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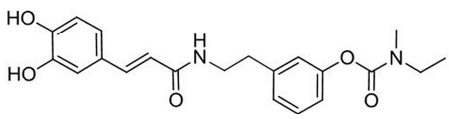

Compound **5**

Inhibitary of ChE (AChE 91.5%, BuChE 23.99% at 5 μM)
Inhibitary of Aβ self-aggregation
Inhibitary of H_2O_2-induced neuronal cell death
Chelator of Copper

Discovery of novel rivastigmine-hydroxycinnamic acid hybrids as multi-targeted agents for Alzheimer's disease

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Abstract:

A series of rivastigmine-caffeic acid and rivastigmine-ferulic acid hybrids were designed, synthesized, and evaluated as multifunctional agents for Alzheimer's disease (AD) *in vitro*. The new compounds exerted antioxidant neuroprotective properties and good cholinesterases (ChE) inhibitory activities. Some of them also inhibited amyloid protein (Aβ) aggregation. In particular, compound **5** emerged as promising drug candidates endowed with neuroprotective potential, ChE inhibitory, Aβ self-aggregation inhibitory and copper chelation properties. These data suggest that compound **5** offers an attractive starting point for further lead optimization in the drug-discovery process against AD.

Key words: rivastigmine; caffeic acid; ferulic acid; hybrid; multifunctional agents; Alzheimer's disease

Introduction

Alzheimer's disease (AD) is the main cause of dementia and one of the great healthcare challenges of the 21st century. In December, 2013, the G8 stated that dementia should be made a global priority and their ambition that a cure or a disease-modifying therapy should be available by 2025 [1]. It starts with mild cognitive impairment, which advances to severe dementia and ultimately death. AD shows highly complex pathological processes that result in the accumulation of abnormal deposits of β-amyloid peptide (Aβ) and hyper-phosphorylated tau protein as well as massive cell death and loss of synapses, especially in the cholinergic system [2].

Given that the pathogenesis of AD is complex and related to the dysfunction of multi-systems, the prevention and treatment of AD have remained elusive. Currently, the therapeutic options for the treatment of AD are limited to three acetylcholinesterase (AChE) inhibitors donepezil, rivastigmine, galantamine and one N-methyl-D-aspartate receptor antagonist, memantine. Unfortunately, these drugs merely stabilize some symptoms of early to mid-stage forms of AD for a limited period of time. The complex interconnection of the molecular events underpinning AD is thought to be one of the explanations why the single-target directed drugs reached clinical trials failed. Thus, multi-target-directed ligands (MTDLs) are considered as an effective way for the treatment of neurodegenerative diseases [3-5].

One hallmark of AD is the presence of senile plaques in the hippocampus. Aβ oligomerization and aggregation are thought to play a key role in AD pathogenesis and progression [6,7]. In addition, increases of damaged cellular macromolecules such as nucleic acids, proteins, lipids has been observed in AD brains. These findings support reactive oxygen species (ROS) plays a pivotal role in AD onset and progression. Therefore, antioxidants should be beneficial therapeutic tools in AD treatment [8]. Small molecules targeting Aβ, ROS and cholinesterase simultaneously might be more effective for the treatment of AD. Actually, many multifunctional agents have been synthesized as therapeutics that interact simultaneously with two or more targets such as AChE, Aβ, tau protein, monoamine oxidase, metal ions, ROS and many others [9-17]. Although the advantages of multi-target are clear, the combination of multi-target "functionalities" in one molecule and the identification of the most appropriate therapeutic targets to select still remain the critical points.

Previously, some group and we reported that hybrids combining hydroxycinnamic acids and ChE inhibitory moieties have potential MTDLs' profile for the therapy of AD [13, 18-21]. For example, T3CA, a tacrine-caffeic acid hybrid has MTDLs' profiles of AChE inhibitor, free radical scavenger, inhibitor of both self- and AChE-induced Aβ aggregation, as well as potent neuroprotectant against H_2O_2- and glutamate- induced cell injury with low toxicity in HT22 cells [19]. Continuing with our interest in the design, synthesis, and

biological evaluation of multifunctional molecules, our work are currently focused on taking advantage of the potential anti-ROS profile of caffeic acid- and ferulic acid- based hybrids, which are endowed with additional anticholinergic properties.

Butyrylcholinesterase (BuChE) plays several roles both in neural and non-neural functions. Recent observations suggest that rather than selective inhibition of AChE, the inhibition of BuChE may be more effective, especially in neurodegenerative diseases such as AD [22, 23]. Several lines of evidence indicate that high cortical levels of BuChE are associated with some important AD hallmarks, such as the extracellular deposition of the Aβ and the aggregation of hyper-phosphorylated tau protein. Consistent with this suggestion, the use of acetyl- and butyryl- cholinesterase inhibitors could improve clinical efficacy with respect to selective AChE inhibitors. On this basis, our study describes the preparation and *in vitro* activities of novel rivastigmine-hydroxycinnamic acid hybrids as inhibitors of AChE and BuChE, ROS scavengers and inhibitors of Aβ aggregation. In addition, the toxicity as well as the neuroprotective activity against H_2O_2- and glutamate-induced cytotoxicity in HT22 cell lines were also assessed.

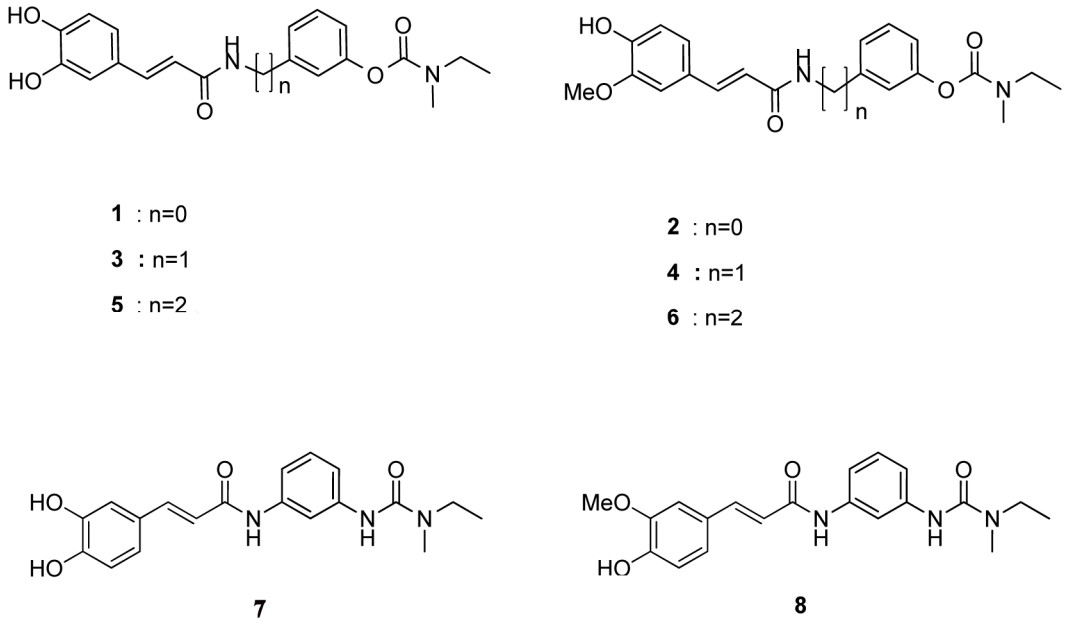
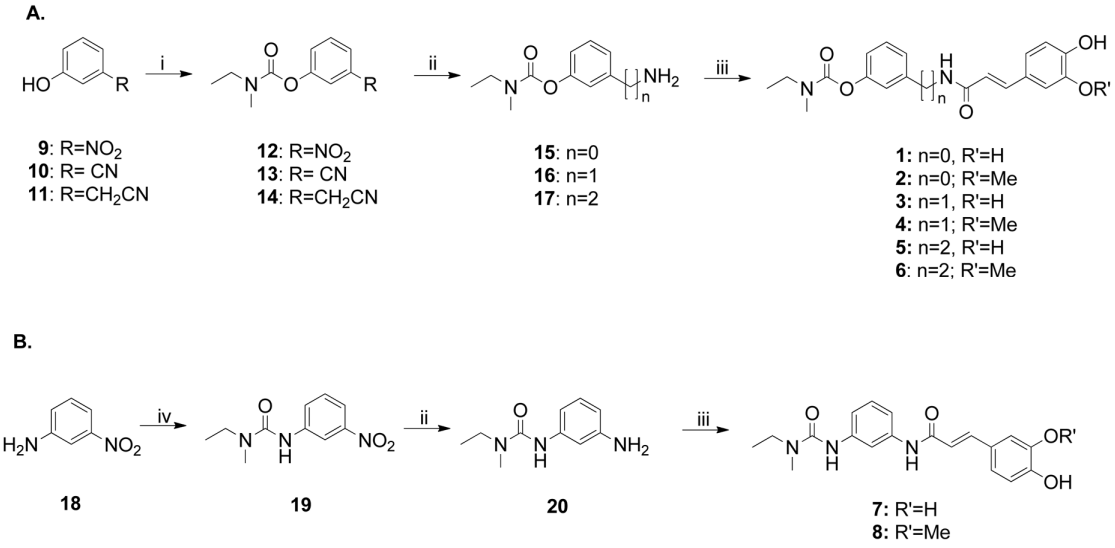


Figure 1 Design of rivastigmine-hydroxycinnamic acid hybrids 1-8.

Chemistry

The small series of N-ethyl-N-methylcarbamoil derivatives **1-6** and the ureido-derivatives **7,8** were synthesized as reported in Scheme 1. The carbamate **12-14**, synthesized by the condensation of N-ethyl-methylcarbamoylchloride with the appropriate 3-substituted-phenol **9-11**, was subjected to a catalytic hydrogenation to give the amine derivative **15-17**. The subsequent condensation of caffeic or ferulic acid with the amine **15-17**, using dicyclohexylcarbodiimide (DCC) as condensing reagent, gave the corresponding amide-product **1-6**. The intermediate **19** was obtained from the 3-nitro-aniline, through the formation of the corresponding isocyanate and subsequent reaction with the N-ethyl methylamine (Scheme 1B). The catalytic hydrogenation of **19** afforded the aniline derivative

20, which subjected to the condensation with caffeic acid or ferulic acid in the presence of DCC, as a condensing reagent, to give the hydroxycinnamamide hybrids **7** and **8**, respectively.



Scheme 1. **Synthesis of rivastigmine-hydroxycinnamic acid hybrids 1-8.** Reagents and conditions: (i) N-ethylmethylcarbamoyl chloride,TEA, 95°C, 15h; (ii) EtOH, Pd/C, H_2, rt, 12h; (iii) DCC, THF, caffeic acid or ferulic acid, 70°C, reflux, 7h; (iv)triphosgene, N-ethylmethylamine,TEA, THF,12h.

Results and discussion

AChE and BuChE inhibitory activity. Synthesized compounds were tested for the inhibitory activity of AChE and BuChE. Donepezil (AChE inhibitor) and rivastigmine (BuChE inhibitor) were used as positive drugs. The data were summarized in Table 1.

Interestingly, all the compounds synthesized have a good affinity against both cholinesterases. Noteworthy, the compounds showed to be more potent than rivastigmine against AChE. Within the brain, BuChE is primarily expressed and secreted by glial cells [24] and it is thought to play a compensatory role in response to the decrease of AChE activity in the brain as AD progresses [25]. Indeed, inhibition of central BuChE activity has also been investigated as a potential therapeutic approach to ameliorate the cholinergic deficit in moderate forms of AD [26]. As showed in Table 1, most compounds (**2**, **3**, **4**, **5**, and **6**) exerted stronger inhibition against BuChE than rivastigmine at 1 μM. Rivastigmine-caffeic acid and rivastigmine-ferulic acid hybrids retained the rivastigmine ChE inhibitory moiety, and conjunction with caffeic acid or ferulic acid seemly enhanced the cholinesterase inhibition effect of the "native" drug (i.e. rivastigmine).

Table 1. Inhibitory effects of compounds 1-8 against AChE and BuChE activity.

Compounds	Inhibition rate % AChE		Inhibition rate % BuChE	
	1 μM	5 μM	1 μM	5 μM
Donepezil	95.19	96.81	14.78	44.56
Rivastigmine	6.77	15.56	61.04	92.99
1	16.96	16.75	49.62	90.96
2	18.56	24.62	98.04	98.34
3	21.24	23.3	80.85	97.00
4	23.83	34.28	87.86	97.86
5	23.42	23.99	76.32	91.95
6	24.43	70.66	72.30	96.59
7	18.37	16.40	13.81	24.43
8	12.58	14.93	19.45	36.60

DPPH radical scavenging activities. Previous studies showed that both caffeic acid and ferulic acid exerted radical scavenging properties and their combination with other drugs retained the native drugs the antioxidant properties [21]. Compounds were tested for their radical scavenging activities by using 1,1-diphenyl-2- picryl-hydrazyl (DPPH) assay. All compounds exerted good radical scavenging activity. In particular, compound **3** and **7** showing 69.7 % and 64.7% of radical scavenging activities, respectively (Table 2).

Inhibition of $A\beta_{1-42}$ self-aggregation. It is well-known that polyphenols, including caffeic acid, inhibit the Aβ self-aggregation [27]. Recently, many evidence suggested that the inhibitory effect on $A\beta_{1-42}$ self-aggregation is strongly related to the non-cholinergic functions of AchE [28]. Previous studies demonstrated that tacrine-caffeic acid and tacrine-ferulic acid hybrids inhibit amyloid protein self-aggregation [13, 18, 29]. In order to evaluate the influence of the tested compounds on amyloid fibrils, a Th-T fluorescence assay was performed. Compounds were tested at 10 μM and the results are reported in Table 2. Results showed that compounds **5** and **7** were able to inhibit the Aβ aggregation with % values of 85.3% and 82.5% respectively, which are comparable to that of Congo red, the positive drug (86.5%). Moreover, the compounds **1** and **3** also showed a significant inhibitory activity. Overall, the data indicated that the rivastigmine-caffeic acid hybrids (**1**, **3**, **5** and **7**) potently inhibited the $A\beta_{1-42}$ self-aggregation, while those with ferulic acid seem to induce only a mild effect.

Table 2. Inhibitory effects of compounds 1-8 on DPPH radical scavenging activities and $A\beta_{1-42}$ self-aggregation.

Compouds (10 μM)	DPPH scavenging abilities (%)	Inhibition of $A\beta_{1-42}$ self-aggregation (%)
Caffeic acid	64.6	NA
Ferulic acid	19.9	NA
1	37.0	73.2
2	19.5	13.2
3	69.7	77.6
4	17.9	3.2
5	51.7	85.3
6	18.1	2.2
7	64.7	82.5
8	24.1	2.2
Congo red	NA	86.5

NA: Not available

Molecular docking compounds 5 and 7 with Aβ. Aβ consists of a hydrophobic C-terminal domain (residues 29-42) and an N-terminal domain (amino acids 10-24) which is responsible for the conversion from a non-toxic α-helical form to a toxic β-sheet structure [30]. It is well-known that molecules able to hinder this conversion could thus prevent the fibril formation. In order to explore the binding modes of the active compounds **5** and **7** with Aβ, a molecular docking experiment was performed. The structure of Aβ used was downloaded from the Protein Data Bank (PDB: 1BA4) [28]. The binding modes of the active compounds and Aβ predicted using DOCK6. As shown in Figure 2, compounds **5** and **7** were locked at the C-terminus hydrophobic area of Aβ. Compound **5** formed five hydrogen bonds with the

residues of Asp1, Asp 7, Asp23, Gly9, and Lys16. Compound **7** showed similar binding modes with Aβ. It formed four hydrogen bonds with the residues Asp1, Asp23, Glu3, and Lys16. Both compounds **5** and **7** formed the hydrophobic interactions with the residues Phe19, Phe20 and Lys16. Moreover, the phenyl group of **5** formed π-cation interaction with the residue of Lys16. These hydrogen bonds and hydrophobic interactions might be favorable for the binding of Aβ and compounds **5** and **7**. This might provide the molecular basis for interpreting the biological data, and some general considerations for future design.

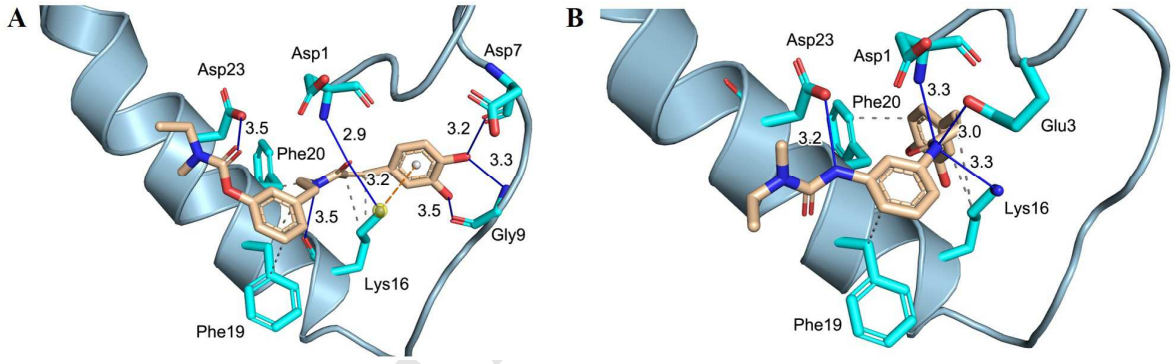


Figure 2 Predicted binding modes of compounds 5 and 7 with Aβ (PDB ID: 1BA4). The blue lines represent hydrogen bonds, the gray dashed lines represent hydrophobic interaction, the orange yellow lines stand for the π-cation interaction. Compounds **5** and **7** were locked at the -terminus hydrophobic area of Aβ (PDB: 1BA4). Compound **5** formed five hydrogen bonds with the residues of Asp1, Asp 7, Asp23, Gly9, and Lys16. Compound **7** showed similar binding modes with Aβ. It formed four hydrogen bonds with the residues Asp1, Asp23, Glu3, and Lys16. Both compounds **5** and **7** formed the hydrophobic interactions with the residues Phe19, Phe20 and Lys16. Moreover, the phenyl group of **5**

formed π-cation interaction with the residue of Lys16.

Effects of compounds on the viability of HT22 cells. Compounds **1-8** (10-100 μM) were submitted to a preliminary evaluation on HT22 cell viability by using MTT reduction in order to evaluate the cytotoxicity . Cells were exposed to compounds for 24 h and then MTT assay was carried out. Data showed that no compound was cytotoxic at 10 μM, but all of the compounds induced cytotoxicity at 100 μM (Figure 3).

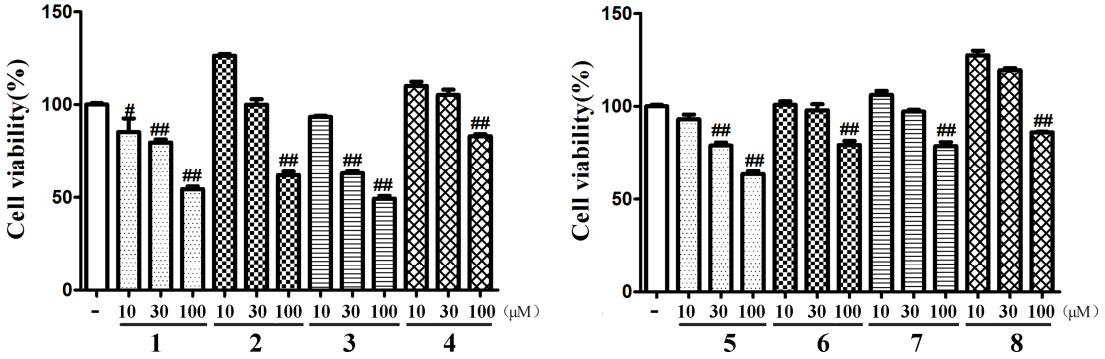


Figure 3 The toxicity of novel rivastigmine-hydroxycinnamic acid hybrids in HT22 cells. HT22 cells were exposure to compounds **1-8** at the indicated concentrations (10-100 μM) for 24 h. Viability of HT22 cells were measured by MTT assay. The data were represented as mean ± SEM, n = 6. $^{**}P< 0.01$ versus glutamate-treated alone cells, $^{\#\#}P< 0.01$ versus control).

Neuroprotective effects against glutamate- and H_2O_2-induced cell death. Given that the compounds showed DPPH radical scavenging activity, the neuroprotective effects against ROS-induced cell death were investigated in two *in vitro* models in HT22 cells, glutamate- and H_2O_2-induced cell death. Novel compounds were tested at 10 μM and 30 μM. The results (Figure 4A) showed that compounds **1** and **7** exerted good neuroprotective effects in glutamate-induced cell death (52.9% and 56.9% cell viability, respectively). Interestingly, all of compounds (except compound **4**) showed a significant protective effect in HT22 cell injury induced by H_2O_2 (Figure 4 B). It is well know that phenolic hydroxyl has strong anti-oxidative effect [31]. This effect may explain, at least in part, the stronger activity of the rivastigmine-caffeic acid hybrids than rivastigmine-ferulic in DPPH test and thus the improved capability to prevent the oxidative stress-induced cell death.

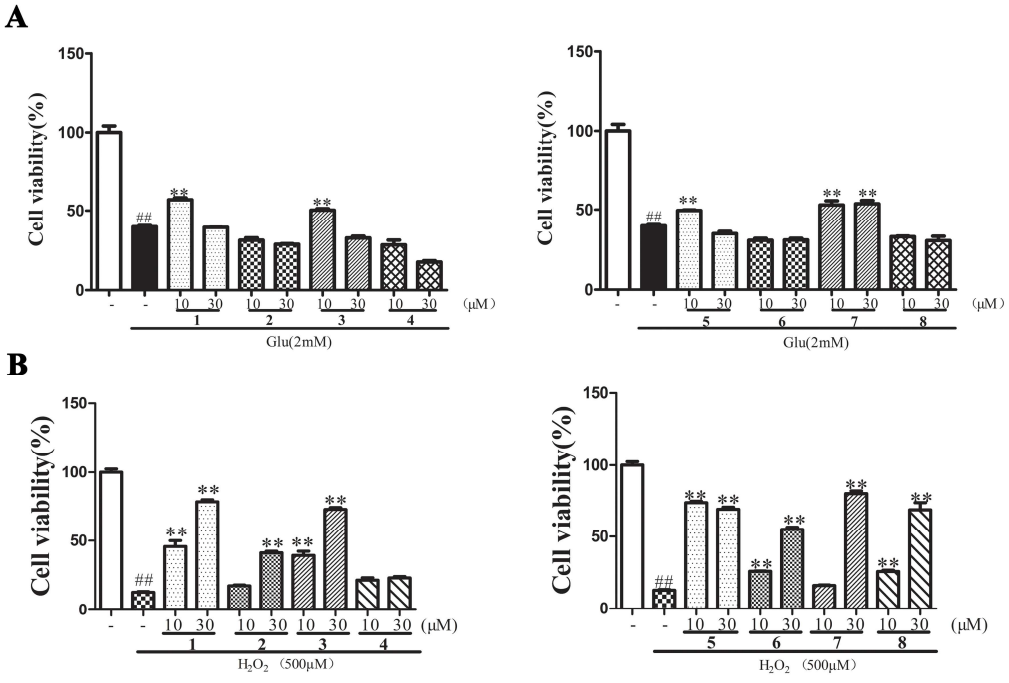


Figure 4 The neuroprotective effects of novel rivastigmine-hydroxycinnamic acid hybrids against glutamate- and H_2O_2-induced cell death in HT22 cells. (A) Neuroprotective effects of compounds on HT22 cells against cell injury induced by glutamate. HT22 cells were pretreated with/without compounds at the indicated concentrations for 30 min and then incubated with/without 2 mM glutamate for 24 h. The data were represented as mean ± SEM, n = 6. $^{**}P< 0.01$ versus glutamate-treated cells, $^{\#\#}P< 0.01$ versus control group). (B) Neuroprotective effects of compounds on HT22 cells against H_2O_2 induced cell death. HT22 cells were pretreated with/without compounds at the indicated concentrations for 30 min and then incubated with/without 500 μM H_2O_2 for 24 h. The data were represented as mean ± SEM, n = 6. $^{**}P< 0.01$ versus glutamate-treated cells, $^{\#\#}P< 0.01$ versus control group).

Copper-Chelating properties of 5. Mounting evidence demonstrated that redox-active metal ions such as Cu^{2+} contribute to the production of ROS [32], and elevated concentrations of Cu^{2+} has been detected by spectroscopic studies in Aβ plaques [33]. Cu^{2+} can interact with Aβ peptide thus promoting Aβ-aggregation in *in vitro* studies [34]. Therefore, modulation of Cu^{2+} in the brain might be benefit to the treatment of AD. Hence, the chelating ability of **5** toward Cu^{2+} was studied by UV–vis spectrometry [35]. As shown in Figure 5, the maximum absorption suffered a bathochromic shift upon the addition of Cu^{2+}, suggesting the formation of complex **5**–Cu^{2+}. According to previous report, phenolic

compounds can chelate with Cu^{2+} to inhibiting Cu^{2+}-induced Aβ aggregation and oxidative stress [36]. Chelating activity of Cu^{2+} of **5** might be due to its two phenolic hydroxyl groups .

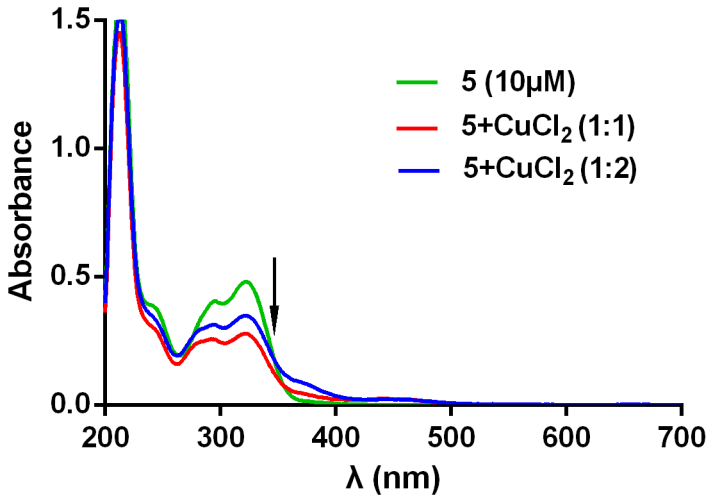


Figure 5 The copper chelating activity of 5. UV spectra of **5** (10 μM) alone (green line), treated with $CuCl_2$ (10 μM) (red line), and treated with $CuCl_2$ (20 μM) (blue line). $CuCl_2$ (dissolved in Milli-Q water) and **5** (dissolved in methanol and diluted in PBS), all agents were dissolved in absolute ethanol, with a final sample volume of 200 μL. The final sample was allowed to stand at room temperature for 30 min, and then the absorption spectrum was recorded at room temperature.

Conclusion

Developing a single molecule being able to strongly interact with structurally distinct targets is not an easy task, even using computer-aided drug design tools [37]. Here we

demonstrated that rivastigmine-hydroxycinnamic acid hybrids showed an interesting MTDL profiles. In particular, the most interesting hybrid **5** was demonstrated to inhibit the hydrolytic activity of ChE, to potentially prevent Aβ self-aggregation, to protect HT22 cells from glutamate and H_2O_2 induced cell death, to scavenge free radicals and to chelate copper . These data support that compound **5** could be an attractive lead compound for further optimization in the drug-discovery process against AD.

Experimental Section

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra of all compounds were obtained with a Bruker Advance 400 spectrometer at 400 MHz in a ~2% solution of $CDCl_3$, CD_3OD-d_4, Acetone-d_6 and DMSO-d_6. Chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane and referenced from solvent references. Coupling constants are reported in Hz. The elemental compositions of the compounds agreed to within ± 0.4% of the calculated value. Chromatographic separation was performed on silica gel columns by flash (Kieselgel 40, 0.040–0.063 mm; Merck). Reactions were followed by thin-layer chromatography (TLC) on Merck aluminum silica gel (60 F_{254}) sheets that were visualised under a UV lamp. Evaporation was performed *in vacuo* (rotating evaporator). Sodium sulphate was always used as the drying agent. Commercially available chemicals were purchased from Sigma-Aldrich.

General procedure for the synthesis of amides (1-8). A solution of the appropriate amine (0.93 mmol) in THF (5mL) was added to a mixture of the proper carboxylic acid (0.93 mmol), and DCC (192 mg, 0.93 mmol) in THF (10 mL). The mixture was stirred at reflux temperature for 7 h. After removal of the solvent, the crude residue was purified by flash chromatography.

(E)-3-(3-(3,4-dihydroxyphenyl)acrylamido)phenyl-(N-ethyl(methyl))carbamate (1)

Compound **1** was synthesized from caffeic acid (167 mg, 0.93 mmol) and 3-aminophenyl-N-ethyl(methyl)carbamate **15** (180 mg, 0.93 mmol). The crude product was purified by flash chromatography using $CHCl_3$/MeOH (9.5:0.5) as the eluent, and subsequent precipitation from AcOEt/*n*-hexane to give compound **1** as a yellow solid (278 mg, 0.78 mmol, 84% yield). mp 193-195 °C; ^{1}H NMR (Acetone-d_6) δ: 1.15 (t, 1.5H, *J* = 7.0 Hz, CH_3 rotamer), 1.24 (t, 1.5H, *J* = 7.0 Hz, CH_3 rotamer), 2.95 (s, 1.5H, CH_3 rotamer), 3.08 (s, 1.5H, CH_3 rotamer), 3.37 (q, 1H, *J* = 7.0 Hz, CH_2 rotamer), 3.49 (q, 1H, *J* = 7.0 Hz, CH_2 rotamer), 6.59 (d, 1H, *J* = 15.4 Hz, CH=), 6.81-6.84 (m, 1H, Ar), 6.87 (d, 1H, *J* = 8.0 Hz, Ar), 6.99 (dd, 1H, *J* = 8.0 , 2.1 Hz, Ar), 7.10 (d, 1H, *J* = 2.1 Hz, Ar), 7.28 (dd, 1H, *J* = 8.4, 8.0 Hz, Ar), 7.47 (d, 1H, *J* = 8.4 Hz, Ar), 7.54 (d, 1H, *J* = 15.4 Hz, CH=), 7.73 (s, 1H, Ar) ppm. ^{13}C NMR (Acetone-d_6) δ: 164.18, 153.83, 152.21, 147.33, 145.39, 141.44, 140.52, 128.97, 127.30, 121.11, 118.61, 116.60, 115.58, 115.51, 114.10, 112.98, 43.68, 33.46 (NCH_2 rotamer), 33.30 (NCH_2 rotamer), 12.63 (CH_3 rotamer), 11.81 (CH_3 rotamer) ppm. Anal. ($C_{19}H_{20}N_2O_5$) Calc%:

C 64.04, H 5.66, N 7.86; Found%: C 64.32, H 5.71, N 7.59.

(E)-3-(3-(4-hydroxy-3-methoxyphenyl)acrylamido)phenyl-(N-ethyl(methyl))carbamate (2). Compound **2** was synthesized from ferulic acid (142 mg, 0.73 mmol) and 3-aminophenyl-N-ethyl(methyl)carbamate **15** (142 mg, 0.73 mmol) as previously described. The crude product was purified by flash chromatography using $CHCl_3$/MeOH (9.5:0.5) as the eluent. The subsequent precipitation from AcOEt/*n*-hexane gave compound **2** as a yellow solid. Compound **2** (124 mg, 0.36 mmol, 46% yield), mp 133-135 °C; ^{1}H NMR (Acetone-d_6) δ: 1.15 (t, 1.5H, *J* = 6.8 Hz, CH_3 rotamer), 1.24 (t, 1.5H, *J* = 6.8 Hz, CH_3 rotamer), 2.95 (s, 1.5H, CH_3 rotamer), 3.08 (s, 1.5H, CH_3 rotamer), 3.38 (q, 1H, *J* = 6.8 Hz, CH_2 rotamer), 3.49 (q, 1H, *J* = 6.8 Hz, CH_2 rotamer), 3.90 (s, 3H, CH_3O), 6.66 (d, 1H, *J* = 15.4 Hz, CH=), 6.81-6.85 (m, 1H, Ar), 6.86 (d, 1H, *J* = 8.0 Hz, Ar), 7.11 (dd, 1H, *J* = 8.0, 2.0 Hz, Ar), 7.21 (d, 1H, *J* = 2.0 Hz, Ar), 7.28 (dd, 1H, *J* = 8.4, 8.0 Hz, Ar), 7.48 (d, 1H, *J* = 8.0 Hz, Ar), 7.60 (d, 1H, *J* = 15.4 Hz, CH=), 7.74 (s, 1H, Ar) ppm. ^{13}C NMR (Acetone-d_6) δ: 165.00, 154.50, 153.15, 149.56, 148.67, 142.25, 141.46, 129.86, 127.99, 122.96, 119.75, 117.50, 116.45, 116.21, 113.86, 111.47, 56.26, 44.58, 34.57 (CH_2 rotamer), 34.20 (CH_2 rotamer), 13.56 (CH_3 rotamer), 12.80 (CH_3 rotamer) ppm. Anal. ($C_{20}H_{22}N_2O_5$) Calc%: C 64.85, H 5.99, N 7.56; Found%: C 64.90, H 5.87, N 7.62.

(E)-3-((3-(3,4-dihydroxyphenyl)acrylamido)methyl)phenyl-(N-ethyl(methyl))carbamate (3). Compound **3** was synthesized from caffeic acid (86 mg, 0.48

mmol) and 3-(aminomethyl)phenyl-N- ethyl(methyl)carbamate **16** (100 mg, 0.48 mmol). The crude product was purified by flash chromatography using $CHCl_3$/MeOH (9.5:0.5) as eluent affording to compound **3** as a yellow solid (66 mg, 0.18 mmol, 37 % yield); mp 188-190 °C; ^{1}H NMR(CD_3OD) δ: 1.17 (t, 1.5H, *J* = 7.0 Hz, CH_3 rotamer), 1.24 (t, 1.5H, *J* = 7.0 Hz, CH_3 rotamer), 2.96 (s, 1.5H, CH_3 rotamer), 3.08 (s, 1.5H, CH_3 rotamer), 3.38 (q, 1H, *J* = 7.0 Hz, CH_2 rotamer), 3.49 (q, 1H, *J* = 7.0 Hz, CH_2 rotamer), 4.48 (s. 2H, CH_2NH), 6.40 (d, 1H, *J* = 15.8 Hz, CH=), 6.76 (d, 1H, *J* = 8.2 Hz, Ar), 6.91 (dd, 1H, *J* = 2.0, 8.2 Hz, Ar), 6.98-7.02 (m, 2H, Ar), 7.06 (s, 1H, Ar), 7.18 (d, 1H, *J* = 7.8 Hz, Ar), 7.34 (dd, 1H, *J* = 7.8, 8.0 Hz, Ar), 7.43 (d, 1H, *J* = 15.8 Hz, CH=) ppm. ^{13}C NMR(CD_3OD) δ: 169.18, 156.39, 153.01, 148.86, 146.74, 142.69, 141.80, 130.46, 128.25, 125.68, 122.19, 122.08, 121.72, 118.13, 116.46, 115.07, 45.15, 43.85, 34.53 (CH_2 rotamer), 34.27 (CH_2 rotamer), 13.40 (CH_3 rotamer), 12.61 (CH_3 rotamer) ppm. Anal. ($C_{20}H_{22}N_2O_5$) Calc%: C 64.85, H 5.99, N 7.56; Found%: C 64.78, H 5.86, N 7.62.

(E)-3-((3-(4-hydroxy-3-methoxyphenyl)acrylamido)methyl)phenyl-(N-ethyl(methyl)) carbamate (4). Compound **4** was synthesized from ferulic acid (93 mg, 0.48 mmol) and 3-(aminomethyl)phenyl-N-ethyl(methyl)carbamate **16** (100 mg, 0.48 mmol). The crude product was purified by flash chromatography using $CHCl_3$/MeOH (9.5:0.5) as the eluent and subsequent precipitation from $CHCl_3$/n-hexane. Coumpound **4** (yellow solid) (65 mg, 0.17 mmol, 35% yield); mp 138-140 °C; ^{1}H NMR($CDCl_3$) δ: 1.19–1.26 (m, 3H, CH_3), 2.98

(s, 1.5H, CH_3rotamer), 3.06 (s, 1.5H, CH_3 rotamer), 3.37–3.49 (m, 2H, CH_2), 3.91 (s, 3H, OCH_3), 4.56 (d, 2H, *J* = 5.2 Hz, CH_2NH), 6.27 (d, 1H, *J* = 15.6 Hz, CH=), 6.90 (d, 1H, *J* = 8.2 Hz, Ar), 7.00–7.08 (m, 4H, Ar), 7.15 (d, 1H, *J* = 7.6 Hz, Ar) 7.32 (dd, 1H, *J* = 7.6, 8.2 Hz, Ar), 7.58 (d, 1H, *J* = 15.6 Hz, CH=) ppm. ^{13}C NMR(CD_3OD) δ: 169.02, 156.27, 152.97, 149.88, 149.23, 142.49, 141.73, 130.41, 128.17, 125.60, 123.23, 121.99, 121.67, 118.48, 116.45, 111.62, 56.35, 45.09, 43.82, 34.48 (CH_2rotamer), 34.22 (CH_2rotamer), 13.37 (CH_3rotamer), 12.58 (CH_3rotamer) ppm. Anal. ($C_{21}H_{24}N_2O_5$) Calc%: C 65.61, H 6.29, N 7.29; Found%: C 65.52, H 6.12, N 7.35.

(E)-3-(2-(3-(3,4-dihydroxyphenyl)acrylamido)ethyl)phenyl-(N-ethyl(methyl))carbamate (5). Compound **5** was synthesized from caffeic acid (81 mg, 0.45 mmol) and 3-(2-aminoethyl)phenyl-N- ethyl(methyl)carbamate **17** (100 mg, 0.45 mmol). The crude product was purified by flash chromatography using $CHCl_3$/MeOH (9.5:0.5) as the eluent and subsequent precipitation from AcOEt/*n*-hexane to afford compound **5** as a yellow solid (66 mg, 0.17 mmol, 38 % yield); mp 78-80 °C; ^{1}H NMR (Acetone-d_6) δ: 1.13 (t, 1.5H, *J* =6.7 Hz, CH_3 rotamer), 1.21 (t, 1.5H, *J* = 6.7 Hz, CH_3 rotamer), 2.85 (t, 2H, *J* = 7.2 Hz, $ArCH_2$), 2.94 (s, 1.5H, CH_3 rotamer), 3.06 (s, 1.5H, CH_3rotamer), 3.36 (q, 1H, *J* = 6.7 Hz, CH_2 rotamer), 3.46 (q, 1H, *J* = 6.7 Hz, CH_2 rotamer), 3.52-3.57 (m, 2H, CH_2N), 6.43 (d, 1H, *J* = 15.8 Hz, CH=), 6.82 (d, 1H, *J* = 8.4 Hz, Ar), 6.92 (dd, 1H, *J* = 1.6, 7.9 Hz, Ar), 6.97 (d, 1H, *J* = 8.4 Hz, Ar), 7.01 (s, 1H, Ar), 7.07-7.08 (m, 2H, Ar), 7.27 (t, 1H, *J* =7.9 Hz,

Ar), 7.42 (d, 1H, *J* = 15.8 Hz, CH=) ppm.^{13}C NMR(Acetone-d_6) δ: 166.80 (CO rotamer), 166.72 (CO rotamer), 152.87, 147.94, 146.29, 141.91, 140.74, 129.82, 128.34, 126.18, 123.05, 121.54, 120.51, 119.56, 116.32, 114.89, 44.55, 41.49 (CH_3 rotamer), 41.36 (CH_3 rotamer), 36.25, 34.34 (CH_2 rotamer), 34.02 (CH_2 rotamer), 13.46 (CH_3 rotamer), 12.69 (CH_3 rotamer) ppm. Anal. ($C_{21}H_{24}N_2O_5$) Calc%: C 65.61, H 6.29, N 7.29; Found%: C 65.35, H 6.12, N 7.41.

(E)-3-(2-(3-(4-hydroxy-3-methoxyphenyl)acrylamido)ethyl)phenyl-(N-ethyl(methyl))carbamate (6). Compound **6** was synthesized from ferulic acid (52 mg, 0.27 mmol) and 3-(2-aminoethyl)phenyl-N-(ethyl(methyl))carbamate **17** (60.00 mg, 0.27 mmol). The crude product was purified by flash chromatography using $CHCl_3$/MeOH (9.8:0.2) as the eluent. Compoud **6 (**yellow solid) (43 mg, 0.11 mmol, 40 % yield); mp 63-65 °C; ^{1}H NMR(Acetone-d_6) δ:1.13 (t, 1.5H, *J* =6.7 Hz, CH_3 rotamer), 1.21 (t, 1.5H, *J* = 6.7 Hz, CH_3 rotamer), 2.86 (t, 2H, *J* = 7.2 Hz, $ArCH_2$), 2.94 (s, 1.5 H, CH_3 rotamer), 3.06 (s, 1.5 H, CH_3 rotamer), 3.36 (q, 1H, *J* = 6.7 Hz, CH_2 rotamer), 3.47 (q, 1H, *J* = 6.7 Hz, CH_2 rotamer), 3.52-3.58 (m, 2H, CH_2N), 3.87 (s, 3H, CH_3O), 6.49 (d, 1H, *J* = 15.6 Hz, CH=), 6.83 (d, 1H, *J* = 8.1 Hz, Ar), 6.99 (d, 1H, *J* = 8.2 Hz, Ar), 7.03 (s, 1H, Ar), 7.06 (dd, 1H, *J* = 1.6, 8.1 Hz, Ar), 7.08 (d, 1H, *J* =7.6 Hz, Ar), 7.15 (d, 1H, *J* = 1.6 Hz, Ar), 7.28 (dd, 1H, *J* = 7.6, 8.2 Hz, Ar), 7.44 (d, 1H, CH=, *J* = 15.6 Hz) ppm.^{13}C NMR(Acetone-d_6) δ: 165.47, 152.02, 148.00, 147.70, 141.20, 139.44, 128.89, 127.42, 125.23, 122.13, 121.64, 119.61, 119.18, 115.18,

110.38, 55.33, 43.64, 40.47, 35.39, 12.60 ppm. Anal. ($C_{22}H_{26}N_2O_5$) Calc%: C 66.32, H 6.58, N 7.03; Found %: C 66.07, H 6.73, N 6.95.

(2*E*)-3-(3,4-dihydroxyphenyl)-*N*-(3-{[ethyl(methyl)carbamoyl]amino}phenyl)prop-2-enamide (7). Compound **7** was synthesized from caffeic acid (109 mg, 0.53 mmol) and 3-(3-aminophenyl)-1-ethyl-1-methylurea **20** (111 mg, 0.53 mmol). The crude product was purified by flash chromatography eluting with $CHCl_3$/MeOH (9.5:0.5). Compound **7** (81 mg, 0.23 mmol, 43 % yield), yellow solid; mp 110-112 °C; ^{1}H NMR(CD_3OD) δ: 1.17 (t, 3H, *J* = 7.2 Hz, CH_3), 3.01 (s, 3H, CH_3), 3.43 (q, 1H, *J* = 7.2 Hz, CH_2), 6.55 (d, 1H, *J* = 15.4 Hz, CH=), 6.78 (d, 1H, *J* = 8.0 Hz, Ar), 6.95 (dd, 1H, *J* = 1.6, 8.0 Hz, Ar), 7.05 (d, 1H, *J* = 1.6 Hz, Ar), 7.11 (d, 1H, *J* = 8.0 Hz, Ar), 7.22 (t, 1H, *J* = 8.0 Hz, Ar), 7.34 (d, 1H, *J* = 8.0 Hz, Ar), 7.51 (d, 1H, *J* = 15.4 Hz, CH=), 7.70 (s, 1H, Ar) ppm. ^{13}C NMR (Acetone-d_6) δ: 165.03, 156.18, 148.19, 146.30, 141.96, 140.62, 129.31, 128.23, 121.84, 119.79, 116.37, 115.86, 114.98, 114.10, 111.84, 43.94, 34.11, 13.19 ppm. Anal. ($C_{19}H_{21}N_3O_4$) Calc%: C 64.21, H 5.96, N 11.82; Found%: C 64.47, H 6.08, N 11.69

(2*E*)-*N*-(3-{[ethyl(methyl)carbamoyl]amino}phenyl)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enamide (8). Compound **8** was synthesized from ferulic acid (92 mg, 0.48 mmol) and from 3-(3-aminophenyl)-1-ethyl-1-methylurea **20** (100 mg, 0.48 mmol). The crude product was purified by flash chromatography eluting with $CHCl_3$/MeOH (9.8:0.2). Compound **8** was obtained as pale yellow solid (71 mg, 0.19 mmol, 40 % yield);

mp 112-115 °C; ^{1}H NMR(CD_3OD) δ: 1.18 (t, 3H, *J* = 7.2 Hz, CH_3), 3.02 (s, 3H, CH_3), 3.44 (q, 1H, *J* = 7.2 Hz, CH_2), 3.91 (s, 3H, OCH_3), 6.55 (d, 1H, *J* = 15.6 Hz, CH=), 6.82 (d, 1H, *J* = 8.2 Hz, Ar), 7.09 (dd, 1H, *J* = 1.8, 8.2 Hz, Ar), 7.12 (d, 1H, *J* = 8.0 Hz, Ar), 7.17 (d, 1H, *J* = 1.8 Hz, Ar), 7.22 (t, 1H, *J* = 8.0 Hz, Ar), 7.35 (d, 1H, *J* = 8.0 Hz, Ar), 7.57 (d, 1H, *J* = 15.6 Hz, CH=), 7.70 (s, 1H, Ar) ppm. ^{13}C NMR (Acetone-d_6) δ: 164.87, 156.00, 149.44, 148.65, 142.15, 141.75, 140.68, 129.29, 128.09, 122.77, 120.15, 116.20, 115.61, 113.88, 111.48, 56.23, 43.90, 34.08, 13.20 ppm. Anal. ($C_{20}H_{23}N_3O_4$) Calc%: C 65.03, H 6.28, N 11.37; Found%: C 65.32, H 6.44, N 11.53.

General procedure for the synthesis of carbamates (12, 13, 14). To a solution of TEA (1 mL, 7.13 mmol) were added N-ethyl-methyl carbomoyl chloride (200 mg, 1.65 mmol) and the opportune phenol **9-11** (1.62 mmol). The mixture was then stirred at 95°C for 15 h, then the reaction mixture was diluted with CH_2Cl_2 and washed with NaOH 1N, dried over Na_2SO_4, and evaporated.

3-nitrophenyl-N-ethyl(methyl)carbamate (12). It was synthesized from 3-nitrophenol **(9)** (225 mg, 1.62 mmol) affording **12** as a brown oil (272 mg, 1.21 mmol, 75 % yield); ^{1}H NMR ($CDCl_3$) δ: 1.21 (t, 1.5H, *J* = 7.2 Hz, CH_3 rotamer), 1.26 (t, 1.5H, *J* = 7.2 Hz, CH_3 rotamer), 3.01 (s, 1.5H, CH_3 rotamer), 3.09 (s, 1.5H, CH_3 rotamer), 3.42 (q, 1H, *J* = 7.2 Hz, CH_2 rotamer), 3.49 (q, 1H, *J* = 7.2 Hz, CH_2 rotamer), 7.47-7.54 (m, 2H, Ar), 8.00-8.02 (m, 1H, Ar), 8.06 (d, 1H, *J* = 7.2 Hz, Ar) ppm.

3-cyanophenyl-N-ethyl(methyl)carbamate (13). It was synthesized from 3-hydroxybenzonitrile **(10)** (193 mg, 1.62 mmol) affording **13** as a brown oil (215 mg, 1.05 mmol, 65 % yield); ^{1}H NMR($CDCl_3$) δ: 1.20 (t, 1.5H, *J* = 7.2 Hz, CH_3 rotamer), 1.25 (t, 1.5H, *J* = 7.2 Hz, CH_3 rotamer), 3.00 (s, 1.5H, CH_3 rotamer), 3.07 (s, 1.5H, CH_3 rotamer), 3.41 (q, 1H, *J* = 7.2 Hz, CH_2 rotamer), 3.47 (q, 1H, *J* = 7.2 Hz, CH_2 rotamer), 7.37-7.39 (m, 1H, Ar), 7.43-7.49 (m, 3H, Ar) ppm.

3-(cyanomethyl)phenyl-N-ethyl(methyl)carbamate (14). It was synthesized from (3-hydroxyphenyl)acetonitrile (**11**) (215 mg, 1.62 mmol) affording **14** as a brown oil (218 mg, 1.00 mmol, 62 % yield); ^{1}H NMR ($CDCl_3$) δ: 1.19 (t, 1.5H, *J* = 7.2 Hz, CH_3 rotamer), 1.25 (t, 1.5H, *J* = 7.2 Hz, CH_3 rotamer), 2.99 (s, 1.5H, CH_3 rotamer), 3.07 (s, 1.5H, CH_3 rotamer), 3.40 (q, 1H, *J* = 7.2 Hz, CH_2 rotamer), 3.47 (q, 1H, *J* = 7.2 Hz,CH_2 rotamer), 3.75 (s, 2H, CH_2CN), 7.08-7.11 (m, 2H, Ar), 7.16 (d, 1H, *J* = 7.6 Hz, Ar), 7.36 (t, 1H, *J* = 7.6 Hz, Ar) ppm.

General procedure for the synthesis of amine (15, 16, 17). The appropriate nitro-derivative **12** or cyano-derivative (**13, 14)** (1.45 mmol) was hydrogenated in EtOH (6 mL) in the presence of 10% Pd-C (225 mg, 2.12 mmol) for 12 h. Then, the catalyst was filtered off, and the solution was evaporated to dryness.

3-aminophenyl-N-ethyl(methyl)carbamate (15). It was synthesized from 3-nitrophenyl-N-ethyl(methyl)carbamate **12** (325 mg, 1.45 mmol) to give **15** as an oil (211 mg, 1.09 mmol,

75 % yield); ^{1}H NMR ($CDCl_3$) δ: 1.16–1.23 (m, 3H, CH_3), 2.97 (s, 1.5H, CH_3 rotamer), 3.04 (s, 1.5H, CH_3 rotamer), 3.37–3.46 (m, 2H, CH_2), 6.44 (s, 1H, Ar), 6.49 (dd, 2H, *J* = 2.2, 8.2 Hz, Ar), 7.10 (t, 1H, *J* = 8.2 Hz, Ar) ppm.

3-(aminomethyl)phenyl-N-ethyl(methyl)carbamate (16). It was synthesized from 3-cyanophenyl-N-ethyl(methyl)carbamate **13** (296 mg, 1.45 mmol) to give **16** as a brown oil (193 mg, 0.93 mmol, 64 % yield); ^{1}H NMR ($CDCl_3$) δ: 1.17-1.25 (m, 3H, CH_3), 2.98 (s, 1.5H, CH_3 rotamer), 3.06 (s, 1.5H, CH_3 rotamer), 3.43 (q, 1H, *J* = 7.2 Hz, CH_2 rotamer), 3.47 (q, 1H, *J* = 7.2 Hz, CH_2rotamer), 3.86 (s, 2H, CH_2NH_2), 6.99 (d, 1H, *J* = 7.6 Hz, Ar), 7.08 (s, 1H, Ar), 7.13 (d, 1H, *J* = 7.8 Hz, Ar), 7.31 (dd, 1H, *J* = 7.6, 7.8 Hz, Ar) ppm.

3-(2-aminoethyl)phenyl-N-ethyl(methyl)carbamate (17). 3-(cyanomethyl)phenyl-N-ethyl(methyl)carbamate **14** (318 mg, 1.45 mmol) was hydrogenated in EtOH (14 mL) e HCl conc. (0.15 mL) in the presence of 10% Pd-C (112 mg, 1.06 mmol) for 4 h. Then, the catalyst was filtered off, and the solution was evaporated to dryness. The residue was diluted with $CHCl_3$ and washed with NaOH 1N and H_2O. The organic layer was dried over Na_2SO_4, and evaporated, to give **17** as a brown oil (222 mg, 1.00 mmol, 69 % yield); ^{1}H NMR ($CDCl_3$) δ: 1.17-1.25 (m, 3H, CH_3), 2.73-2.77 (m, 2H, CH_2), 2.93-2.99 (m, 2H, CH_2), 2.98 (s, 1.5H, CH_3 rotamer), 3.06 (s, 1.5H, CH_3 rotamer), 3.38-3.49 (m, 2H, CH_2), 6.91-6.99 (m, 2H, Ar), 7.02 (d, 1H, *J*= 7.6 Hz, Ar), 7.26-7.30 (m, 1H, Ar) ppm.

1-ethyl-1-methyl-3-(3-nitrophenyl)urea (19). The 3-nitroaniline **18** (1.00 g, 7.24 mmol)

dissolved in THF (10 ml) was dropped slowly to a stirred solution of triphosgene (2.15 g, 7.24 mmol) in THF (5 mL), then TEA (1.46 g, 15.20 mmol) was added slowly to the reaction mixture. The solution was stirred for 1h and then evaporated. THF (10 mL) and N-ethylmethylamine (10 mmol) were added directly to the residue. The reaction mixture was stirred at rt for 12h, then the solvent was evaporated and the crude mixture was diluted with AcOEt and washed with H_2O. The organic layer was dried over Na_2SO_4 and evaporated. The crude product was purified by precipitation from AcOEt/*n*-hexane to obtain **19** as a yellow solid (1.32 g, 5.94 mmol, 68 % yield); ^{1}H NMR ($CDCl_3$) δ: 1.22 (t, 3H, *J* = 7.2 Hz, CH_3), 3.04 (s, 3H, CH_3), 3.44 (q, 1H, *J* = 7.2 Hz, CH_2), 6.62 (br s, 1H, NH), 7.42 (dd, 1H, *J*= 8.4, 8.0 Hz, Ar), 7.83-7.86 (m, 2H, Ar), 8.22 (s, 1H, Ar) ppm.

3-(3-aminophenyl)-1-ethyl-1-methylurea (20). It was synthesized from 1-ethyl-1-methyl-3-(3-nitrophenyl)urea **19** (323 mg, 1.45 mmol). Hydrogenation of **19** in EtOH (6 mL) in the presence of 10% Pd-C for 12 h afforded a crude mixture which was filtered off. The solution was evaporated to dryness to give **20** (212 mg, 1.10 mmol, 76 % yield) as a yellow solid; ^{1}H NMR($CDCl_3$) δ: 1.17-1.25 (m, 3H, CH_3), 2.98 (s, 1.5H, CH_3 rotamer), 3.06 (s, 1.5H, CH_3 rotamer), 3.43 (q, 1H, *J* = 7.2 Hz, CH_2 rotamer), 3.47 (q, 1H, *J* = 7.2 Hz, CH_2 rotamer), 3.86 (s, 2H, CH_2NH_2), 6.99 (d, 1H, *J* = 7.6 Hz, Ar), 7.08 (s, 1H, Ar), 7.13 (d, 1H, *J* = 7.8 Hz, Ar), 7.31 (dd, 1H, *J* = 7.6, 7.8 Hz, Ar) ppm.

Biological assay.

Materials. Dulbecco's modified Eagle's medium (DMEM), and fetal bovineserum (FBS) were purchased from Gibico-BRL (Grand Island, NY, USA). Trypsin, $A\beta_{1-42}$, dimethyl sulfoxide (DMSO), thiazolyl blue tetrazolium bromide (MTT), DPPH, AChE, BuChE, Donepezil, Rivatigamine and all other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless stated otherwise. Test compounds were dissolved in DMSO and stored at -20 ℃.

Cell culture and treatment. HT22 cells were maintained in DMEM supplemented with 10% (v/v) FBS and incubated at 37 °C under 5% CO_2. To study the protective effect of test compounds on glutamate or H_2O_2-induced neuronal death and their cell toxicity, cells were seeded in 96-well plates (10,000 cells/well). Cells in the control group were treated with vehicle alone.

ChE inhibitory assay. The method of Ellman et al [38] was followed. AChE stock solution was prepared by dissolving 1000 units of lyophilized powder (Sigma Chemical) in 0.1 M phosphate buffer (pH 8.0) containing Triton X-100, 0.1%. BuChE stock solution was prepared by dissolving 100 units of lyophilized powder (Sigma Chemical) in aqueous gelatin solution (0.1%w/v). Enzymes stock solutions were diluted before use to reach an activity ranging between 0.13 and 0.100 AU/min in the final assay conditions. Five different concentrations of each compound were used to obtain inhibition of AChE or BuChE activity between 20% and 80%. The assay solution consisted of 118 μL 0.1 M phosphate buffer (pH

8.0), with the addition of 40μM DTNB (Ellman's reagent), 2 μl AChE or BuChE, 20 μL compound and 20 μM acetylthiocholine iodide (ATi). The final assay volume was 200 μL. Initial rate assays were performed at 37 °C with a Jasco V-530 double beam spectrophotometer: the rate of increase in the absorbance at 412 nm was followed for 5 min. Test compounds were added to the assay solution and preincubated with the enzyme for 60 min, according to the kinetic type, followed by the addition of substrate. Assays were done with a blank containing all components except AChE or BuChE to account for nonenzymatic reaction. The reaction rates were compared, and the percent inhibition due to the presence of the test compound was calculated. Each concentration was analyzed in triplicate, and IC_{50} values were determined graphically from the logconcentration-inhibition curves.

DPPH assay. Compounds were added into a 1 mM ethanol solution of DPPH (final concentration was 100 μM) to make final concentrations of 10 μM. The reaction tubes were wrapped in aluminum foil and kept at 25-26 °C for 60 min in dark. All measurements were done under dim light. Spectrophotometric measurements were operated at 517 nm by ultraviolet spectrophotometer. The capacity to scavenge free radicals was measured as follow: capacity = (A1 – A2) / A1 *100%, where A1 and A2 are the absorbance of the control treatment and the twelve compounds or caffeic acid in 517 nm, respectively.

Thioflavin T (Th-T) based fluorometric assay. The Th-T fluorescence method was performed to determine that compounds can restrain amyloid fibril formation. Prepare $A\beta_{1-42}$

oligomers: 100 μL of 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) were add to 0.1 mg of $A\beta_{1-42}$, and incubate for 60 min at room temperature. Then the solution were incubate for 10 min at 0°C, followed by drying in vacuum freezing dryer for 60 min. Freeze drying $A\beta_{1-42}$ powder were dissolved in DMSO, making the final concentration of 2.3 mM, Stored in - 80 ° C, avoid light. Before use, use 0.215 M PBS (pH 8.0) dilute to the required concentration. After incubated at 37℃ for 24 h, the solutions containing 10 μM $A\beta_{1-42}$ oligomers plus 1 μM compounds or 0.215 M PBS were added to 50 mM glycine-NaOH buffer (pH 8.5) containing 1.67 μM Th-T in a final volume of 200 μL. Fluorescence were monitored with excitation at 446 nm and emission at 490 nm. A time scan of fluorescence was performed and the intensity values reached at the plateau (around 300 s) were averaged after subtracting the background fluorescence from 1.5 μM Th-T and PBS. The percent inhibition of $A\beta_{1-42}$ aggregation due to the presence of test compounds were calculated by the following expression: $100 - (IF_i/IF_0*100)$ where IF_i and IF_0 are the fluorescence intensities obtained for $A\beta_{1-42}$ or $A\beta_{1-42}$ plus test compound in the presence, respectively.

Molecular docking study. Dock6 was employed to identify the potential binding of compounds **5** and **7** to Aβ. The initial structure of A*β* was taken from the NMR structure (PDB ID: 1BA4) [28]. The potential ligand binding site was defined as in our previous study.[22] All compounds and Aβ were optimized using AMBER14SB force filed, and then structures of compound were protonated with the program Dock Prep. The Aβ sphere was

generated using sphgen model in UCSF Chimera. DOCK6 was used to perform half-flexible docking. APLIP online (https://projects.biotec.tu-dresden.de/plip-web/plip/index) was employed to identify the binding poses of compounds **5** and **7** for Aβ. All docked poses of compound **5** and **7** were ranked on the basis of the binding docking energie. For each compound, the lowest energy conformation was chosen for binding modes analyses.

Assessment of cell viability by MTT assay. The cell viability was determined by MTT assay as previously described [39]. Briefly, cells were cultured in 96-well plates and exposed to different concentration of compounds for indicated times. Then, the cells were incubated at 37 °C for 3 h with MTT (0.5 mg/ml) and lysed in DMSO after discarding the supernatants. The optical density was measured at 570 nm with spectrometer and all data were expressed as percentage of control.

Metal-chelating study. The experiments were performed as reported previously [34]. The metal chelation was monitored spectrophotometrically using a UV–vis spectrophotometer. Typically, with the exception of $CuCl_2$ (dissolved in Milli-Q water) and **5** (dissolved in methanol and diluted in PBS), all agents were dissolved in absolute ethanol, with a final sample volume of 200 μL. The final sample was allowed to stand at room temperature for 30 min, and then the absorption spectrum was recorded at room temperature.

Statistical analysis. All quantitative data and experiments described in this study were repeated at least three times. The data were presented as the means ± standard error of

multiple independent experiments. Statistical analyses between two groups were performed by unpaired Student's t-test. Differences among groups were tested by one-way analysis of variance (ANOVA). Following ANOVA analyses, the Tukey's test was used and $P< 0.05$ was accepted to be statistically significant.

Conflict of interest

The authors declare no competing financial interest.

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Highlights

1.Compound **5** exerted good radical scavenging activity and significant neuroprotective effect.

2.Compounds **5** inhibit the Aβ aggregation with % values of 85.3%.

3.Compound **5** exerted stronger inhibition against BuChE than rivastigmine at 1 μM.

4.Compound **5** can form chelate with Cu^{2+}.