, J = 22.7 Hz, 1H, OCH), 3.95 (d, J = 4.7 Hz, 2H, OCH_2), 2.67 (m, 6H, 3 $\times$ NCH_2), 2.46 (d, J = 17.1 Hz, COCH_3), 1.05 (t, J = 7.1 Hz, 6H, 2 $\times$ CH_3). ^{13}C NMR (100 MHz, CDCl_3) δ 202.6, 165.1, 164.9, 132.3, 114.0, 107.7, 101.5, 70.5, 65.6, 55.6, 47.3 (2C), 26.1, 11.3 (2C). HR-ESI-MS: Calcd. for $\text{C}_{15}\text{H}_{23}\text{NO}_4$ $[\text{M}+\text{H}]^+$: 282.1661, found: 282.1698.

(S)-1-(4-(3-(4-benzylpiperidin-1-yl)-2-hydroxypropoxy)-2-hydroxyphenyl)ethan-1-one (**6a**). Light yellow oil, 84.7% yield, 98.2% HPLC purity. ^1H NMR (400 MHz, CDCl_3) δ 12.74 (s, 1H, ph-OH), 7.64 (d, J = 8.9 Hz, 1H, Ar-H), 7.30 (t, J = 7.4 Hz, 2H, 2 $\times$ Ar-H), 7.22 (d, J = 7.1 Hz, 1H, Ar-H), 7.16 (d, J = 7.4 Hz, 2H, 2 $\times$ Ar-H), 6.50 (d, J = 8.9 Hz, 1H, Ar-H), 6.44 (s, 1H, Ar-H), 4.08 (m, 1H, OCH), 4.01 (d, J = 5.8 Hz, 2H, OCH_2), 3.00 (d, J = 11.2 Hz, 1H, 1/2 NCH_2), 2.83 (d, J = 11.2 Hz, 1H, 1/2 NCH_2), 2.56 (d, J = 5.2 Hz, 5H, NCH_2 , COCH_3), 2.49 (d, J = 13.4, 6.8 Hz, 2H, NCH_2), 2.29 (d, J = 11.5 Hz, 1H, 1/2 ph CH_2), 1.98 (d, J = 11.4 Hz, 1H, 1/2 ph CH_2), 1.68 (d, J = 13.0 Hz, 2H, CH_2), 1.32 (d, J = 10.1 Hz, 3H, CH, CH_2). ^{13}C NMR (100 MHz, CDCl_3) δ 202.7, 165.3, 165.2, 140.5, 132.4, 129.2 (2C), 128.3 (2C), 126.0, 114.1, 108.0, 101.6, 70.7, 65.2, 60.5, 55.6, 52.7, 43.1, 37.8, 32.3, 32.0, 26.3. HR-ESI-MS: Calcd. for $\text{C}_{23}\text{H}_{29}\text{NO}_4$ $[\text{M}+\text{H}]^+$: 384.2130, found: 384.2172.

(S)-1-(4-(3-(4-benzylpiperazin-1-yl)-2-hydroxypropoxy)-2-hydroxyphenyl)ethan-1-one (**6b**). Light yellow oil, 78.4% yield, 98.0% HPLC purity. ^1H NMR (400 MHz, CDCl_3) δ 12.73 (s, 1H, ph-OH), 7.64 (d, J = 8.8 Hz, 1H, Ar-H), 7.32 (t, J = 12.1 Hz, 5H, 5 $\times$ Ar-H), 6.49 (d, J = 8.9 Hz, 1H, Ar-H), 6.44 (s, 1H, Ar-H), 4.12 (m, 1H, OCH), 4.01 (d, J = 5.1 Hz, 2H, OCH_2), 3.55 (s, 2H, NCH_2), 2.75 (s, 2H, NCH_2), 2.58 (m, 11H, 4 $\times$ NCH_2 , COCH_3). HR-ESI-MS: Calcd. for $\text{C}_{22}\text{H}_{28}\text{N}_2\text{O}_4$ $[\text{M}+\text{H}]^+$: 385.2083, found: 385.2126.

(S)-1-(2-hydroxy-4-(2-hydroxy-3-(4-isopropylpiperazin-1-yl)propoxy)phenyl)ethan-1-one (**6c**). Light yellow oil, 86.2% yield, 97.6% HPLC purity. ^1H NMR (400 MHz, CDCl_3) δ 7.59 (d, J = 8.9 Hz, 1H, Ar-H), 6.44 (d, J = 8.9 Hz, 1H, Ar-H), 6.39 (s, 1H, Ar-H), 4.07 (d, J = 4.4 Hz, 1H, OCH), 3.97 (d, J = 8.6 Hz, 2H, OCH_2), 2.67 (m, 3H, NCH_2 , NCH), 2.49 (m, 11H, 4 $\times$ NCH_2 , COCH_3), 1.03 (d, J = 6.5 Hz, 6H, 2 $\times$ CH_3). HR-ESI-MS: Calcd. for $\text{C}_{18}\text{H}_{28}\text{N}_2\text{O}_4$ $[\text{M}+\text{H}]^+$: 337.2083, found: 337.2117.

(*S*)-1-(2-hydroxy-4-(2-hydroxy-3-(4-(pyrimidin-2-yl)piperazin-1-yl)propoxy)phenyl)ethan-1-one (**6d**). Light yellow oil, 72.7% yield, 97.4% HPLC purity. ^1H NMR (400 MHz, CDCl_3) δ 12.59 (s, 1H, ph-OH), 8.18 (d, J = 4.6 Hz, 2H, 2 $\times$ Ar-H), 7.51 (d, J = 8.9 Hz, 1H, Ar-H), 6.36 (m, 3H, 3 $\times$ Ar-H), 4.08 (m, 1H, OCH), 3.94 (m, 2H, OCH_2), 3.74 (s, 4H, 2 $\times$ NCH_2), 2.99 (q, J = 7.2 Hz, 2H, NCH_2), 2.61 (m, 2H, NCH_2), 2.46 (m, 2H, NCH_2), 2.42 (s, 3H₂, COCH_3). HR-ESI-MS: Calcd. for $\text{C}_{19}\text{H}_{24}\text{N}_4\text{O}_4$ $[\text{M}+\text{H}]^+$: 373.1831, found: 373.1866.

(*S*)-1-(2-hydroxy-4-(2-hydroxy-3-(piperidin-1-yl)propoxy)phenyl)ethan-1-one (**6e**). Light yellow oil, 85.8% yield, 98.8% HPLC purity. ^1H NMR (400 MHz, CDCl_3) δ 12.68 (s, 1H, ph-OH), 7.60 (d, J = 8.9 Hz, 1H, Ar-H), 6.45 (d, J = 8.9 Hz, 1H, Ar-H), 6.39 (s, 1H, Ar-H), 4.15 (d, J = 8.7 Hz, 1H, OCH), 3.98 (d, J = 4.7 Hz, 2H, OCH_2), 2.69 (d, J = 3.6 Hz, 2H, NCH_2), 2.55 (m, 7H, 2 $\times$ NCH_2 , COCH_3), 1.64 (d, J = 5.0 Hz, 4H, 2 $\times$ CH_2), 1.48 (d, J = 5.3 Hz, 2H, CH_2). HR-ESI-MS: Calcd. for $\text{C}_{16}\text{H}_{23}\text{NO}_4$ $[\text{M}+\text{H}]^+$: 294.1661, found: 294.1705.

(*S*)-1-(4-(3-(benzyl(ethyl)amino)-2-hydroxypropoxy)-2-hydroxyphenyl)ethan-1-one (**6f**). Light yellow oil, 88.1% yield, 97.3% HPLC purity. ^1H NMR (400 MHz, CDCl_3) δ 12.72 (s, 1H, ph-OH), 7.63 (d, J = 8.9 Hz, 1H, Ar-H), 7.30 (d, J = 3.3 Hz, 4H, 4 $\times$ Ar-H), 7.23 (m, 1H, Ar-H), 6.49 (d, J = 8.9 Hz, 1H, Ar-H), 6.44 (s, 1H, Ar-H), 4.22 (d, J = 7.6 Hz, 1H, OCH), 4.03 (d, J = 9.7 Hz, 2H, OCH_2), 3.78 (d, J = 13.5 Hz, 1H, 1/2 NCH_2), 3.68 (d, J = 13.5 Hz, 1H, 1/2 NCH_2), 2.79 (m, 4H, 2 $\times$ NCH_2), 2.55 (s, 3H, COCH_3), 1.12 (t, J = 7.0 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, CDCl_3) δ 202.7, 165.1, 132.5, 132.3, 129.0, 128.5, 127.4, 107.9, 101.5, 70.6, 65.8, 58.2, 55.6, 47.8, 26.3, 11.7. HR-ESI-MS: Calcd. for $\text{C}_{20}\text{H}_{25}\text{NO}_4$ $[\text{M}+\text{H}]^+$: 344.1817, found: 344.1862.

(*S*)-1-(4-(3-(diethylamino)-2-hydroxypropoxy)-2-hydroxyphenyl)ethan-1-one (**6g**). Light yellow oil, 88.9% yield, 98.5% HPLC purity. ^1H NMR (400 MHz, CDCl_3) δ 7.59 (d, J = 8.9 Hz, 1H, Ar-H), 6.44 (d, J = 8.9 Hz, 1H, Ar-H), 6.38 (m, 1H, Ar-H), 4.01 (m, 1H, OCH), 3.96 (d, J = 5.1 Hz, 2H, OCH_2), 2.61 (d, J = 12.8 Hz, 6H, 3 $\times$ NCH_2), 2.51 (s, 3H, COCH_3), 1.04 (t, J = 7.1 Hz, 6H, 2 $\times$ CH_3). HR-ESI-MS: Calcd. for $\text{C}_{15}\text{H}_{23}\text{NO}_4$ $[\text{M}+\text{H}]^+$: 282.1661, found: 282.1687.

Biological activity

Inhibition experiments of AChE and BuChE. AChE and BuChE inhibitory potency of the target compounds were tested by slightly modified Ellman assay [25]. Briefly, for AChE inhibition assays, a reaction mixture (100 μL) included substrate ATC (1 mmol/L, 30 μL), phosphate-buffered solution (0.1 M $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$, PH = 8.0, 40 μL), diverse concentrations (DMSO < 1%) of test compounds (20 μL) and 10 μL enzyme (eeAChE 0.45 U/mL) was incubated at 37°C for 15 min. After that 5,5'-dithiobis-2-nitrobenzoic acid (DTNB, 0.2%, 30 μL) was added to the mixture. Using a Varioskan Flash Multimode Reader at 412 nm to determine the activities. The inhibition percent was calculated by the following expression: $(1 - A_i/A_c) \times 100$, in which A_i and A_c are the absorbance obtained for AChE in the presence and absence of inhibitors after subtracting the background. All samples were performed in triplicate. The procedure of BuChE assay was similar as described above using BTC and BuChE instead of ATC and AChE.

Antioxidant activity assay. The antioxidant activity was assessed by the oxygen radical absorbance capacity fluorescein (ORAC-FL) assay. The detailed procedure referenced our previous work [27, 28].

Recombinant human MAO-A and MAO-B inhibition studies [31]. Recombinant human MAO-A and MAO-B, purchased from Sigma-Aldrich, were prealiquoted and stored at -80 °C. The test compounds were dissolved in DMSO (2.5 mM) and diluted with potassium phosphate buffer (100 mM, pH 7.40, containing KCl 20.2 mM) to a final volume of 500 µL composed of different concentrations of test compounds (0-100 µM) and kynuramine (45 µM for MAO-A and 30 µM for MAO-B). The reactions were triggered via the addition of the enzyme (7.5 µg/mL) and then incubated for 30 min at 37°C. After that 400 µL NaOH (2N) and 1000 µL water were added to terminate the reactions and finally the mixtures were centrifuged at 16000g for 10 min. The reactions were quantified on a Varioskan Flash Multimode Reader (PerkinElmer) based on the fluorescence of the supernatant with excitation and emission wavelengths at 310 nm and 400 nm, respectively. IC₅₀ values were calculated using GraphRad Prism 5. IC₅₀ values were determined in triplicate and expressed as mean ± SD.

Metal chelation studies. The UV absorption of the target compound, alone or in the presence of CuCl₂, ZnCl₂, FeSO₄, and AlCl₃, was recorded with Shimadzu UV-2450 spectrophotometer with wavelength ranging from 200 to 600 nm after incubating for 30 min at room temperature [27]. The final concentrations of tested compound and metals were 37.5 µM, and the final volume of reaction mixture was 1 mL. The spectra of the metal alone and the compound alone numerically subtracted from the spectra of the mixture to give the difference UV-vis spectra due to complex formation. The procedure referenced our previous work.

BACE-1 inhibition studies. BACE-1 in vitro inhibition assay was tested as described in the assay procedure by utilizing the β-secretase Inhibitor Screening Assay Kit [32]. Reaction mixture comprised of test compound (2.5 µL) or Donepezil (control), BACE-1 enzyme (15 µL) and the substrate was incubated for 1 hour at 25 °C. The fluorescence signal was recorded at an excitation wavelength of 340 nm and an emission wavelength 490 nm by microplate reader (EnSpire Multimode; PerkinElmer). The inhibition percentage was calculated by the equation: Percent inhibition (%) = 100 - [AF_i/AF₀] × 100%, where AF_i and AF₀ mean the fluorescence intensities obtained for BACE-1 in the presence and absence of an inhibitor, respectively.

Neuroprotective effect. The neuroprotective effects were evaluated with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The detailed procedure referenced our previous work [27, 28].

In vitro blood-brain barrier permeation assay. The parallel artificial membrane permeation assay (PAMPA) was used to evaluate the blood-brain barrier penetration of compounds [33, 34]. The donor microplate (PVDF membrane, pore size 0.45 µm) and acceptor microplate were both from Millipore. The 96-well UV plate (COSTAR) was from Corning Incorporated. According to the detailed procedure in our previous work, we concluded that compounds with Pe values above 3.44 × 10⁻⁶ cm/s could cross the blood- brain barrier.

Declarations

Supporting Information

The representative ^1H , ^{13}C NMR and HR-ESI-MS spectra for the synthesized compounds were available in supporting information.

Notes

The authors declare no competing financial interest.

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References

1. Barnett R (2019) Alzheimer's disease. *Lancet* 393(10181):1589
2. Patterson C (2018) World Alzheimer Report 2018—the state of the art of dementia research: new frontiers. Alzheimer's Disease International (ADI), London, pp 1–48
3. Alzheimer's Disease International (2019) *World Alzheimer Report 2019: Attitudes to dementia*. Alzheimer's Disease International, London
4. Wang YJ (2014) Alzheimer disease: Lessons from immunotherapy for Alzheimer disease. *Nat Rev Neurol* 10(4):188–189
5. Jagust W (2018) Imaging the evolution and pathophysiology of Alzheimer disease. *Nat Rev Neurosci* 19(11):687–700
6. Anand P, Singh B (2013) A review on cholinesterase inhibitors for Alzheimer's disease. *Arch Pharm Res* 36(4):375–399
7. Greig NH, Utsuki T, Yu Q, Zhu X, Holloway HW, Perry T, Lee B, Ingram DK, Lahiri DK (2001) A new therapeutic target in Alzheimer's disease treatment: attention to butyrylcholinesterase. *Curr Med Res Opin* 17(3):159–165
8. Hardy J, Selkoe DJ (2002) The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* 297(5580):353–356
9. Nalivaeva NN, Turner AJ (2019) Targeting amyloid clearance in Alzheimer's disease as a therapeutic strategy. *Br J Pharmacol* 176(18):3447–3463
10. Burki T (2018) Alzheimer's disease research: the future of BACE inhibitors. *Lancet* 391(10139):2486
11. Kumar D, Ganeshpurkar A, Kumar D, Modi G, Gupta SK, Singh SK (2018) Secretase inhibitors for the treatment of Alzheimer's disease: Long road ahead. *Eur J Med Chem* 148:436–452

12. <http://integrity.thomson-pharma.com>
13. Dixon SJ, Stockwell BR (2014) The role of iron and reactive oxygen species in cell death. *Nat Chem Biol* 10(1):9–17
14. Liu Y, Nguyen M, Robert A, Meunier B (2019) Metal Ions in Alzheimer's Disease: A Key Role or Not? *Acc Chem Res* 52(7):2026–2035
15. Wang L, Yin YL, Liu XZ, Shen P, Zheng YG, Lan XR, Lu CB, Wang JZ (2020) Current understanding of metal ions in the pathogenesis of Alzheimer's disease. *Transl Neurodegener* 9:10
16. Schedin-Weiss S, Inoue M, Hromadkova L, Teranishi Y, Yamamoto NG, Wiehager B, Bogdanovic N, Winblad B, Sandebring-Matton A, Frykman S, Tjernberg LO (2017) Monoamine oxidase B is elevated in Alzheimer disease neurons, is associated with γ -secretase and regulates neuronal amyloid β -peptide levels. *Alzheimers Res Ther* 9(1):57
17. <https://www.alzforum.org/therapeutics/rasagiline>
18. de Freitas Silva M, Dias KST, Gontijo VS, Ortiz CJC, Viegas C Jr (2018) Multi-target directed drugs as a modern approach for drug design towards Alzheimer's disease: An update. *Curr Med Chem* 25(29):3491–3525
19. Cavalli A, Bolognesi ML, Minarini A, Rosini M, Tumiatti V, Recanatini M, Melchiorre C (2008) Multi-target-directed ligands to combat neurodegenerative diseases. *J Med Chem* 51(3):347–372
20. Zhang P, Xu S, Zhu Z, Xu J (2019) Multi-target design strategies for the improved treatment of Alzheimer's disease. *Eur J Med Chem* 176:228–247
21. Legoabe LJ, Petzer A, Petzer JP (2015) 2-acetylphenol analogs as potent reversible monoamine oxidase inhibitors. *Drug Des Devel Ther* 9:3635–3644
22. Zhu G, Wang K, Shi J, Zhang P, Yang D, Fan X, Zhang Z, Liu W, Sang Z (2019) The development of 2-acetylphenol-donepezil hybrids as multifunctional agents for the treatment of Alzheimer's disease. *Bioorg Med Chem Lett* 29(19):126625
23. Sang Z, Wang K, Wang H, Wang H, Ma Q, Han X, Ye M, Yu L, Liu W (2017) Design, synthesis and biological evaluation of 2-acetyl-5-O-(amino-alkyl)phenol derivatives as multifunctional agents for the treatment of Alzheimer's disease. *Bioorg Med Chem Lett* 27(22):5046–5052
24. Domínguez JL, Fernández-Nieto F, Castro M, Catto M, Paleo MR, Porto S, Sardina FJ, Brea JM, Carotti A, Villaverde MC, Sussman F (2015) Computer-aided structure-based design of multitarget leads for Alzheimer's disease. *J Chem Inf Model* 55(1):135–148
25. Sang Z, Qiang X, Li Y, Yuan W, Liu Q, Shi Y, Ang W, Luo Y, Tan Z, Deng Y (2015) Design, synthesis and evaluation of scutellarein-*O*-alkylamines as multifunctional agents for the treatment of Alzheimer's disease. *Eur J Med Chem* 94:348–366
26. Rosenberry TL, Sonoda LK, Dekat SE, Cusack B, Johnson JL (2008) Analysis of the reaction of carbachol with acetylcholinesterase using thioflavin T as a coupled fluorescence reporter. *Biochemistry* 47(49):13056–13063

27. Sang Z, Wang K, Shi J, Liu W, Cheng X, Zhu G, Wang Y, Zhao Y, Qiao Z, Wu A, Tan Z (2020) The development of advanced structural framework as multi-target-directed ligands for the treatment of Alzheimer's disease. *Eur J Med Chem* 192:112180
28. Sang Z, Wang K, Shi J, Cheng X, Zhu G, Wei R, Ma Q, Yu L, Zhao Y, Tan Z, Liu W (2020) Apigenin-rivastigmine hybrids as multi-target-directed ligands for the treatment of Alzheimer's disease. *Eur J Med Chem* 187:111958
29. Sang Z, Wang K, Wang H, Wang H, Ma Q, Han X, Ye M, Yu L, Liu W (2017) Design, synthesis and biological evaluation of 2-acetyl-5-O-(amino-alkyl)phenol derivatives as multifunctional agents for the treatment of Alzheimer's disease. *Bioorg Med Chem Lett* 27(22):5046–5052
30. Wang XL, Bian ZX, Wang XG (2007) The IR spectra of complexes of N-(phenyl, ferrocenyl)methyl-beta-hydroxyethylamine with Ni(II), Cu(II), Zn(II), Cd(II) and Hg(II). *Guang Pu Xue Yu Guang Pu Fen Xi* 27(11):2221–2223
31. Sang Z, Wang K, Zhang P, Shi J, Liu W, Tan Z (2019) Design, synthesis, in-silico and biological evaluation of novel chalcone derivatives as multi-function agents for the treatment of Alzheimer's disease. *Eur J Med Chem* 180:238–252
32. Zhao XJ, Gong DM, Jiang YR, Guo D, Zhu Y, Deng YC (2017) Multipotent AChE and BACE-1 inhibitors for the treatment of Alzheimer's disease: Design, synthesis and bio-analysis of 7-amino-1,4-dihydro-2H-isoquinolin-3-one derivatives. *Eur J Med Chem* 138:738–747
33. Di L, Kerns EH, Fan K, McConnell OJ, Carter GT (2003) High throughput artificial membrane permeability assay for blood-brain barrier. *Eur J Med Chem* 38:223
34. Sang Z, Wang K, Bai P, Wu A, Shi J, Liu W, Zhu G, Wang Y, Lan Y, Chen Z, Zhao Y, Qiao Z, Wang C, Tan Z (2020) Design, synthesis and biological evaluation of novel O-carbamoyl ferulamide derivatives as multi-target-directed ligands for the treatment of Alzheimer's disease. *Eur J Med Chem* 194:112265
35. Pérez-Cruz F, Vazquez-Rodriguez S, Matos MJ, Herrera-Morales A, Villamena FA, Das A, Gopalakrishnan B, Olea-Azar C, Santana L, Uriarte E (2013) Synthesis and electrochemical and biological studies of novel coumarin-chalcone hybrid compounds. *J Med Chem* 56:6136–6145

Figures

Figure 1

The structure of compounds in clinical phase II/III trials and lead compound DDDT-15.

Figure 2

Design strategy of 2-acetylphenol-O-alkylhydroxyethylamine derivatives

Figure 3

Kinetic study of EeAChE inhibition by compound 5c.

Figure 4

(A) The UV spectrum of compound 5c in methanol was investigated using CuCl_2 , FeSO_4 , ZnCl_2 and AlCl_3 ; (B) The molar ratio method was used to evaluate the stoichiometry of complex- Cu^{2+} with ascending amounts of CuCl_2 (ranging from 3.75 to 112.5 μM).

Figure 5

(A) Representation of compound 5c (green stick) interacting with residues in the binding site of TcAChE (code ID: 1EVE). (B) 3D docking model of compound 5c (green stick) with AChE. (C) 2D schematic diagram of docking model of compound 5c with AChE. (D) Representation of donepezil (green stick) interacting with residues in the binding site of TcAChE (code ID: 1EVE).

Figure 7

(A) The cell viability on PC12 cells, **p < 0.01, *p < 0.05 vs control. (B) The cell viability (%) of compound 5c on H2O2-induced PC12 cell injury using MTT assay. Three independent experiments were carried out in triplicate. Data were expressed as mean ± SD and percentage of control value. ##p < 0.01 vs control; **p < 0.01, *p < 0.05 vs H2O2 group.

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