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Design, Synthesis and Biological Evaluation of 1-Phenyl-Ethanone Derivatives for Multi-Targeted Treatment of Alzheimer's Disease

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Abstract

Alzheimer's disease (AD) is a progressive neurodegenerative disease leading to the irreversible loss of brain neurons and cognitive abilities. Multiple factors, such as acetylcholinesterase (AChE), metal ions and amyloid-β (Aβ) have been considered play an important role in the pathogenesis of AD. In this work, AChE and metal ions, both of which are also associated with the deposition of Aβ in the brain, were selected as targets simultaneously. 22 compounds were rationally designed by hybridizing AChE inhibitor rivastigmine and metal chelator 2-hydroxyacetophenone, in a hoping that these compounds could be as a substrate and inhibitor of AChE, while the subsequent enzymatic hydrolysis products by AChE could be as a metal ion chelator. Thus these 22 compounds were synthesized and their biological activities against AD were evaluated *in vitro*. The results showed that compound **w8** presented the best inhibitory activity of AChE (IC_{50}=31.9 μM), and the representing enzymatic hydrolysis products **7f** exhibted the metal chelating function. Furthermore, both **7f** and one of the targeted compound **w15** could inhibit the aggregation of Aβ.

Keywords: Alzheimer's disease; Amyloid-β; AChE inhibitor; Metal chelators; Drug design

Introduction

Alzheimer's disease (AD), the leading cause of dementia in the elderly, is a complex neurodegenerative disorder [1,2]. Since multiple factors, such as reduced acetylcholine (ACh) level, metal dyshomeostasis, oxidative stress and amyloid-β (Aβ) aggregation, have been considered to play important roles in the pathogenesis of AD, it is difficult to shed desirable therapeutic effect for single-target strategy [3,4]. Thus, Multi-Target-Directed Ligand, which is rationally designed to hit multiple targets for a particular disease to improve pharmacological profiles, raise as a potentially more effective strategy for AD treatment [5-7].

Until now, clinical available drugs approved for AD are mainly AChE inhibitors [8,9], which improve the ACh level, one of the important neurotransmitters responsible for cognition in the central cholinergic system. Some experiments also find that the level of metal ions in AD patients are 3-7 folds higher than that of healthy individuals [10], it indicated that the dyshomeostasis of biometals (Fe, Cu, Zn) in the brain may contribute to AD pathology [11]. Clioquinol (a classic metal chelator used as antifungal drug and antiprotozoal drug), had been tested to treat AD [12,13]. The phase II clinical trials suggested that clioquinol could halt cognitive decline in AD, possibly owing to its ability to chelate copper and zinc ions. Therefore, decreasing the level of metal ions in brain by using metal chelator represents another rational therapeutic approach for the treating of AD.

Furthermore, both AChE and metal ions are associated with Aβ, which plays a central role in the pathogenesis of AD [14-18]. Recent evidence indicated certain links between Aβ and AChE [19]. AChE could form a complex with Aβ, which changes the conformation of Aβ and then promotes the aggregation Aβ [20,21]. While metal ions binding to soluble Aβ via histidine residues could greatly accelerate Aβ nucleated aggregation [22,23], which is enhanced under mild acidic conditions similar to that present in aging and AD brains. Thus, simultaneously inhibition of AChE and chelation of metal ions, both of which also contribute to the same result of decelerating the Aβ aggregation with different mechanisms, may form synergistic effect in the treatment of AD [24].

Considering the above, we focused on multi-target-directed ligands integrated AChE inhibitors and metal chelators, which not only reduce the hydrolysis of ACh and decrease the levels of metal ions in brain but also slow down the aggregation of Aβ. Rivastigmine, an AChE inhibitor approved for the treatment of AD, could be cleavaged by AChE to release metabolites NAP226-90 in brain (Figure 1) [25], which inspired us to design a molecular skeleton that could be as a substrate and inhibitor of AChE first, and then the subsequent hydrolysis products by AChE could be as a metal ion chelator. Based on this design strategy, 1-phenyl-ethanone derivatives were designed by hybridizing AChE inhibitor rivastigmine and metal chelator 2-hydroxyacetophenone (Figure 2). The 1-phenyl-ethanone derivatives were expected to have multifunctional with effects inhibiting AChE and chelating metal ion, furthermore to have the synergy functions on the inhibition of Aβ formation.

According to the classical drug design methods such as the principle of bioisosteres and scaffold hopping, a series of 1-phenyl-ethanone derivatives had been designed by changing group R_1, R_2 and R_3. After screening by the Rule of Five, 22 compounds were picked up (Figure 2) and then predicted the blood brain barrier (BBB) permeability by Discovery Studio 2.1. The results showed that all the compounds had good permeability through the BBB (Figure 3). Therefore, a series of 1-phenyl-ethanone derivatives were synthesized and their biological activities against AD were evaluated *in vitro*.

Results and Discussion

Chemistry

With hydroxyl acetophenone **1** as starting material, the carbonyl was reduced by sodium borohydride first to form **2**, and then **2**

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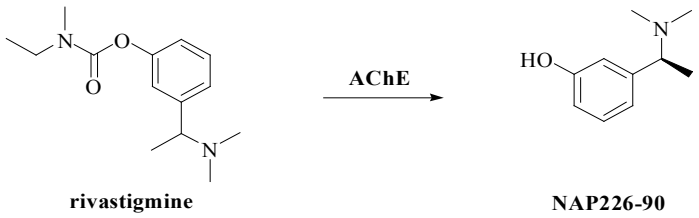

Figure 1: The enzymatic hydrolysis of Rivastigmine by AChE.

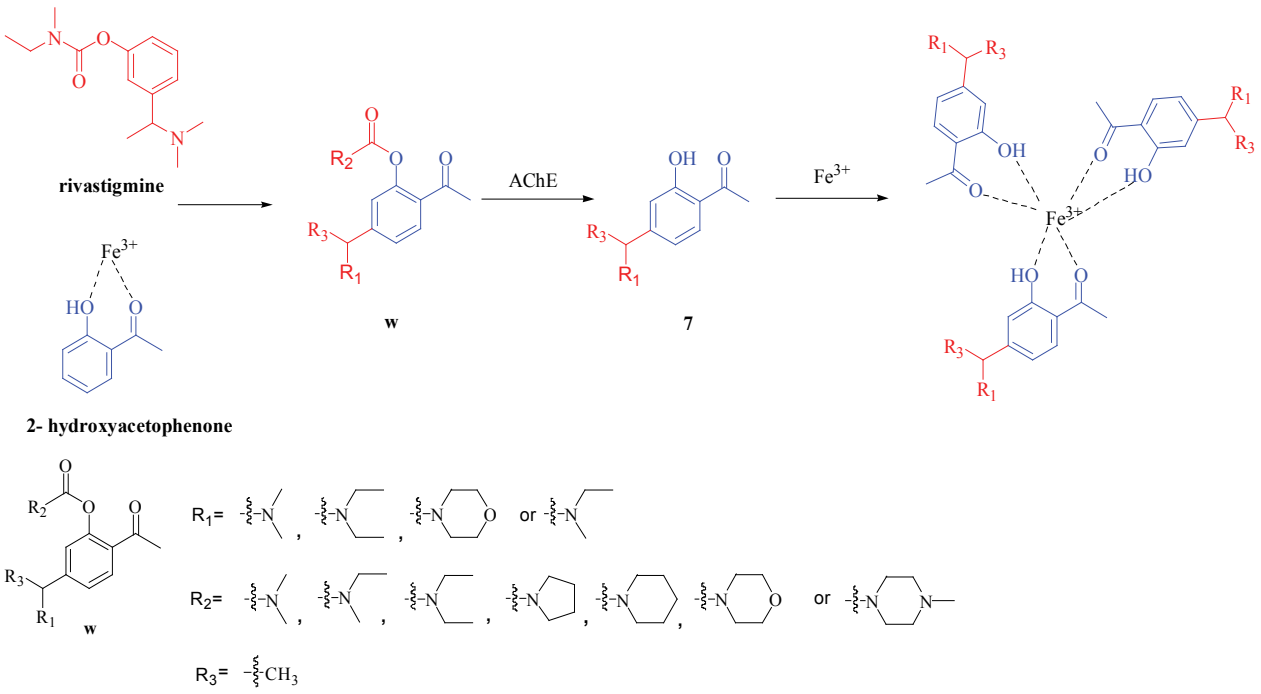

Figure 2: The design and functions of targeted molecular skeleton.

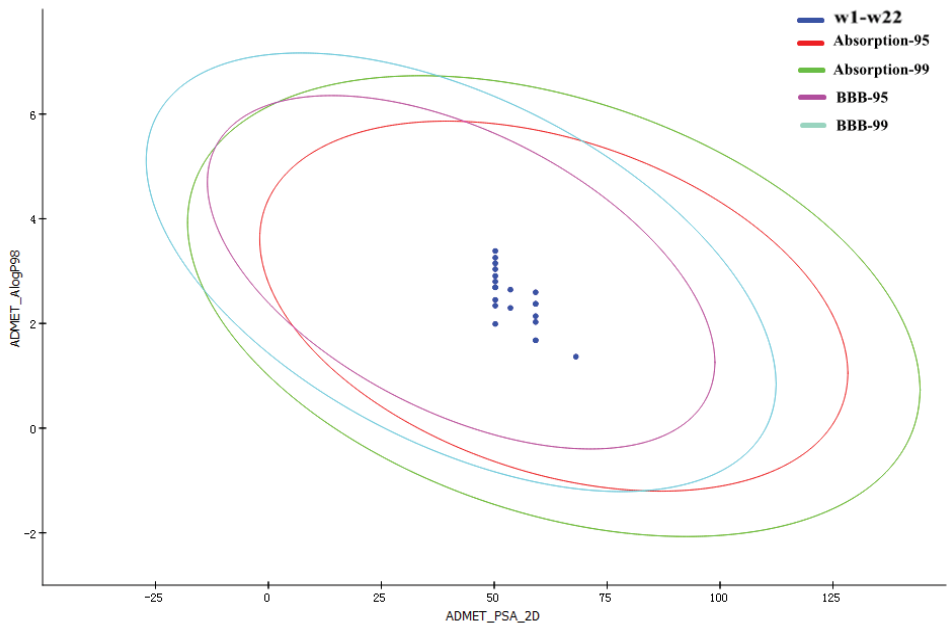

Figure 3: The prediction of BBB permeability of designed compounds.

was acetylated into **3** with acetic anhydride for the protection of phenolic hydroxyl. By chlorination of **3** with thionyl chloride and further condensation of **4** with different secondary amines, a series of compounds **5** were obtained. After acetylation of **5**, the produced critical intermediates **6** through the Fries Rearrangement enabled the preparation of **7**. Finally, the targeted compounds **w1~w22** were synthesized by esterification of **7** with different carbamyl chlorides. The synthetic route was shown in Figure 4.

Reagents and condition: a. $NaBH_4$, THF, 25°C, 3 h; b. KOH, 0°C, 2 h; c. DMF, $SOCl_2$, DCM, 25°C, 2 h; d. KI, R_1, acetonitrile, reflux; e. Na_2CO_3, DCM, 0°C; f. $AlCl_3$, nitrobenzene, 150°C, 5 h; g. DMAP, TEA, THF, reflux, 4 h.

In vitro biological evaluation

Metal chelating effect: The increase levels of iron, zinc and particularly copper on brain was reported to actively contribute to the formation of amyloid plaques by generating more reactive oxygen species through the Aβ (1-42) metal complex [26]. We once published a review concerning the drug-like metal chelating agents [27]. Many drug-like metal chelating agents were found to be able to reverse Aβ aggregation, dissolve amyloid plaques, and delay the AD-related cognitive impairment. In this presented work, compounds **7** were designed as active ingredients for metal chelating, so that randomly, compound **7f** was picked to test the metal-chelating effect.

The metal-chelating effect of compound **7f** was studied by ultra violet (UV) spectrometry with wavelength ranging from 200 to 400 nm.[27] Upon the addition of Cu^{2+}, Fe^{2+} and Zn^{2+} to the **7f** methanol solution, the maximum absorption wavelength or absorption intensity happened to change, representing the formation of **7f**-Cu^{2+}, **7f**-Fe^{2+} and **7f**-Zn^{2+} (Figure 5). In detail, when Zn^{2+} was added into the solution **7f**, the UV absorption intensity was no obvious changing, only the maximum absorption wavelength shifted slightly. When Fe^{2+} and Zn^{2+} were added into solution **7f** respectively, the maximum absorption wavelength had no obvious change, while the UV absorption intensity increased obviously. This data revealed that metal-chelating form of **7f**-Cu^{2+} was different from **7f**-Fe^{2+} and **7f**-Zn^{2+}.

Aβ aggregation inhibition: Compounds **7f** and **w15** were tested for their ability to inhibit self-induced Aβ (1~42) aggregation by thioflavin T-based fluorometric assay, [28] with curcumin (Cur) as reference compounds. The fluorescence intensities of five tested groups, respectively **7f**, **w15**, Aβ itself, Cur, and blank, were recorded in Table 1. The morphology of Aβ (1~42) aggregation was transferred for imaging by transmission electron microscopy with 2 μm and 0.5 μm (Figure 6). The fluorescence intensities of **w15** was stronger than **7f** hydrolyzed from **w15** by AChE, but slightly weaker than the positive the control compound Cur. The results revealed that compounds **w15** and **7f** had considerable potencies inhibiting Aβ aggregation.

AChE enzymatic hydrolysis: For confirming that the synthesized compound **w1~w22** could be hydrolyzed to **7** by AChE, the enzymatic hydrolysis of compound **w13** by AChE was investigated *in vitro*, the hydrolysis products after 0.1 h, 0.5 h, 1 h, 3 h, 6 h was detected respectively by HPLC with **w13** and **7f** as external standard (Figure 7). The results indicated that **w13** could be hydrolyzed to **7f** by AChE, moreover the hydrolysis was intensified with the increasing of hydrolysis time.

AChE inhibitory activity: The AChE inhibitory activity of 1-phenyethanone derivatives **w1~w22** were tested by spectrophotometric method [29], using rivastigmine as the reference. The percentages of AChE inhibition and IC_{50} values of all tested compounds were summarized in Table 2.

As shown in Table 1, compounds **w1, w8, w10, w22,** exhibited moderate AChE inhibitory activities in comparison with rivastigmine, wherein compound **w8** displayed most potentially with an IC_{50} value of 31.9 μM**.** Unexpectedly, except the above four compounds, all the rest compounds displayed poor AChE inhibitory activities in this test. It may be attributed to that **w1~w22** are harder to across the narrow aromatic gorge which linked the central site and peripheral site in AChE.[30]. According to results, it was supposed that when R_1 and R_2 were short or small secondary amines, such as N-dimethylamine, N-ethylmethylamine or pyrrolidine, the targeted compounds exhibited better AChE inhibitory activities.

Molecular docking study: In an attempt to understand the molecular interaction between **w8** and AChE, a molecular docking study was built on previous work,[31] which was performed by Discovery Studio 4.0/CDOCKER protocol using the crystal structure of E2020/AChE complex (PDB ID: 1EVE) as the template. Docking and

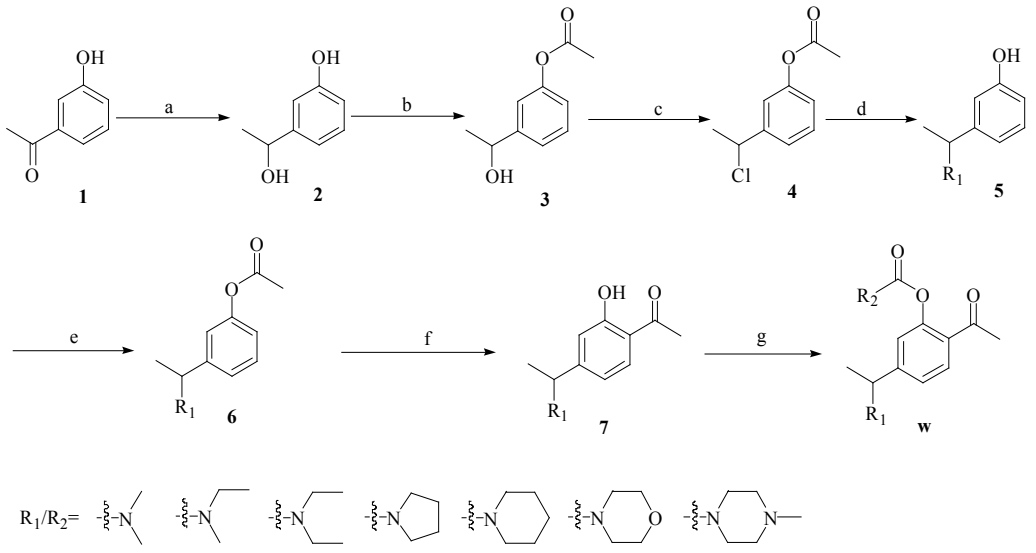

Figure 4: The synthetic route of **w1~w22**.

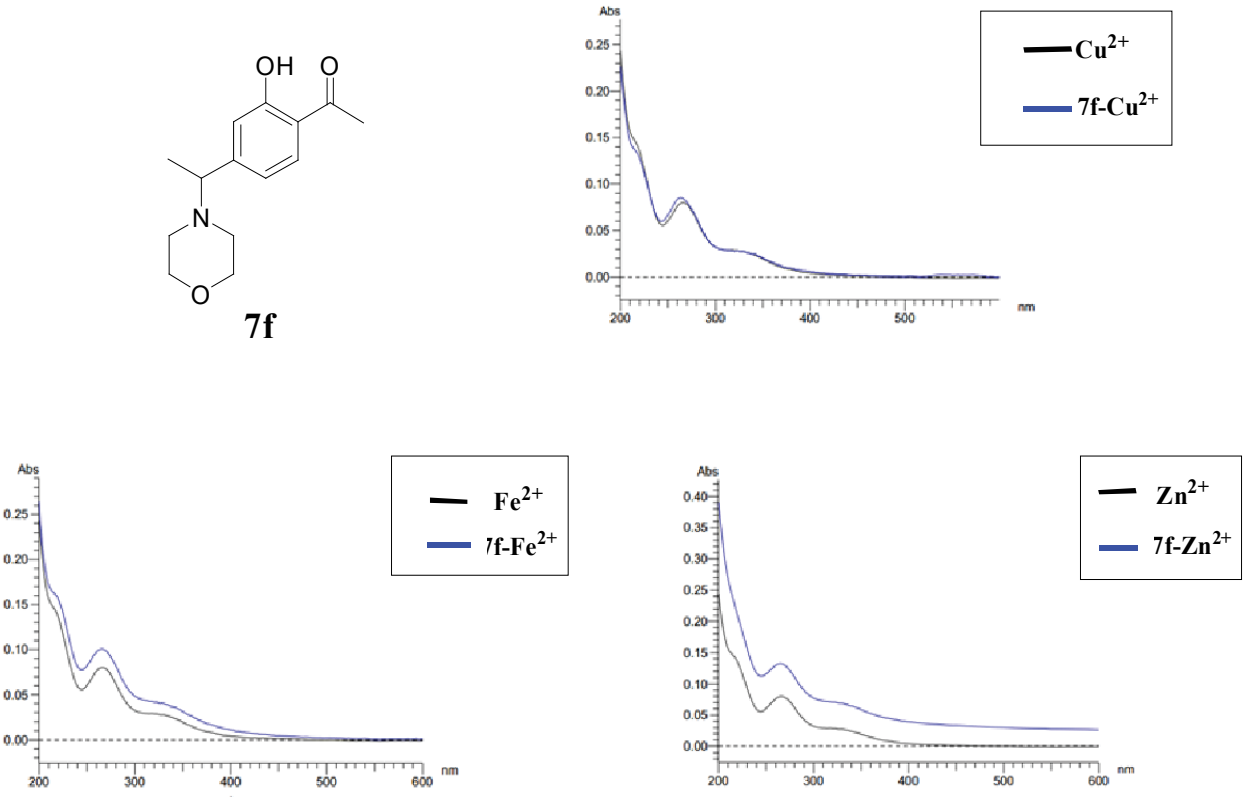

Figure 5: The absorption spectra of **7f** (25 μM) chelate Copper, Iron and Zinc ions.

	1	2	3	Average	Absolute	Inhibition%
Blank	24958	24928	24772	24886		
Aβ itself	31044	33188	35388	33206.67	8320.67	0
Cur	29563	28837	28692	29030.67	4144.67	50.19
7f	31202	31228	29030	30486.67	5600.67	32.69
w15	32061	29730	28208	29999.67	5113.67	38.55

Table 1: The results of the ability to inhibit self-induced Aβ aggregation.

subsequent scoring studies were performed using default parameters. It was disclosed that the binding pattern of **w8** into the AChE was similar to the crystal structure of rivastigmine/AChE (PDB ID:1GQR). As shown in Figure 8, hydrogen bond interactions were observed between amide group and Gly118/Ser200 with distance of 2.15 Å and 1.83 Å, respectively. Besides, the charged nitrogen also made a hydrogen bond interaction with Tyr121 with a distance of 2.68 Å. In addition, the benzene ring formed π-π stacking with Trp334. Moreover, same as the binding pattern of rivastigmine, the carbamate group of **w8** was very close to the esteratic site (Ser200, Glu327 and His440), which was beneficial to the enzymatic hydrolysis of carbamate group, and then resulted in a 'flattening' of the carbamate moiety over the esteratic site, producing the prolonged inhibition of AChE.

Conclusion

In summary, a series of 1-phenyl-ethanone derivatives have been successfully designed, synthesized and their biological activities were evaluated in respect to metal chelating, AChE inhibiting and Aβ aggregation inhibiting for the treatment of AD. Among which, several targeted compounds exhibited AChE inhibiting acitivity, although it was somewhat decreased compared with ravastigmine. It was also proved that 1-phenyl-ethanone derivatives could be hydrolyzed by AChE to release OH-metabolite chelating Cu^{2+}, Fe^{2+} and Zn^{2+}. Meanwhile the tested 1-phenyl-ethanone derivative and its hydrolysis product showed the ability to inhibit the Aβ aggregation. These results validated the possibility of our molecular design strategy, while improvement of AChE activities of 1-phenyl-ethanone derivatives would be the important point in the next stage of the anti-AD drug development with multi-targeted strategy.

Experimental Section

Chemical synthesis

All solvents used were analytical grade. Melting points were recorded on a Buchi apparatus without correction, IR spectra were recorded on a Bruker VECTOR 22 FTIR spectrophotometer. ^{1}H NMR spectra were recorded on a Bruker Avance III 500M instrument (chemical shifts are expressed as δ values relative to TMS as internal standard). Mass spectra (MS) were recorded on an Esquire-LC-00075 spectrometer.

3-(1-hydroxy-ethyl)-phenol (2): A solution of 150 g compound **1** in 500 mL THF was cooled to 0°C with a salt-ice bath and sodium borohydride (51.0 g, 1.3 mol) was added in batches. After that, the reaction was stirred at room temperature for 3 h, and then was quenched

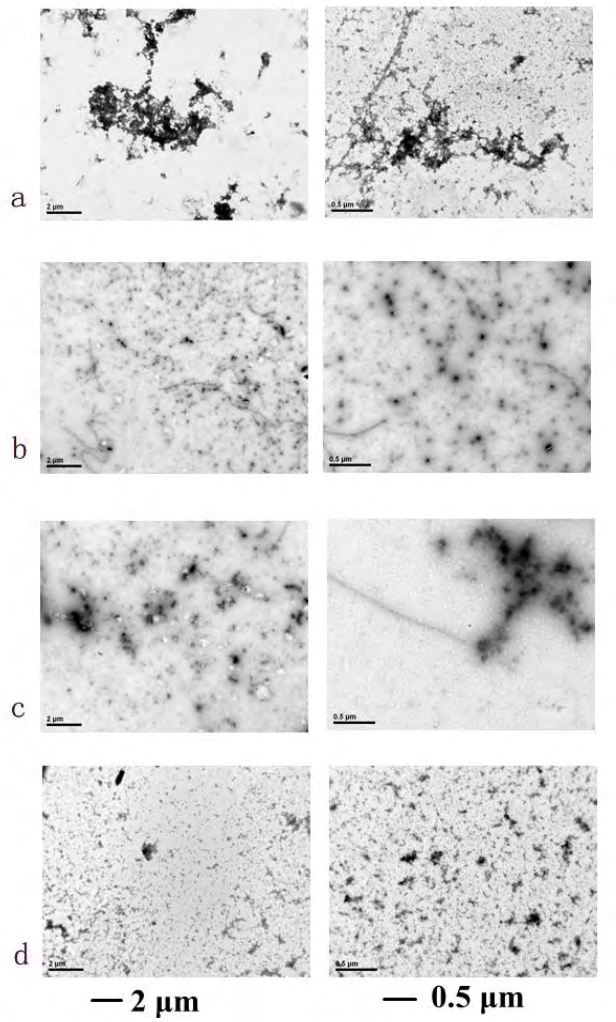

Figure 6: Transmission electron micrographys of Aβ aggregation inhibition. **a.** Aβ (25 μM) diluted with 10 mM phosphate buffer. **b.** Aβ (25 μM) mixed with Cur (20 μM) in 1% final DMSO concentration. **c.** Compound **7f** mixed with Aβ (25 μM) in 1% final DMSO concentration. **d. w15** mixed with Aβ (25 μM) in 1% final DMSO concentration.

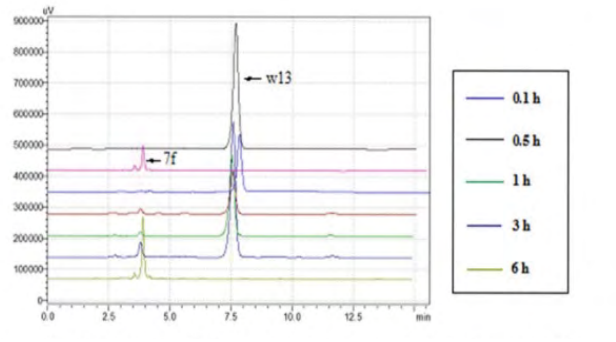

Figure 7: The HPLC profiles of **w13** and **7f** in the hydrolysis with AChE.

with water. The aqueous solution was adjusted pH to 5~6 and extracted with ethyl acetate. The organic layer was dried over Na_2SO_4, and the residue was crystallized with ethyl acetate to give **2** (132.6 g, 72.5%).

3-(1-hydroxyl) ethyl benzoic acid ethyl ester (3): Compound **2** (126.6 g, 0.9 mol) was dissolved in potassium hydroxide solution (5.5 $mol \cdot L^{-1}$, 500 mL) and cooled to 0°C. Acetic anhydride (113 mL, 1.2 mol) was slowly dripped into the solution with stirring. The mixture was stirred at room temperature for 2 h and then extracted with ethyl acetate. The organic layer was successively washed with saturated sodium bicarbonate, saturated sodium chloride, water and dried over Na_2SO_4, at last ethyl acetate was removed to give **3** (125.5 g, 76.3%).

3-(1-chlorine) ethyl benzoic acid ethyl ester (4): Compound **3** (125.5 g, 0.7 mol) was dissolved in the solution of distilled DCM (500 mL) and DMF (123 mL, 1.6 mol). Purified $SOCl_2$ was added slowly into the solution (67 mL, 1.1 mol) and the reaction was stirred at room temperature for 2 h. Then the mixture was slowly added into crushed ice, adjusted pH to 7~8. The organic layer was separated, and the aqueous layer was extracted three times with chloroform. The combined organic layer was washed with water and dried over Na_2SO_4. The organic solvent was evaporated, and then the residue was chromatographed on silica gel using petroleum ether to give compound **4** (127.0 g, 91.4%).

General procedure A for the syntheses of compounds (5a~5g): Taking **5a** as an example: Compounds **4** (12.0g, 60 mmol), KI (80 mmol), K_2CO_3 in acetonitrile (200 mL), and N-dimethylamine hydrochloride (200 mmol) were mixed together, the mixture was stirred at 45°C for 24 h. The solvent was removed, then the residues were dissolved in water and the solution was acidified to pH 4~5. The aqueous solution was washed three times with ethyl acetate and adjusted to pH 8, then extracted with ethyl acetate. After removal of ethyl acetate, compound **5a** (4.0 g, 40.1%) was obtained.

General procedure B for the synthesis of compounds (6a~6g): Taking **6a** as an example: **5a** (3.8 g, 23.0 mmol) and sodium carbonate (1.5 g, 14.0 mmol) were dissolved in DCM. Then acetic anhydride (2.6 mL, 28 mmol) was added slowly in the above mixture at 0°C. The reaction mixture was stirred at room temperature and monitored by TLC. When the reaction was completed, dichloromethane was removed under vacuum. The solution of the residue was dissolved in water, acidified to pH 4~5 and stirred for another 0.5 h. Followed by the aqueous solution was washed with ethyl acetate, adjusted to pH 8 with Na_2CO_3, and then extracted with ethyl acetate, dried over Na_2SO_4, at last ethyl acetate was removed to give to give compound **6a** (4.3 g, 90.2%).

General procedure C for the synthesis of compounds (7a~7g): Taking **7a** as an example: **6a** (3.9 g, 19.0 mmol) and aluminium trichloride (12.6 g, 95.0 mmol) were dissolved in nitrobenzene (20 mL). Acetic anhydride was dissolved in nitrobenzene (20 mL) also, and added dropwise to the above reaction, stirred for 0.5 h at 150°C. Then the solution of Na_2CO_3 was added in slowly to adjust pH to 8. The aqueous solution was extracted with ethyl acetate, after dried over Na_2SO_4, ethyl acetate was removed to give compound **7a** (2.3 g, 58.1%).

General procedure D for the syntheses of compounds (w1~w22): Taking **w1** as an example: The compounds **7a** (13 mmol), DMAP (160.0 mg, 1.3 mmol), dimethylcarbamoyl chloride and TEA (156 mmol) were dissolved in THF (100 mL). Then the mixture was stirred at reflux for 2 h. When the reaction was completed, acidified to pH 4~5 with and washed with ethyl acetate. The aqueous layer was adjusted to pH 9 and extracted with ethyl acetate. After dried over Na_2SO_4, ethyl acetate was removed to give compound **w1**. yield 90.2%. ^1H NMR (400 MHz, $CDCl_3$): δ 7.74 (1H, d, J=8.0 Hz), 7.23 (1H, dd, J_1=8.0 Hz, J_2=1.2 Hz), 7.11 (1H, d, J=1.2 Hz), 3.30 (1H, q, J=6.4 Hz), 3.15 (3H, s), 3.05 (3H, s), 2.55 (3H, s), 2.22 (6H, s), 1.36 (3H, d, J=6.4 Hz). MS ($[M+H]^+$) m/z calcd for $C_{15}H_{22}N_2O_3$ 279.2; found 279.2.

Compound	R_1	R_2	Inhibition[a] (%)	IC_{50} (μM)
Rivastigmine	--	--	31.0[b]	1.3
w1			30.3	94.3
w2			18.7	--
w3			17.1	--
w4			9.8	--
w5			18.5	--
w6			14.6	--
w7			18.0	--
w8			44.9	31.9
w9			11.2	--
w10			30.2	61.8
w11			12.7	--
w12			6.2	--
w13			9.9	--
w14			6.8	--
w15			7.9	--
w16			6.9	--
w17			11.9	--
w18			4.4	--
w19			17.4	--
w20			21.9	--
w21			14.4	--
w22			24.9	80.3

Table 2: Inhibition of AChE activity and value of IC_{50}.

a: Inhibition of AChE activity of **w1~w22** were measured in 50 mM; **b:** Inhibition of AChE activity of rivastigmine was measured in 1μM

Figure 8: The molecular docking of representative compound **w8** with AChE.

Ethyl-methyl-carbamic acid 2-acetyl-5-(1-dimethylamino-ethyl)-phenyl ester (w2): Compound **w2** was obtained from compound **7a** and N-ethyl-N-methylcarbamoyl chloride by general procedure D; yield 90.2%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.65 (1H, d, J=8.0 Hz), 7.14 (1H, d, J=8.0 Hz), 7.02 (1H, d, J=6.8 Hz), 3.45 (1H, q, J=6.8 Hz), 3.35 (1H, q, J=6.8 Hz), 3.24 (1H, m), 3.03 (1.5H, s), 2.92 (1.5H, s), 2.47 (3H, s), 2.14 (6H, s_2), 1.29 (3H, d, J=6.0 Hz), 1.21 (1.5H, t, J=7.2 Hz), 1.12 (1.5H, t, J=7.2 Hz). MS ([M+H]$^+$) m/z calcd for $C_{16}H_{24}NO_2$ 293.2; found 293.2.

Diethyl-carbamic acid 2-acetyl-5-(1-dimethylamino-ethyl)-phenyl ester (w3): Compound **w3** was obtained from compound **7a** and diethylcarbamyl chloride by general procedure D; yield 90.4%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.71 (1H, d, J=8.0 Hz), 7.21 (1H, d, J=8.0 Hz), 7.09 (1H, s), 3.50 (2H, q, J=7.2 Hz), 3.38 (2H, q, J=7.2 Hz), 3.26 (1H, q, J=6.8 Hz), 2.54 (3H, s), 2.21 (6H, s), 1.35 (3H, d, J=6.8 Hz), 1.29 (3H, t, J=7.2 Hz), 1.21 (3H, t, J=7.2 Hz). MS ([M+H]$^+$) m/z calcd for $C_{17}H_{26}N_2O_3$ 307.2; found 307.2.

Diethyl-carbamic acid 2-acetyl-5-(1-dimethylamino-ethyl)-phenyl ester (w4): Compound **w4** was obtained from compound **7a** and 1-pyrrolidinecarbonyl chloride by general procedure D; yield 89.7%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73 (1H, d, J=8.0 Hz), 7.22 (1H, d, J=8.0 Hz), 7.14 (1H, s), 3.62 (2H, t, J=6.8 Hz), 3.49 (2H, t, J=6.8 Hz), 3.29 (1H, q, J=6.8 Hz), 2.56 (3H, s), 2.21 (6H, s), 1.98 (4H, m), 1.36 (3H, d, J=6.4 Hz). MS ([M+H]$^+$) m/z calcd for $C_{17}H_{24}N_2O_3$ 307.2; found 305.2.

Piperidine-1-carboxylic acid 2-acetyl-5-(1-dimethylamino-ethyl)-phenyl ester (w5): Compound **w5** was obtained from compound **7a** and piperidine-1-carbonyl chloride by general procedure D; yield 90.6%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73 (1H, d, J=8.0 Hz), 7.22(1H, dd, J_1=8.0 Hz, J_2=1.6 Hz), 7.10 (1H, d, J=1.6 Hz), 3.65 (2H, m), 3.52 (2H, m), 3.29 (1H, q, J=6.8 Hz), 2.55 (3H, s), 2.21 (6H, s), 1.67 (6H, m), 1.36 (3H, d, J=6.8 Hz). MS ([M+H]$^+$) m/z calcd for $C_{18}H_{26}N_2O_3$ 307.2; found 319.2.

Synthesis of Morpholine-4-carboxylic acid 2-acetyl-5-(1-dimethylamino-ethyl)- phenyl ester (w6): Compound **w6** was obtained from compound **7a** and morpholine-4-carbonyl chloride by general procedure D; yield 91.8%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75 (1H, d, J=8.0 Hz), 7.25 (1H, dd, J_1=8.0 Hz, J_2=1.2 Hz), 7.12 (1H, d, J=1.2 Hz), 3.78 (5H, m), 3.72 (1H, m), 3.56 (2H, t, J=4.4 Hz), 3.27 (2H, t, J=4.4 Hz), 2.55 (3H, s), 2.21 (6H, s), 1.35 (3H, d, J=6.4 Hz). MS ([M+H]$^+$) m/z calcd for $C_{17}H_{24}N_2O_4$ 307.2; found 321.2.

Dimethyl-carbamic acid 2-acetyl-5-(1-diethylamino-ethyl)-phenyl ester (w7): Compound **w7** was obtained from compound **7c** and dimethylcarbamoyl chloride by general procedure D; yield 88.6%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73 (1H, d, J=8.0 Hz), 7.30 (1H, dd, J_1=8.0 Hz, J_2=1.2 Hz), 7.16 (1H, d, J=1.2 Hz), 3.82 (1H, q, J=6.8 Hz), 3.15 (3H, s), 3.03 (3H, s), 2.55 (3H, s), 2.52 (4H, m), 1.32 (3H, d, J=6.8 Hz), 0.99 (6H, t, J=6.8 Hz). MS ([M+H]$^+$) m/z calcd for $C_{17}H_{26}N_2O_3$ 307.2; found 307.2.

Ethyl-methyl-carbamic acid 2-acetyl-5-(1-diethylamino-ethyl)-phenyl ester (w8): Compound **w8** was obtained from compound **7c** and N-ethyl-N-methylcarbamoyl chloride by general procedure D; yield 88.7%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.72 (1H, d, J=8.0 Hz), 7.29 (1H, dd, J_1=8.0 Hz, J_2=1.2 Hz), 7.15 (1H, d, J=8.0 Hz), 3.81 (1H, q, J=6.4 Hz), 3.53 (q, 1H, J=6.8 Hz, -$CONCH_2CH_3$), 3.42 (q, 1H, J=6.8 Hz, -$CONCH_2CH_3$), 3.12 (s, 1.5H), 3.00 (1.5H, s), 2.55 (3H, s), 2.53 (4H, m), 1.32 (3H, d, J=6.8 Hz), 1.19 (3H, m), 0.99 (6H, t, J=6.8 Hz). MS ([M+H]$^+$) m/z calcd for $C_{18}H_{28}N_2O_3$ 321.2; found 321.2.

Diethyl-carbamic acid 2-acetyl-5-(1-diethylamino-ethyl)-phenyl ester (w9): **w9** was obtained from compound **7c** and diethylcarbamyl chloride by general procedure D; yield 89.3%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.71 (1H, dd, J_1=8.0 Hz, J_2=2.4 Hz), 7.28 (1H, dd, J_1=8.0 Hz, J_2=1.6 Hz), 7.13 (1H, d, J=1.2 Hz), 3.81 (1H, m), 3.49 (2H, m), 3.38 (2H, m), 2.54 (3H, s), 2.52 (4H, m), 1.30 (6H, m), 1.21 (3H, m), 0.98 (6H, m,). MS ([M+H]$^+$) m/z calcd for $C_{19}H_{30}N_2O_3$ 335.2; found 335.2.

Pyrrolidine-1-carboxylic acid 2-acetyl-5-(1-diethylamino-ethyl)-phenyl ester (w10): Compound **w10** was obtained from compound **7c** and 1-pyrrolidinecarbonyl chloride by general procedure D; yield 87.5%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73 (1H, d, J=8.0 Hz), 7.29 (1H, dd, J_1=8.0 Hz, J_2=1.6 Hz), 7.14 (1H, d, J=1.2 Hz), 3.81 (1H, q, J=6.8 Hz), 3.62 (2H, t, J=6.8 Hz), 3,49 (2H, t, J=6.8 Hz), 2.55 (3H, s), 2.53 (4H, m), 1.97 (4H, m), 1.31 (3H, d, J=6.4 Hz), 0.99 (6H, t, J=7.2 Hz). MS ([M+H]$^+$) m/z calcd for $C_{19}H_{28}N_2O_3$ 335.2; found 335.2.

4-Methyl-piperazine-1-carboxylic acid 2-acetyl-5-(1-diethylaminoethyl)-phenyl ester (w11): Compound **w11** was obtained from compound **7c** and 4-Methyl-piperazine-1-carbonyl chloride by general procedure D; yield 80.2%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73 (1H, d, J=8.0 Hz), 7.32 (1H, d, J=8.0 Hz), 7.16 (1H, s), 3.82 (1H, q, J=6.8 Hz), 3.74 (2H, m), 3.60 (2H, m), 2,54 (3H, s), 2.50 (8H, m), 2.36 (s, 3H, -NCH_3), 1.32 (3H, d, J=6.8 Hz), 0.99 (6H, t, J=7.2 Hz,). MS ([M+H]$^+$) m/z calcd for $C_{20}H_{31}N_3O_3$ 362.2; found 362.2.

Morpholine-4-carboxylic acid 2-acetyl-5-(1-diethylamino-ethyl)-phenyl ester (w12): Compound **w12** was obtained from compound **7c** and morpholine-4-carbonyl chloride by general procedure D; yield 90.2%. ^{1}H NMR (400 MHz, $CDCl_3$): δ7.74 (1H, d, J=8.0 Hz), 7.32 (1H, dd, J_1=8.0 Hz, J_2=1.2 Hz), 7.17 (1H, d, J=1.6 Hz), 3.78 (4H, m), 3.83 (1H, m), 3.72 (2H, m), 3.60 (2H, m), 2.54 (3H, s), 2.52 (4H, m), 1.32 (3H, d, J=6.4 Hz), 0.99 (6H, t, J=7.2 Hz). MS ([M+H]$^+$) m/z calcd for $C_{19}H_{28}N_2O_4$ 349.2; found 349.2.

Dimethyl-carbamic acid 2-acetyl-5-(1-morpholin-4-yl-ethyl)-phenyl ester (w13): Compound **w13** was obtained from compound **7f** and dimethylcarbamoyl chloride by general procedure D; yield 90.4%.^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73 (1H, d, J=8.0 Hz), 7.25 (1H, dd, J_1=8.0 Hz, J_2=1.6 Hz), 7.12 (1H, d, J=2.0 Hz), 3.69 (4H, m), 3.33 (1H, q, J=6.8 Hz,), 3.15 (3H, s), 3.03 (3H, s), 2.54 (3H, s), 2.49 (2H, m), 2.37 (2H, m), 1.33 (3H, d, J=6.8 Hz,). MS ([M+H]$^+$) m/z calcd for $C_{17}H_{24}N_2O_4$ 3321.2; found 321.2.

Ethyl-methyl-carbamic acid 2-acetyl-5-(1-morpholin-4-yl-ethyl)-phenyl ester (w14): Compound **w14** was obtained from compound **7f** and N-ethyl-N-methylcarbamoyl chloride by general procedure D; yield 89.9%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.72 (1H, d, J=8.0 Hz), 7.25 (1H, d, J=8.0 Hz), 7.11 (1H, d, J=10.0 Hz), 3.69 (4H, m), 3.53 (1H, q, J=7.2 Hz), 3.41 (1H, q, J=7.2 Hz), 3.33 (1H, q), 3.12 (1.5H, s), 3.00 (1.5H, s), 2.54 (3H, s), 2.49 (2H, m), 2.38 (2H, m), 1.33 (3H, d, J=6.4 Hz), 1.27 (1.5H, t, J=7.2 Hz), 1.21 (1.5H, t, J=7.2 Hz). MS ([M+H]$^+$) m/z calcd for $C_{18}H_{26}N_2O_4$ 3321.2; found 335.2.

Diethyl-carbamic acid 2-acetyl-5-(1-morpholin-4-yl-ethyl)-phenyl ester (w15): Compound **w15** was obtained from compound **7f** and diethylcarbamyl chloride by general procedure D; yield 91.2%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.63 (1H, d, J=8.0 Hz), 7.18 (1H, d, J=8.0 Hz), 7.02 (1H, s), 3.47 (4H, m), 3.40 (2H, q, J=7.2 Hz), 3.23 (3H, m), 2.35 (5H, m), 2.22 (2H, m), 1.19 (3H, d, J=6.8 Hz), 1.16 (3H, t, J=7.2 Hz), 1.03 (3H, t, J=6.8 Hz,). MS ([M+H]$^+$) m/z calcd for $C_{19}H_{28}N_2O_4$ 349.2; found 349.2.

Pyrrolidine-1-carboxylic acid 2-acetyl-5-(1-morpholin-4-yl-ethyl)-phenyl ester (w16): Compound **w16** was obtained from compound **7f** and 1-pyrrolidinecarbonyl chloride by general procedure

D; yield 91.2%; yield 87.5%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.66 (1H, d, J=8.0 Hz), 7.18 (1H, d, J=8.0 Hz), 7.08 (1H, s), 3.62 (4H, m), 3.55 (2H, t, J=6.4 Hz), 3.42 (2H, t, J=6.4 Hz), 3.22 (1H, m), 2.48 (3H, s), 2.42 (2H, m), 2.36 (2H, m), 1.90 (4H, m), 1.26 (3H, d, J=6.0 Hz,). MS ([M+H]$^+$) m/z calcd for $C_{19}H_{26}N_2O_4$ 347.2; found 347.2.

Piperidine-1-carboxylic acid 2-acetyl-5-(1-morpholin-4-yl-ethyl)-phenyl ester (w17): Compound **w17** was obtained from compound **7f** and piperidine-1-carbonyl chloride by general procedure D; yield 88.6%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73 (1H, d, J=8.0 Hz), 7.25 (1H, d, J=8.0 Hz), 7.11 (1H, s), 3.69 (4H, m), 3.66 (2H, m), 3.52 (2H, m), 3.33 (1H, q, J=6.4 Hz), 2.54 (3H, s), 2.49 (2H, m), 2.39(2H, m), 1.68 (6H, m), 1.33 (3H, d, J=6.4 Hz). MS ([M+H]$^+$) m/z calcd for $C_{20}H_{28}N_2O_4$ 360.4; found 360.4.

Morpholine-4-carboxylic acid 2-acetyl-5-(1-morpholin-4-yl-ethyl)-phenyl ester (w18): Compound **w18** was obtained from compound **7f** and morpholine-4-carbonyl chloride by general procedure D; yield 90.0%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75 (1H, d, J=8.0 Hz), 7.27 (1H, d, J=8.0 Hz), 7.14 (1H, s), 3.79 (4H, m), 3.69 (6H, m), 3.58 (2H, m), 3.35 (1H, q, J=6.4 Hz), 2.55 (3H, s), 2.49 (2H, m), 2.38 (2H, m), 1.34 (3H, d, J=6.4 Hz). MS ([M+H]$^+$) m/z calcd for $C_{19}H_{26}N_2O_5$ 363.2; found 363.2.

Ethyl-methyl-carbamic acid 2-acetyl-5-[1-(ethyl-methyl-amino)-ethyl]-phenyl ester (w19): Compound **w19** was obtained from compound **7b** and N-ethyl-N-methylcarbamoyl chloride by general procedure 4.7.1; yield 91.6%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73 (1H, d, J=8.0 Hz), 7.24 (1H, dd, J_1=8.0 Hz, J_2=1.6 Hz), 7.11 (1H, d, J=8.0 Hz), 3.54 (2H, m), 3.41 (1H, q, J=6.8 Hz,), 3.11 (1.5H, s), 3.00 (1.5H, s), 2.54 (3H, s), 2.49 (1H, m), 2.36 (1H, m), 2.19 (3H, s), 1.33 (3H, d, J=6.8 Hz), 1.28 (1.5H, t, J=6.8 Hz), 1.20 (1.5H, t, b, J=6.8 Hz), 1.02 (3H, t, J=6.8 Hz,). MS ([M+H]$^+$) m/z calcd for $C_{17}H_{26}N_2O_3$ 307.2; found 307.2.

Ethyl-methyl-carbamic acid 2-acetyl-5-(1-pyrrolidin-1-yl-ethyl)-phenyl ester (w20): Compound **w20** was obtained from compound **7d** and N-ethyl-N-methylcarbamoyl chloride by general procedure D; yield 87.7%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.72 (1H, d, J=8.0 Hz), 7.25 (1H, dd, J_1=8.0 Hz, J_2=1.2 Hz), 7.13 (1H, d, J=6.8 Hz), 3.52 (1H, q, J=6.8 Hz), 3.41 (1H, q, J=6.8 Hz), 3.22 (1H, q, J=6.8 Hz), 3.11 (1.5H, s), 3.00 (1.5H, s), 2.54 (3H, s), 2.49 (2H, m), 2.39 (2H, m), 1.78 (4H, m), 1.38 (3H, d, J=6.8 Hz), 1.28 (1.5H, m), 1.20 (1.5H, m). MS ([M+H]$^+$) m/z calcd for $C_{18}H_{26}N_2O_3$; found 319.2.

Ethyl-methyl-carbamic acid 2-acetyl-5-(1-piperidin-1-yl-ethyl)-phenyl ester (w21): Compound **w21** was obtained from compound **7e** and N-ethyl-N-methylcarbamoyl chloride by general procedure D; yield 86.9%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.72 (d, 1H, J=8.0 Hz, ArH), 7.24 (d, 1H, J=8.0 Hz, ArH), 7.09 (d, 1H, J=8.4 Hz, ArH), 3.52 (m, 3H, -$CHCH_3$, -CH_2CH_3), 3.43 (m, 3H, -NCH_3), 2.54 (s, 3H, -$COCH_3$), 2.37 (m, 4H, piperidine), 1.54 (m, 4H, piperidine), 1.40 (m, 2H, piperidine), 1.34 (d, 3H, J=6.8 Hz, -$CHCH_3$), 1.20 (m, 3H, -CH_2CH_3). MS ([M+H]$^+$) m/z calcd for $C_{19}H_{28}N_2O_3$; found 332.2.

Ethyl-methyl-carbamic acid 2-acetyl-5-[1-(4-methyl-piperazin-1-yl)-ethyl]-phenyl ester (w22): Compound **w22** was obtained from compound **7g** and N-ethyl-N-methylcarbamoyl chloride by general procedure 4.7.1; yield 85.3%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.71 (1H, d, J=8.0 Hz), 7.24 (1H, d, J=8.0 Hz), 7.10 (1H, d, J=9.2 Hz), 3.52 (1H, m), 3.39 (2H, m), 3.11 (1.5H, s), 3.00 (1.5H, s), 2.53 (3H, d, J=2.0 Hz), 2.47 (8H, m), 2.32 (3H, d, J=2.0 Hz), 1.33 (3H, dd, J_1=6.4 Hz, J_2=2.8 Hz), 1.29 1.5H, (m), 1.20 (1.5H, m). MS ([M+H]$^+$) m/z calcd for $C_{19}H_{29}N_3O_3$; found 348.2.

Spectrophotometric measurement of complex with Cu^{2+} and Fe^{2+}

All compounds were tested as metal chelators, using difference UV-Vis spectra recorded in methanol at 298 K with wavelength ranging from 210 nm to 400 nm. Numerical subtraction of the spectra of the metal alone and the compound alone from the spectra of the mixture obtained the difference UV-Vis spectra due to complex formation. A fixed amount of **9f** (25 μmol·L^{-1}) was mixed with growing amounts of copper ion (1.25 μmol·L^{-1} to 50 μmol·L^{-1}) and tested the difference UV-Vis spectra to investigate the ratio of ligand/metal in the complex.

Study of AChE hydrolysis

Compound **w13** 20 μL (3 mol·L^{-1}) was added into PBS buffer (pH 7.4) 160 μL, 0.05% (v/v) Triton X-100 10 μL and rat AChE homogenate, was incubated at 37°C. After 0.1 h, 0.5 h, 1 h, 3 h, and 6 h, 20 μL was taken out from the incubating mixture with a pipette gun, extracted with ethyl acetate, combined with organic layer, dried by anhydrous sodium sulfate and evaporated solvent. The residue was dissolved in acetonitrile and analyzed with HPLC. HPLC analyses was conducted on Dikma Technologies C18 (symmetry: 250 mm × 4.6 μm) column at 35°C, eluent: acetonitrile: 0.1% ethylamine aqueous solution=6:4, flow rate: 1 mL/min. detection at 254 nm.

Conflict of Interest

The authors declare no competing financial interest.

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