Accepted Manuscript

Nature-based molecules combined with rivastigmine: A symbiotic approach for the synthesis of new agents against Alzheimer's disease

Giulia Nesi, Qiuhe Chen, Simona Sestito, Maria Digiacomo, Xiaohong Yang, Shengnan Wang, Rongbiao Pi, Simona Rapposelli

PII: S0223-5234(17)30798-5

DOI: 10.1016/j.ejmech.2017.10.006

Reference: EJMECH 9798

To appear in: *European Journal of Medicinal Chemistry*

Received Date: 14 April 2017

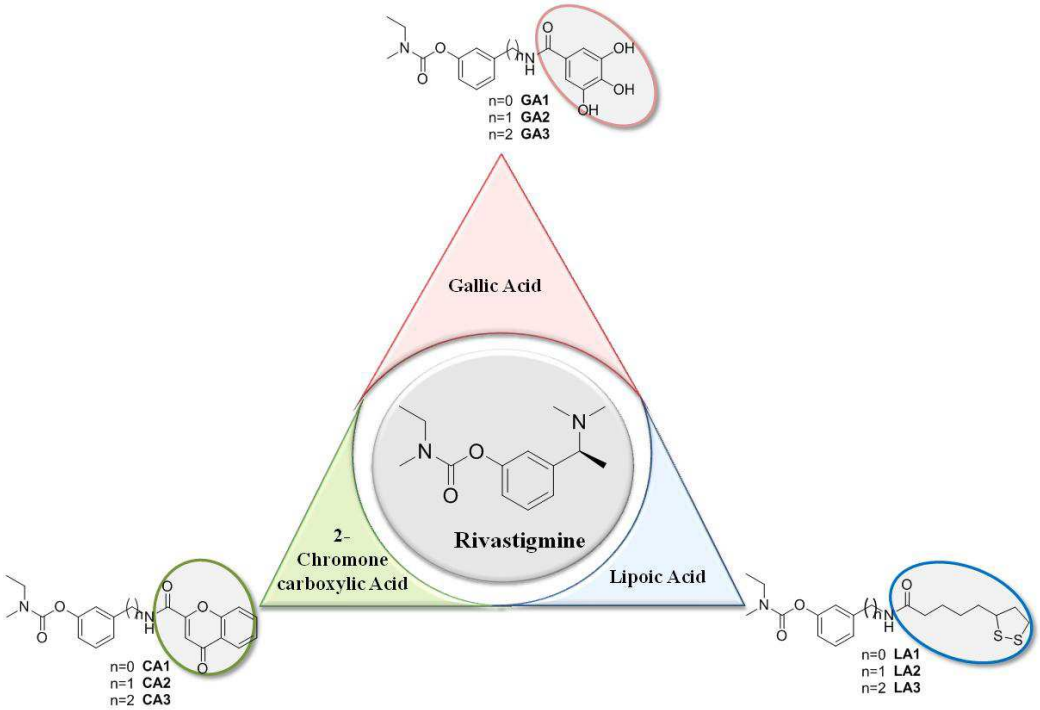
Revised Date: 31 July 2017

Accepted Date: 2 October 2017

Please cite this article as: G. Nesi, Q. Chen, S. Sestito, M. Digiacomo, X. Yang, S. Wang, R. Pi, S. Rapposelli, Nature-based molecules combined with rivastigmine: A symbiotic approach for the synthesis of new agents against Alzheimer's disease, *European Journal of Medicinal Chemistry* (2017), doi: 10.1016/j.ejmech.2017.10.006.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Graphical abstract



ACCEPTED MANUSCRIPT

Nature-based molecules combined with rivastigmine: a symbiotic approach for the synthesis of new agents against Alzheimer's disease.

Giulia Nesi[a,1], Qiuhe Chen[b,c1], Simona Sestito[a], Maria Digiacomo[a], Xiaohong Yang[b], Shengnan Wang[b], Rongbiao Pi[b,c,d]* and Simona Rapposelli[a]*

[a]*Department of Pharmacy, University of Pisa, Via Bonanno 6, 56126 Pisa (Italy)*

[b]*Department of Pharmacology& Toxicology, School of Pharmaceutical Sciences, Sun Yat-Sen University, Guangzhou 5100060(China)*

[c]*International Joint Laboratory (SYSU-PolyU HK) of Novel Anti-dementia Drugs of Guangdong, Guangzhou 510006, China;*

[d]*Guangdong Province Key Laboratory of Brain Function and Disease, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou 510080, China.*

Abstract

Starting from nature as original source, new potential agents with pleiotropic activities have been synthesized and evaluated as neuroprotective agents. In this work, novel nature-based hybrids, combining antioxidant motifs with rivastigmine, have been designed and synthesized. The biological results revealed that the new compounds inhibit both AChE and BuChE. In particular, lipoic acid hybrids **LA1, LA2, LA3** resulted to be the most potent inhibitors of BuChE showing IC_{50} values ranging from 340 to 378 nM. Analogously, all the compounds were able to inhibit the self β-amyloid$_{1-42}$ aggregation. The gallic acid hybrid **GA2** as well as the 2-chromonecarboxylic acid hybrids **CA1** and **CA2** prevented the self-mediated Aβ aggregation with percentages of inhibition ranging from 53% to 59%. Finally, some of them also show potent neuroprotective effects against glutamate-induced cell death and low toxicity in HT22 cells.

Keywords: Rivastigmine, Gallic acid, Lipoic acid, neuroprotective agents, Multifunctional agents, Alzheimer's disease

1. Introduction

Alzheimer's disease (AD) represents the most common form of neurodegenerative dementia. “Dementia” describes a set of symptoms including memory loss and difficulties in thinking, problem-solving or language. Several molecular events have been implicated in the complex AD pathophysiology; therefore, to date, many therapeutic strategies are available [1]. Unfortunately, only a few treatments have been proved to be able to reduce AD symptoms, but none of them result able to halt the disease progression [2], thus the search for new potential drugs is still strongly pursued.

The hallmarks of AD, such as Aβ deposition in senile plaques and neurofibrillary tangles (NFT), are strongly intertwined with the production of reactive oxygen species (ROS) and with the onset of oxidative stress (OS), which are both considered as the common effectors of the neurodegenerative cascade [3]. Several studies reported an increase of OS markers, such as DNA, RNA, lipid and protein oxidation, and a simultaneous decrease of the level of antioxidants or detoxifying enzymes in patients with AD [4, 5]. Although the specific mechanisms behind the altered redox balance are not entirely clear, researchers demonstrated that OS contributes to the Aβ aggregation and to phosphorylation of tau protein, thus constituting a trigger event in AD pathogenesis [6, 7]. Recently, Zhao et al. reported that amyloid deposition and neurofibrillary tangles improve the levels of oxidative stress markers inducing an apparent vicious circle of AD-pathogenesis [8], thus confirming the key role played by OS in the AD onset and progression.

In this context, supported by numerous *in vitro* and *in vivo* studies on animal models of AD, natural antioxidants could be considered as promising therapeutic tools for design new therapeutic agents for AD [9, 10]. Many studies revealed that nature-based antioxidants, such as polyphenols, have pleiotropic activity including antioxidant, neuroprotective and anti-Aβ aggregating properties. In particular, polyphenols showed to reduce cognitive impairments and Aβ aggregation [11]. Nevertheless, while in animal models the antioxidant agents proved to be very effective, human trial results are still disappointing.

In last decade, the multitarget-directed-ligand (MTDL) strategy has become a rich source of inspiration for many researchers, thus representing the main rational and successful approach to apply in the search of new agents for the treatment of diseases with a complex ethiopathology (e.g. neurodegenerative, cardiovascular diseases, and cancers). Many multitarget strategies have been developed with the aim to improve the bioavailability of antioxidants in order to promote the direct targeting in the brain [12], but also to reduce the possible side effects of native molecules [13]. For these reasons, many multifunctional compounds containing antioxidant compounds have been rationally designed [14, 15].

In this scenario, in last few years, we investigated some hybrid compounds obtained by the conjugation of cholinesterase inhibitors (ChEi), such as tacrine or rivastigmine, with well-known antioxidant molecules, such as caffeic acid and ferulic acid [15-18]. These compounds showed balanced properties of AChE inhibitor, free radical scavenger, inhibitor of both self- and AChE-induced Aβ-aggregation, as well as potent neuroprotective effects against H_2O_2- and glutamate-induced cell injury, with low toxicity in HT22 cells.

The positive results obtained encouraged us to further explore the nature-based compounds (e.g. traditional medicines) which could be appropriately combined with AChE-inhibitors. In particular, herein we describe the design and synthesis of new molecules obtained by the symbiotic combination of rivastigmine with antioxidant agents such as gallic acid (**GA**), lipoic acid (**LA**) and 2-chromonecarboxylic acid (**CCA**). Synthesized compounds were then investigated *in vitro* to evaluate both AChE and BuChE inhibition, ROS scavengers and Aβ aggregation. In addition, the capability to cross the BBB, the toxicity, as well as the neuroprotective activity against glutamate-induced cytotoxicity, have also been assessed in HT22 cell lines.

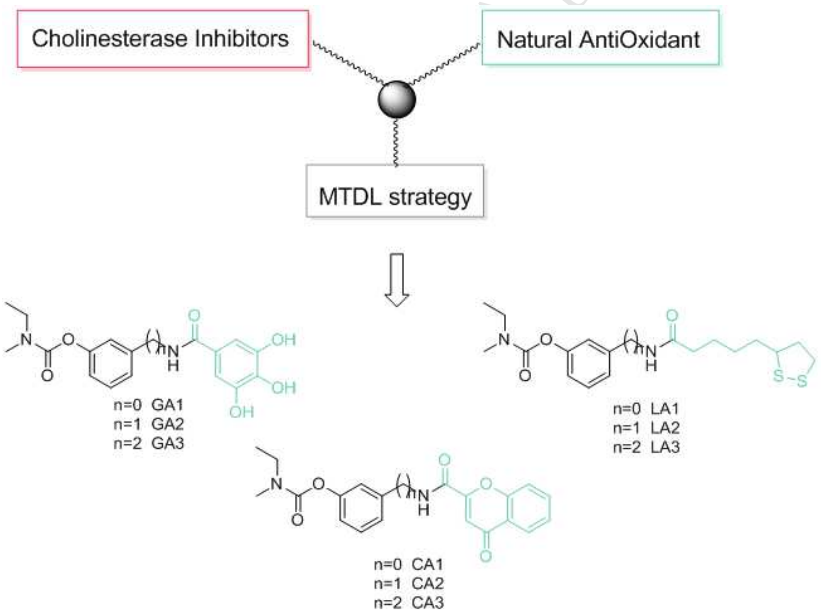


Figure 1. Multitarget-directed-ligand (MTDL) approach for new multifunctional antioxidant hybrids.

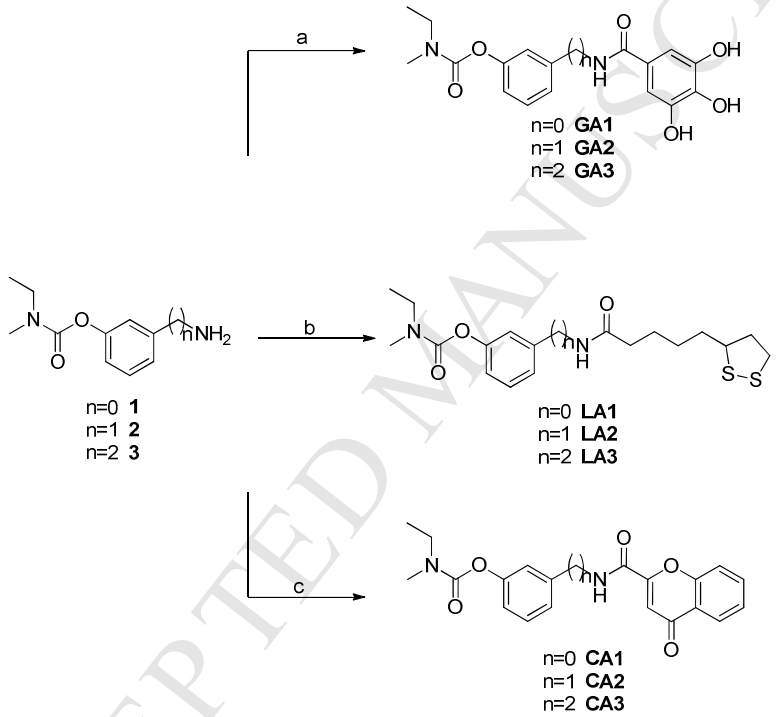
2. Results and discussion

2.1. Chemistry

Scheme 1 reports the general synthetic procedure followed for the synthesis of final compounds. Briefly, the amines **1-3** previously described [19], were obtained by two sequential reactions

between the appropriate phenol and the N-ethyl-methyl carbamoyl chloride in the presence of Et_3N, followed by a catalytic reduction of the appropriate N-ethyl-methylcarbamate.

Subsequently, the condensation of **1-3** with gallic acid in the presence of dicyclohexyl carbodiimide (DCC) as a condensing reagent, yielded the final compounds **GA1-3**. The compounds **LA1-3** were prepared by the reaction of lipoic chloride, freshly prepared by treatment of lipoic acid with $SOCl_2$, and the amine-derivatives **1-3** in the presence of Et_3N. Finally, the condensation between the amines **1-3** and the 4-oxo-4H-1-benzopyran-2-carboxylic acid in the presence of (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) and Et_3N gave the compounds **CA1-3**, in modest yields (lower than 50%).



Scheme 1. Synthesis of final compounds. Reagents and conditions: a) Gallic acid, CH_2Cl_2/DMF, DCC, DMAP, r.t., 12h; b) i) Lipoic acid, $SOCl_2$, DCM, 0°C, 4 h; (ii) TEA, DCM/DMF, 0°C, 4h; c) 4-Oxo-4H-1-benzopyran-2-carboxylic acid, BOP, TEA, r.t., 12h.

2.2 Biological evaluation

Cholinergic neurotransmission damage is believed to be one of the major causes of memory impairment associated with AD [20]. A recent study performed by Norberg et al. [21] showed that, during the progression of AD, the levels of AChE decrease while BuChE levels are likely to increase. This compensatory role of BuChE in response to the decrease of AChE activity [22] could explain, at least in part, the efficacy proven by rivastigmine (BuChE inhibitor) in clinical trials. Consistently, the new synthesized compounds, were firstly tested for their inhibitory activity against both AChE and BuChE, using Donepezil and rivastigmine as positive drugs. The data are

summarized in Table 1. All the GA-compounds **GA1, GA2, GA3** and the CA-derivatives **CA1, CA2, CA3** elicited a slight affinity against AChE, even if they resulted to be more active than the native drug rivastigmine. Moreover, all the compounds showed to inhibit BuChE with a significant % of inhibition values and many of them revealed to be more active than the positive drugs. In particular, lipoic acid hybrids **LA1, LA2, LA3** resulted to be 9-fold more active than donepezil and 2-fold more active than rivastigmine. Analogously, also the CA-derivatives and the GA3 compound exerted a good inhibition against BuChE. Therefore, we further detected the IC_{50} for the most promising compounds for BuChE. As showed in Table 1, LA1-3 showed the best IC_{50} ranging from 340 and 378 nM.

Table 1. Percentage inhibition values of ChEs and $A\beta_{1-42}$ self-induced aggregation, DPPH radical scavenging activities (IC_{50}, μM) and BBB Prediction.

Compd	% inhibition of AChE (%) ± SEM[a]	% inhibition of BuChE (%) ± SEM[a]	IC_{50} of BuchE (nM)	Inhibition of Aβ self-aggregation (%)[b]	DPPH radical scavenging activities (IC_{50}, μM)[d]	Permeability in PAMPA-BBB assay[e]	
	500nM	500nM				Pe ($x10^{-6}$cm s^{-1})	Prediction
GA1	23.56±0.2	7.98±0.4	Nd	47.34±0.1	10.62±0.5	3.08±0.1	CNS+/-
GA2	5.53±3.2	2.39±0.4	Nd	51.83±1.3	12.88±0.1	4.86±0.1	CNS+
GA3	3.48±0.1	58.84±0.0	452±121	27.11±1.3	11.65±0.4	5.14±0.0	CNS+
LA1	0.51±0.8	77.43±1.1	356±79	46.28±1.4	4.53±0.2	10.32±1.0	CNS+
LA2	3.66±1.3	77.31±0.0	378±133	51.77±6.6	4.30±0.1	27.79±1.3	CNS+
LA3	-1.48±0.2	76.68±0.7	340±130	49.84±0.0	4.88±1.0	12.12±0.3	CNS+
CA1	9.03±1.0	53.77±0.8	507±179	63.79±7.1	>100μM	Nd	Nd
CA2	12.71±0.4	37.11±2.4	650±210	55.23±3.9	>100μM	Nd	Nd
CA3	22.08±0.6	49.07±2.7	511±157	67.36±0.9	>100μM	Nd	Nd
Rivastigmine	1.92±0.8	30.08±0.1	678±230			16.32±0.3	CNS+
Donepezil	90.55±0.5	8.21±1.7	Nd				
Curcumin[c]				51.77 ± 3.33			
Trolox					23.49±0.1		

[a]SEM: Standard Error of the mean. [b] Inhibition of self-mediated $A\beta_{1-42}$ aggregation. The thioflavin-T fluorescence method was used, and the measurements were carried out in the presence of 10 μM inhibitor. [c] % of inhibition for Curcumin (positive drug) (20μM) reported by W. Luo et al.[23]. [d]IC_{50} was defined as the concentration resulting in 50% scavenging activity. [e]Range of Permeability of PAMPA-BBB Assays (*Pe*, 10^{-6} cm s^{-1})[24]: Compounds of high BBB permeation (CNS+) *Pe*>4.7; Compounds of uncertain BBB permeation (CNS+/-) 4.7>*Pe*>1.8; Compounds of low BBB permeation (CNS-) *Pe*<1.8. nd = not determined.

ACCEPTED MANUSCRIPT

Since many evidence suggested that polyphenols inhibit the Aβ self-aggregation, we performed a Thioflavin-T fluorescence assay to evaluate the influence of the tested compounds on amyloid fibrils. The measurements were carried out in the presence of 10μM of tested compounds using curcumin as reference drugs. Curcumin, a natural polyphenolic molecule originally isolated from turmeric, has been shown to block aggregation and fibril formation[25]. The results collected (Table 1) showed that all the compounds were able to inhibit the Aβ aggregation. In particular the gallic acid hybrids **GA2** as well as the 2-chromonecarboxylic acid hybrids **CA1** and **CA2** prevented the self-mediated Aβ aggregation with percentages of inhibition ranging from 53% to 59%, which were comparable to curcumin (51%) (Table1). Compound **CA3** exhibited the highest inhibitory activity of 66.44% at 10μM.

On the basis of the encouraging results, we then decided to test also the radical scavenging properties of the synthesised compounds in order to evaluate if the combination of antioxidant motifs with the native drug rivastigmine could affect the antioxidant properties. The evaluation was performed by using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay, with trolox as reference compound. As expected, the analysis of the results clearly showed that the GA- and LA- derivatives proved to be good scavengers with IC_{50} values ranging from 4.30 (LA2) to 12.88 (GA2). On the contrary, none of CCA hybrids (**CA1, CA2, CA3**) exhibit significant antioxidant activities.

The new hybrid compounds were also subjected to an additional assay to evaluate the chelating properties. The complexation ability of the new compounds towards Cu^{2+} was studied by UV–vis spectroscopy and the most interesting results were illustrated in Figure 2. The GA-hybrids induced the most marked isosbestic shift of the maximum absorption upon the addition of $CuCl_2$, suggesting that compounds **GA1-3** form a complex with Cu^{2+}. Similarly, also derivatives **LA1** and **LA2** were able to induce a moderate isosbestic shift while the other new synthesized compounds resulted to be inactive (data not shown).

ACCEPTED MANUSCRIPT

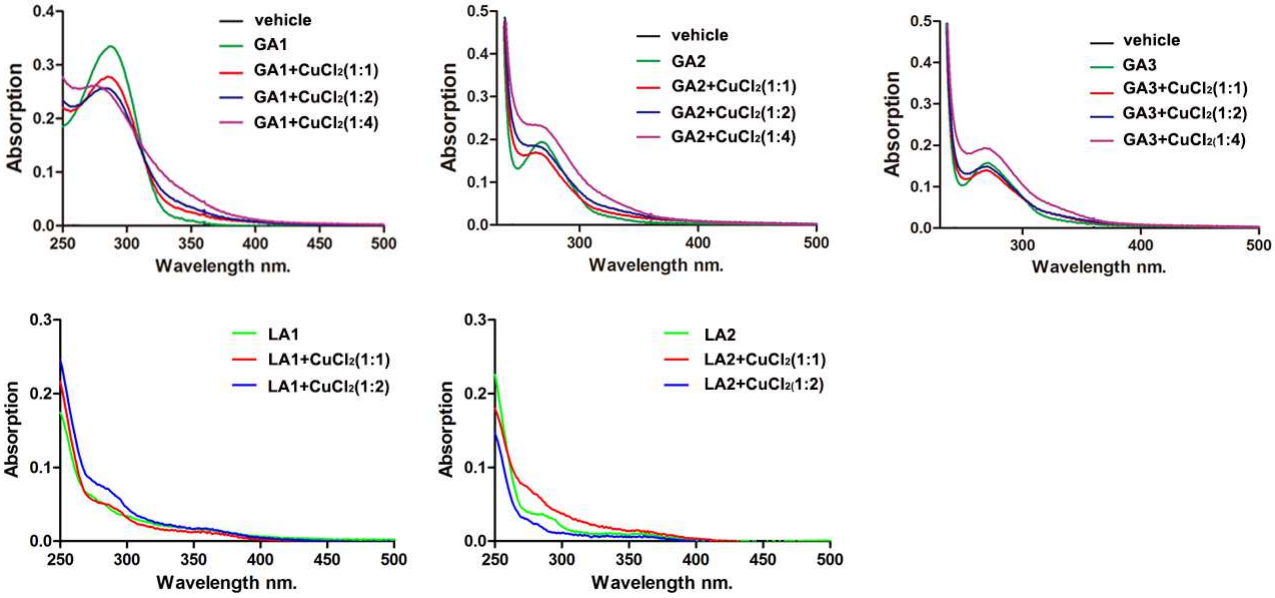


Figure 2. The copper chelating activity of compounds. UV spectra of compounds (10 μM) alone (green line), treated with $CuCl_2$ (10 μM) (red line), and treated with $CuCl_2$ (20 μM) (blue line).

We then performed a preliminary evaluation on HT22 cell viability in order to evaluate the cytotoxicity of the new molecules using the colorimetric MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide] assay (Figure 3). No cytotoxic effects were observed for gallic acid derivative **GA2** and for lipoic acid hybrids **LA1**, **LA2**, **LA3** at 30, 50 and 100μM concentrations whereas analogues **GA1** and **GA3** appeared to be cytotoxic only at the highest concentrations (100 μM). On the contrary CCA-derivatives showed to be cytotoxic, in particular **CA2** induced a cytotoxic effect at the lowest concentration (30 μM).

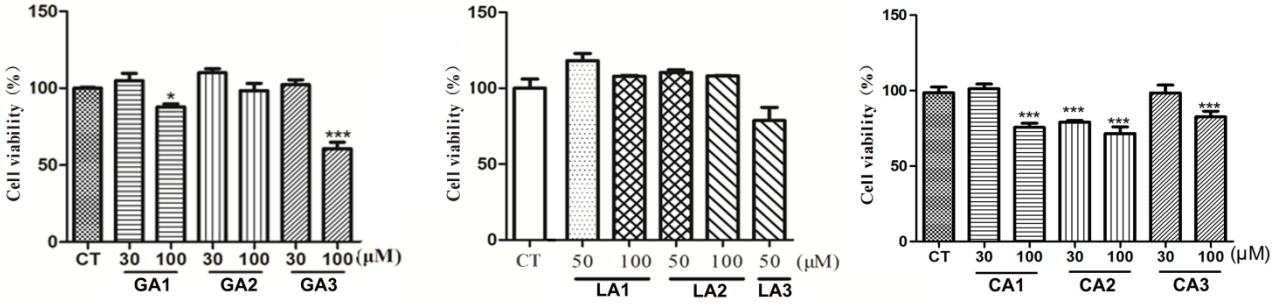


Figure 3. Cell toxicity of tested compounds on HT22 cells. Cells were treated with tested compounds for 24h, then cell viability was determined using the MTT assay (n = 3). $^{*}P < 0.05$ versus control; $^{***}P < 0.001$ versus control.

The neuroprotective effects of the new molecules were tested in HT22 cell against glutamate-induced neuronal death, at different concentrations (3, 10 and 30 μM). As reported in Figure 4, compounds **GA1** and **CA2** produced a significant neuroprotection at 10 μM while compound **GA2** displayed an appreciable neuroprotective effect only at the higher concentration (30μM). Derivatives **GA3**, **CA1**, **CA3**, **LA1**, **LA2** and **LA3** resulted to be inactive in this model of neurotoxicity.

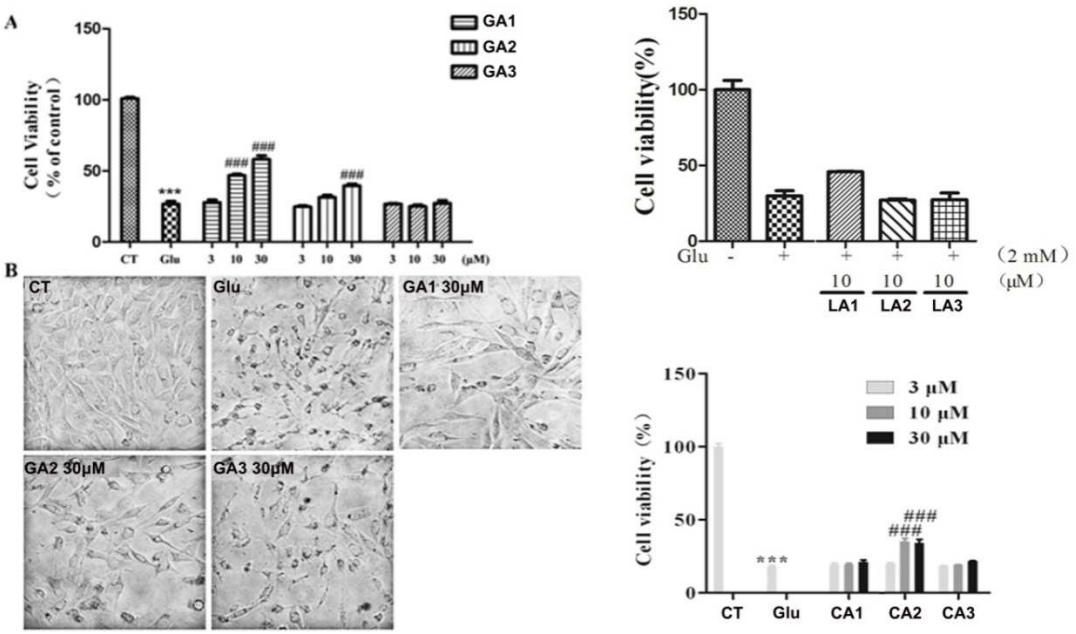


Figure 4. Neuroprotective effects of tested compounds on HT22 cells. After pretreated with tested compounds for 30 min, cell were incubated with 2 μM glutamate for 24 h. Cell viability was determined using the MTT assay (n = 3). $^{***}P< 0.001$ versus control; $^{###}P< 0.001$ versus glutamate-treated group.

For the most active compounds described herein we performed a PAMPA-BBB assay to evaluate the ability of the compounds to cross the BBB. This method developed by Di et al.[26] is capable of measuring the permeability by passive diffusion of small molecules through an artificial lipid membrane. This assay allowed us to classify each hybrids as BBB permeable (CNS+) or BBB impermeable (CNS-). Results indicated that gallic acid derivatives **GA2, GA3** and lipoic hybrids **LA1, LA2** and **LA3** possessed a high capability to penetrate into the brain (value from 4.86±0.1 for **GA2** to 27.79±1.3 for **LA2**) while **GA1** showed a slight BBB permeation ability (3.08±0.1).

3. Conclusion

It is increasingly accepted that Aβ deposition in the brain as well as the production of reactive oxygen species (ROS) and the onset of oxidative stress (OS) are all important features in Alzheimer's disease (AD). Recently, a great number of studies on multi-target ligands have been published [27-29]. In the present study, we performed the design and synthesis of new molecules obtained by the symbiotic combination of rivastigmine skeleton with natural antioxidant agents such as gallic acid (GA), lipoic acid (LA) and 2-chromonecarboxylic acid (CCA).The results discussed here show that this strategy has allowed us to obtain new compounds with pleiotropic activities that could provide a useful starting point to combine the anti-oxidants and anti-aggregating properties of nature-based agents with the anticholinesterase activity of drugs that are currently used as unique therapeutic tools to treat AD. Interestingly, GA2 derivative revealed to be an effective neuroprotective agent in in vitro glutamate-induced neurotoxicity model with a high % of inhibition of Ab aggregation and an appreciable scavengers properties, even if it results to be avoid of ChE inhibitory activity. On the contrary, the lipoic acid hybrid compounds **LA1-3** show to be the most interesting derivatives with pleiotropic activities. Consequently, further investigations in different in vitro models will be of help in elucidating the importance of their multitarget profile in neuroprotection.

4. EXPERIMENTAL SECTION

4.1 Synthesis and characterization

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 a Bruker Advance 400 spectrometer at 400 MHz in a ~2% solution of $CDCl_3$, CD_3OD-d_4. Data for ^{1}H NMR spectra are reported as follows: chemical shift (δ ppm) (multiplicity, coupling constant (Hz), integration). Multiplicities are reported as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, or combinations thereof. Chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane and referenced from solvent references. ATR-FTIR experiments were conducted using a Ftir agilent 620 spectrometer equipment with a DLaTGS detector. Spectra were the result of 32 scans with the resolution 4 cm^{-1} in the spectral range of 4000-500 cm^{-1}. The 95% purity of tested compounds was confirmed by combustion analysis. Chromatographic separation was performed on silica gel columns by flash (Kieselgel 40, 0.040–0.063 mm; Merck). Merck gel plates (60 F254) were used for analytical TLC. UV light was used to examine the spots. Evaporation was performed *in vacuo* (rotating evaporator). Sodium sulfate was always used as the drying agent. Commercially available chemicals were purchased from Sigma-Aldrich.

4.1.1 General procedure for synthesis of compounds GA1-3

To a solution of gallic acid (116 mg, 0.68 mmoli) in THF (2 mL) was added dropwise a solution of appropriate amine **1-3** (0.68 mmoli) and *N*,*N*′-Dicyclohexylcarbodiimide (DCC) (62.1 mg, 0.68 mmol) in THF (2 mL). The mixture was stirred 7 h at r.t., then, the organic layer was dried and concentrated.

4.1.1.1. ***3-(3,4,5-trihydroxybenzamido)phenyl-ethyl(methyl)carbamate*** **GA1**. Compound **GA1** was synthesized from 3-aminophenyl-ethyl(methyl)carbamate **1** (132 mg, 0.68 mmol). The crude product was purified by flash chromatography using *n*-Hexane/AcOEt (90:10) as the eluent to give compound **GA1**(94 mg, 0.27 mmol, 40 % yield) as a yellow oil; IR: 3305 υ (O-H of phenol), 1598 δ (N-H of amide) cm^{-1}. ^1H NMR (CD_3OD-d_4) δ: 1.18 (t, 1.5 H, *J* = 6.8 Hz, Me, rotamer); 1.25 (t, 1.5 H, *J* = 6.8 Hz, Me, rotamer); 2.98 (s, 1.5 H, Me, rotamer); 3.10 (s, 1.5 H, Me, rotamer); 3.39 (q, 1 H, *J* = 6.8 Hz, CH_2, rotamer); 3.51 (q, 1 H, *J* = 6.8 Hz, CH_2, rotamer); 6.85 (d, 1 H, *J* = 7.2 Hz, Ar); 6.95 (s, 2 H, Ar); 7.30-7.34 (m, 1 H, Ar); 7.47 (d, 1H, *J* = 8.4 Hz, Ar); 7.54 (s, 1H, Ar) ppm. ^{13}C NMR (CD_3OD-d_4) δ: 169.1, 156.3, 153.0, 146.7, 141.2, 138.6, 130.2, 126.5, 118.7, 118.4, 115.5, 108.2, 45.1, 34.5 (rotamer), 34.3 (rotamer), 13.4 (rotamer), 12.6 (rotamer) ppm. Anal. ($C_{17}H_{18}N_2O_6$) C, H, N % Calcd: 58.96 (C), 5.24 (H), 8.09 (N). % Found: 59.03 (C), 5.09 (H), 8.36 (N).

4.1.1.2 ***3-((3,4,5-trihydroxybenzamido)methyl)phenyl-ethyl(methyl)carbamate*** **GA2**. Compound **GA2** was synthesized from 3-(aminomethyl)phenyl-ethyl(methyl)carbamate **2** (142 mg, 0.68 mmol). The crude product was purified by flash chromatography using *n*-Hexane/AcOEt (90:10) as the eluent to give compound **GA1** (112 mg, 0.31 mmol, 45 % yield) as a yellow oil; IR: 3341 υ (O-H of phenol), 1572 δ (N-H of amide) cm^{-1}. ^1H NMR (CD_3OD-d_4) δ: 1.18 (t, 1.5 H, *J* = 7.0 Hz, Me, rotamer); 1.25 (t, 1.5 H, *J* =7.0 Hz, Me, rotamer); 2.97 (s, 1.5 H, Me, rotamer); 3.09 (s, 1.5 H, Me, rotamer); 3.38 (q, 1 H, *J* = 7.0 Hz, CH_2, rotamer); 3.47 (q, 1 H, *J* = 7.0 Hz, CH_2, rotamer); 4.52 (s, 2 H, CH_2NH_2); 6.88 (s, 2 H, Ar); 6.99 (d, 1 H, *J* = 7.2 Hz, Ar); 7.07 (s, 1 H, Ar); 7.20 (d, 1 H, *J* = 7.6 Hz, Ar); 7.32-7.36 (m, 1 H, Ar) ppm. ^{13}C NMR (CD_3OD-d_4) δ: 170.5, 156.4, 152.9, 146.8, 142.2, 138.7, 130.4, 125.6, 125.5, 121.9, 121.5, 107.8, 45.1, 44.0, 34.5 (rotamer), 34.3 (rotamer), 13.4 (rotamer), 12.6 (rotamer) ppm. Anal. ($C_{18}H_{20}N_2O_6$) C, H, N % Calcd: 59.99 (C), 5.59 (H), 7.77 (N). % Found: 60.13 (C), 5.48 (H), 7.95 (N).

4.1.1.3 ***3-(2-(3,4,5-trihydroxybenzamido)ethyl)phenyl-ethyl(methyl)carbamate*** **GA3.** Compound **GA3** was synthesized from 3-(2-aminoethyl)phenyl-ethyl(methyl)carbamate **3** (131mg, 0.68 mmol). The crude product was purified by flash chromatography using *n*-Hexane/AcOEt/MeOH (90:10:0.4) as the eluent to give compound **GA3** (107 mg, 0.29 mmol, 42 % yield) as a yellow oil;

IR: 3325 υ (O-H of phenol), 1607 δ (N-H of amide) cm^{-1}. ^{1}H NMR (CD_3OD-d_4) δ: 1.17 (t, 1.5 H, *J* = 7.2 Hz, Me, rotamer); 1.24 (t, 1.5 H, *J* = 7.2 Hz, Me, rotamer); 2.89 (t, 2 H, *J* = 6.4 Hz, CH_2); 2.97 (s, 1.5 H, Me, rotamer); 3.05 (t, 2 H, *J* = 6.4 Hz, CH_2); 3.09 (s, 1.5 H, Me, rotamer); 3.44 (q, 1 H, *J* = 7.2 Hz, CH_2, rotamer); 3.52 (q, 1 H, *J* = 7.2 Hz, CH_2, rotamer); 6.95 (d, 1 H, *J* = 8.4 Hz, Ar); 6.99 (s, 1 H, Ar) 7.06 (s, 2 H, Ar); 7.08 (d, 1 H, *J* = 7.6 Hz, Ar); 7.28-7.32 (m, 1 H, Ar) ppm. ^{13}C NMR (CD_3OD-d_4) δ: 171.1, 156.4, 153.0, 146.3, 142.0, 139.2, 130.5, 127.0, 123.4, 123.0, 120.9, 110.2, 56.1, 45.2, 34.5 (rotamer), 34.3 (rotamer), 33.0, 13.4 (rotamer), 12.6 (rotamer) ppm. Anal. ($C_{19}H_{22}N_2O_6$) C, H, N % Calcd: 60.95 (C), 5.92 (H), 7.48 (N). % Found: 61.10 (C), 6.21 (H), 7.33 (N).

4.1.2 General procedure for synthesis of compounds LA1-3

A suspension of lipoic acid (128 mg, 0.62 mmol) in DCM (3.0 mL), under N_2 atmosphere, was cooled to 0 °C and treated with thionyl chloride (0.07 mL, 0.99 mmol). The mixture was stirred at 0 °C for 4 h, then the solvent was removed under reduced pressure to give the acid chloride as a yellow solid. A solution of the acid chloride in DCM (2.0 mL) was added to a solution of the appropriate amine **1-3** (0.52 mmol) and Et_3N (0.07 mL, 0.52 mmol) in DCM (2.0 mL), cooled to 0 °C. The mixture was stirred at 0 °C for 4 h, diluted with DCM and washed with saturated aqueous $NaHCO_3$. The organic phase was dried over Na_2SO_4 and the solvent evaporated.

4.1.2.1. ***3-{[5-(1,2-dithiolan-3-yl)pentanoyl]amino}phenyl-ethyl(methyl)carbamate*** **LA1**. Compound **LA1** was synthesized from 3-aminophenyl-ethyl(methyl)carbamate **1** (100 mg, 0.52 mmol). The crude product was purified by flash chromatography using $CHCl_3$/MeOH (98:2) as the eluent to give compound **LA1** (86 mg, 0.26 mmol, 50 % yield) as a yellow oil; ^{1}H NMR ($CDCl_3$) δ: 1.19 (t, 1.5 H, *J* = 7.0 Hz, Me, rotamer), 1.24 (t, 1.5 H, *J* = 7.0 Hz, Me, rotamer), 1.41-1.52 (m, 2 H, CH_2), 1.61–1.74 (m, 4 H, CH_2), 1.86–1.94 (m, 1 H, CH_2), 2.21 (t, 2 H, *J* = 7.2 Hz, CH_2), 2.41–2.49 (m, 1 H, CH_2), 2.99 (s, 1.5 H, Me, rotamer), 3.06 (s, 1.5 H, Me, rotamer), 3.07–3.20 (m, 2 H, CH_2), 3.38–3.49 (m, 2 H, CH_2), 3.53-3.60 (m, 1 H, CH), 6.76 (d, 1 H, *J* = 7.6 Hz, Ar), 7.00-7.04 (m, 1 H, Ar), 7.14-7.18 (m, 1 H, Ar), 7.43 (s, 1 H, Ar), 7.96 (br d, 1 H, *J* = 6.4 Hz, NH) ppm. ^{13}C NMR ($CDCl_3$) δ: 171.4, 155.1, 155.0, 151.6, 139.5, 129.2, 116.9, 114.1, 56.5, 44.3, 40.3, 38.6, 37.0, 34.8, 34.4 (CH_2, rotamer), 34.0 (CH_2, rotamer), 29.0, 25.2, 13.3 (CH_3, rotamer), 12.6 (CH_3, rotamer) ppm. Anal. ($C_{18}H_{26}N_2O_3S_2$) C, H, N, S % Calcd: 56.51 (C), 6.85 (H), 7.32 (N), 16.76 (S). % Found: 56.23 (C), 7.09 (H), 7.36 (N), 16.89 (S).

4.1.2.2. ***3-({[5-(1,2-dithiolan-3-yl)pentanoyl]amino}methyl)phenyl-ethyl(methyl)carbamate*** **LA2.** Compound **LA2** was synthesized from 3-(aminomethyl)phenyl-ethyl(methyl)carbamate **2** (108 mg,

0.52 mmol). The crude product was purified by flash chromatography using $CHCl_3$/MeOH (98:2) to give compound **LA2** (88.0 mg, 0.25 mmol, 49 % yield) as a yellow oil; ^{1}H NMR ($CDCl_3$) δ: 1.17 (t, 1.5 H, *J* = 7.0 Hz, Me, rotamer), 1.22 (t, 1.5 H, *J* = 7.0 Hz, Me, rotamer), 1.39–1.51 (m, 2 H, CH_2), 1.60–1.72 (m, 4 H, CH_2), 1.84–1.91 (m, 1 H, CH_2), 2.19 (t, 2 H, *J* = 7.4 Hz, CH_2), 2.39– 2.47 (m, 1 H, CH_2), 2.96 (s, 1.5 H, Me, rotamer), 3.05 (s, 1.5 H, Me, rotamer), 3.07–3.19 (m, 2 H, CH_2), 3.38 (q, 1 H, *J* = 7.0 Hz, CH_2, rotamer), 3.45 (q, 1 H, *J* = 7.0 Hz, CH_2, rotamer), 3.51–3.58 (m, 1 H, CH), 4.37 (d, 2 H, *J* = 5.6 Hz, CH_2NH), 6.02 (br s, 1 H, NH), 6.97-7.03 (m, 2 H, Ar), 7.07 (d, 1 H, *J* = 7.6 Hz, Ar), 7.26–7.3 (m, 1 H, Ar) ppm. ^{13}C NMR ($CDCl_3$) δ: 172.7, 154.5, 151.9, 139.8, 129.7, 124.8, 121.4, 121.1, 56.5, 44.3, 43.5, 40.4, 38.6, 36.6, 34.8, 34.4 (CH_2, rotamer), 34.0 (CH_2 , rotamer), 29.0, 25.5, 13.4 (Me, rotamer), 12.6 (Me, rotamer) ppm. Anal. ($C_{19}H_{28}N_2O_3S_2$) C, H, N, S % Calcd: 57.54 (C), 7.12 (H), 7.06 (N), 16.17 (S). % Found: 57.23 (C), 7.31 (H), 7.20 (N), 16.33 (S).

4.1.2.3. ***3-(2-{[5-(1,2-dithiolan-3-yl)pentanoyl]amino}ethyl)phenyl-ethyl(methyl)carbamate*** **LA3.** Compound **LA3** was synthesized from 3-(2-aminoethyl)phenyl-ethyl(methyl)carbamate **3** (94.0 mg, 0.52 mmol). The crude product was purified by flash chromatography using $CHCl_3$/MeOH (98:2) to give compound **LA3** (110 mg, 0.27 mmol, 52 % yield) as a pale yellow oil; ^{1}H NMR ($CDCl_3$) δ: 1.18 (t, 1.5 H, *J* = 7.2 Hz, Me, rotamer), 1.23 (t, 1.5 H, *J* = 7.2 Hz, Me, rotamer), 1.41-1.45 (m, 2H, CH_2), 1.60-1.68 (m, 4H, CH_2), 1.88-1.91 (m, 1H, CH_2), 2.13 (t, 2H, *J* = 7.6 Hz, CH_2), 2.40-2.49 (m, 1H, CH_2), 2.80 (t, 2H, *J* = 6.8 Hz, CH_2), 2.98 (s, 1.5 H, Me, rotamer), 3.06 (s, 1.5 H, Me, rotamer), 3.09-3.19 (m, 2H, CH_2), 3.39-3.59 (m, 5H, CH, CH_2), 6.94-7.01 (m, 3H, Ar), 7.26-7.30 (m, 1H, Ar) ppm. ^{13}C NMR($CDCl_3$) δ: 173.0, 151.8, 140.5, 129.4, 129.6, 125.8, 122.5, 120.0, 56.6, 44.3, 40.4 (CH_2, rotamer), 40.4 (CH_2, rotamer), 38.6, 36.5, 35.6, 34.8 (Me, rotamer), 34.4 (Me, rotamer), 34.0, 29.8, 29.0, 13.4, 12.7 ppm. Anal. ($C_{20}H_{30}N_2O_3S_2$) C, H, N % Calcd: 58.50 (C), 7.36 (H), 6.82 (N), 15.62 (S). % Found: 58.77 (C), 7.45 (H), 6.92 (N), 15.81 (S).

4.1.3. General procedure for synthesis of compounds CA1-3

To a stirred solution of Chromone-2-carboxylic acid (98 mg, 0.51 mmol) and (Benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (296 mg, 0.60 mmol) in DCM (8.0 mL) were added the appropriate amine **1-3** (100 mg, 0.51 mmol) and then Et_3N (0.20 ml). The resulting mixture was stirred at r.t. for 12 h, then it was diluted with DCM and washed with a solution of HCl 10%, $NaHCO_3$ 0.5M and water. The organic layer was dried over Na_2SO_4 and the solvent evaporated.

4.1.3.1 ***3-{[(4-oxo-4H-chromen-2-yl)carbonyl]amino}phenyl-ethyl(methyl)carbamate*** **CA1.** Compound **CA1** was synthesized from 3-aminophenyl-ethyl(methyl)carbamate **1** (70 mg, 0.31

mmol). The crude product was purified by flash chromatography using $CHCl_3$/MeOH (98:2) as the eluent to give compound **CA1**(53 mg, 0.15 mmol, 47 % yield) as a white solid; m.p. 155-160 °C.^{1}H NMR ($CDCl_3$) δ: 1.19-1.27 (m, 3 H, Me); 3.01 (s, 1.5 H, Me, rotamer); 3.08 (s, 1.5 H, Me, rotamer); 3.40-3.52 (m, 2H, CH_2); 6.94-6.98 (m, 1 H, Ar); 7.37 (dd, 1H, *J* = 8.4, 8.0 Hz, Ar); 7.48-7.54 (m, 2H, Ar); 7.59-7.62 (m, 2 H, Ar); 7.79 (dd, 1H, *J* = 8.0, 7.6 Hz, Ar); 8.25 (d, 1H, *J* = 8.0 Hz, Ar); 8.63 (s, 1H, Ar) ppm. ^{13}C NMR ($CDCl_3$) δ: 178.1; 157.1; 155.3; 154.5; 152.2; 137.2; 134.9; 130.0; 126.4; 126.3; 124.5; 119.1; 119.0; 118.2; 117.3; 114.4; 112.9; 44.3; 34.4 (rotamer); 34.00 (rotamer); 13.4 (rotamer); 12.6 (rotamer) ppm. Anal. ($C_{20}H_{18}N_2O_5$) C, H, N % Calcd: 65.57 (C), 4.95 (H), 7.65 (N). % Found: 65.79 (C), 4.98 (H), 7.96 (N).

4.1.3.2 ***3-({[(4-oxo-4H-chromen-2-yl)carbonyl]amino}methyl)phenyl-ethyl(methyl)carbamate*** **CA2.** Compound **CA2** was synthesized from 3-(aminomethyl)phenyl-ethyl(methyl)carbamate **2** (65 mg, 0.31 mmol). The crude product was purified by flash chromatography using $CHCl_3$/MeOH (95:5) as the eluent to give compound **CA2** (41 mg, 0.11 mmol, 35 % yield); m.p. 165-170 °C. ^{1}H NMR ($CDCl_3$) δ: 1.17–1.26 (m, 3 H, Me); 2.98 (s, 1.5 H, Me, rotamer); 3.06 (s, 1.5 H, Me, rotamer); 3.41 (q, 1H, *J* = 6.8 Hz, CH_2, rotamer); 3.47 (q, 1H, *J* = 6.8 Hz, CH_2, rotamer); 4.65 (d, 2H, *J* = 6.0 Hz, CH_2); 7.05-7.10 (m, 1H, Ar); 7.11-7.14 (m, 1H, Ar); 7.20-7.22 (m, 2H, Ar); 7.37 (t, 1H, *J* = 8.0 Hz, Ar); 7.43-7.47 (m, 1H, Ar); 7.52 (d, 1H, *J* = 8.0 Hz, Ar); 7.70-7.74 (m, 1H, Ar); 8.22 (dd, 1H, *J* = 8.0, 1.6 Hz, Ar) ppm. ^{13}C NMR ($CDCl_3$) δ: 178.3; 159.4; 155.4; 154.7; 152.0; 138.5; 134.7; 129.9; 126.1; 126.1; 125.0; 124.4; 121.7; 121.6; 118.3; 112.5; 44.2; 43.7; 34.4 (rotamer); 34.0 (rotamer); 13.3 (rotamer); 12.6 (rotamer) ppm. Anal. ($C_{21}H_{20}N_2O_5$) C, H, N % Calcd: 66.31 (C), 5.30 (H), 7.36 (N). % Found: 66.57 (C), 5.46 (H), 7.39 (N).

4.1.3.3. ***3-({[(4-oxo-4H-chromen-2-yl)carbonyl]amino}ethyl)phenyl-ethyl(methyl)carbamate*** **CA3.** Compound **CA3** was synthesized from 3-(aminoethyl)phenyl-ethyl(methyl)carbamate **3** (69 mg, 0.31 mmol). The crude product was purified by flash chromatography using $CHCl_3$/MeOH (98:2) as the eluent to give compound **CA3** (55 mg, 0.14 mmol, 45 % yield) as a white solid; m.p. 134-139 °C. ^{1}H NMR ($CDCl_3$) δ: 1.13 (t, 1.5H, *J* = 7.2 Hz, Me, rotamer); 1.21 (t, 1.5H, *J* = 7.2 Hz, Me, rotamer); 2.92-2.95 (m, 2H, CH_2); 2.93 (s, 1.5 H, Me, rotamer); 3.04 (s, 1.5 H, Me, rotamer); 3.33 (q, 1H, *J* = 7.2 Hz, CH_2, rotamer); 3.42 (q, 1H, *J* = 7.2 Hz, CH_2, rotamer); 3.72 (q, 2H, *J* = 6.8 Hz, CH_2); 7.00-7.02 (m, 2H, Ar); 7.06 (d, 1H, *J* = 7.6 Hz, Ar); 7.14-7.18 (m, 2H, Ar, NH); 7.31 (dd, 1H, *J* = 7.6, 8.0 Hz, Ar); 7.40 (dd, 1H, *J* = 7.6, 8.0 Hz, Ar); 7.51 (d, 1H, *J* = 8.4 Hz, Ar); 7.68 (t, 1H, *J* = 8.4 Hz, Ar); 8.17 (dd, 1H, *J* = 7.6, 1.6 Hz, Ar) ppm. ^{13}C NMR ($CDCl_3$) δ: 178.3; 159.4; 155.4; 154.8; 151.9; 139.9; 134.5; 129.7; 126.0; 126.0; 125.8; 124.4; 122.5; 122.5; 120.3; 118.5; 112.1; 44.2; 41.0; 35.3; 34.3 (rotamer) 33.9 (rotamer); 13.3 (rotamer); 12.5 (rotamer) ppm. Anal.

($C_{22}H_{22}N_2O_5$) C, H, N % Calcd: 66.99 (C), 5.62 (H), 7.10 (N). % Found: 66.82 (C), 5.55 (H), 7.16 (N).

Supporting information

*Experimental procedures covering the spectral characterization of compounds **GA1-3, LA1-3** and **CA1-3** and the biological methods.

AUTHOR INFORMATION

***Corresponding authors:** Rongbiao Pi (pirb@mail.sysu.edu.cn) and Simona Rapposelli (simona.rapposelli@farm.unipi.it)

Author contributions: [1] Both authors contributed equally. G.N., S.S. and M.D. synthesized the compounds. Q.C., X.Y. and S.W. performed most of the biological work. S.R. and R.P. conceived the idea and coordinated the project. G.N. Q.C., S.R. and R.P. wrote the main manuscript text. All authors reviewed the manuscript.

Notes: The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This study was supported by Guangdong Provincial International Cooperation Project of Science & Technology (No. 2013B051000038), National Natural Science Foundation of China (No. 31371070 and No. 81671264) and the Fundamental Research Funds for the Central Universities (No.15ykjc08b) to R. Pi. The authors also thank the COST action CA15135 (Multitarget Paradigm for Innovative Ligand Identification in the Drug Discovery Process MuTaLig) for support.

ABBREVIATIONS USED

GA, gallic acid; LA, lipoic acid; CCA, 2-chromonecarboxylic acid; HT22, hippocampal neuronal cell line; OS, oxidative stress; MTDL, multitarget-directed-ligand; DPPH, 1,1-diphenyl-2-picryl-hydrazyl; MTT, [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide];

REFERENCES

ACCEPTED MANUSCRIPT

[1] A. Uflacker, P.M. Doraiswamy, Alzheimer's Disease: An Overview of Recent Developments and a Look to the Future, Focus, 15 (2017) 13-17.

[2] N.S. Buckholtz, Perspective: in search of biomarkers, Nature, 475 (2011) S8-S8.

[3] M.P. Mattson, Pathways towards and away from Alzheimer's disease, Nature, 430 (2004) 631-639.

[4] W.R. Markesbery, M.A. Lovell, Damage to lipids, proteins, DNA, and RNA in mild cognitive impairment, Archives of Neurology, 64 (2007) 954-956.

[5] L. Minghetti, A. Greco, M. Puopolo, M. Combrinck, D. Warden, A.D. Smith, Peripheral reductive capacity is associated with cognitive performance and survival in Alzheimer's disease, Journal of neuroinflammation, 3 (2006) 4.

[6] M. Dumont, M.F. Beal, Neuroprotective strategies involving ROS in Alzheimer disease, Free radical biology and medicine, 51 (2011) 1014-1026.

[7] J. Hroudová, N. Singh, Z. Fišar, K.K. Ghosh, Progress in drug development for Alzheimer's disease: an overview in relation to mitochondrial energy metabolism, European journal of medicinal chemistry, 121 (2016) 774-784.

[8] Y. Zhao, B. Zhao, Oxidative stress and the pathogenesis of Alzheimer's disease, Oxidative Medicine and Cellular Longevity, 2013 (2013).

[9] C. Mancuso, T.E. Bates, D.A. Butterfield, S. Calafato, C. Cornelius, A.D. Lorenzo, A.T. Dinkova Kostova, V. Calabrese, Natural antioxidants in Alzheimer's disease, Expert opinion on investigational drugs, 16 (2007) 1921-1931.

[10] G. Nesi, S. Sestito, M. Digiacomo, S. Rapposelli, Oxidative Stress, Mitochondrial Abnormalities and Proteins Deposition: Multitarget Approaches in Alzheimer's Disease, Curr Top Med Chem, (2017).

[11] C. Ramassamy, Emerging role of polyphenolic compounds in the treatment of neurodegenerative diseases: a review of their intracellular targets, European journal of pharmacology, 545 (2006) 51-64.

[12] A. Tewari, V. Mahendru, A. Sinha, F. Bilotta, Antioxidants: The new frontier for translational research in cerebroprotection, Journal of Anaesthesiology Clinical Pharmacology, 30 (2014) 160.

[13] F. Di Domenico, E. Barone, M. Perluigi, D.A. Butterfield, Strategy to reduce free radical species in Alzheimer's disease: an update of selected antioxidants, Expert review of neurotherapeutics, 15 (2015) 19-40.

[14] Y. Bansal, O. Silakari, Multifunctional compounds: Smart molecules for multifactorial diseases, European Journal of Medicinal Chemistry, 76 (2014) 31-42.

[15] R. Pi, X. Mao, X. Chao, Z. Cheng, M. Liu, X. Duan, M. Ye, X. Chen, Z. Mei, P. Liu, Tacrine-6-ferulic acid, a novel multifunctional dimer, inhibits amyloid-β-mediated Alzheimer's disease-associated pathogenesis in vitro and in vivo, PLoS One, 7 (2012) e31921.

[16] X. Chao, X. He, Y. Yang, X. Zhou, M. Jin, S. Liu, Z. Cheng, P. Liu, Y. Wang, J. Yu, Y. Tan, Y. Huang, J. Qin, S. Rapposelli, R. Pi, Design, synthesis and pharmacological evaluation of novel tacrine–caffeic acid hybrids as multi-targeted compounds against Alzheimer's disease, Bioorganic & Medicinal Chemistry Letters, 22 (2012) 6498-6502.

[17] M. Digiacomo, Z. Chen, S. Wang, A. Lapucci, M. Macchia, X. Yang, J. Chu, Y. Han, R. Pi, S. Rapposelli, Synthesis and pharmacological evaluation of multifunctional tacrine derivatives against several disease pathways of AD, Bioorganic and Medicinal Chemistry Letters, 25 (2015) 807-810.

[18] Z. Chen, M. Digiacomo, Y. Tu, Q. Gu, S. Wang, X. Yang, J. Chu, Q. Chen, Y. Han, J. Chen, G. Nesi, S. Sestito, M. Macchia, S. Rapposelli, R. Pi, Discovery of novel rivastigmine-hydroxycinnamic acid hybrids as multi-targeted agents for Alzheimer's disease, European Journal of Medicinal Chemistry, 125 (2017) 784-792.

[19] Z. Chen, M. Digiacomo, Y. Tu, Q. Gu, S. Wang, X. Yang, J. Chu, Q. Chen, Y. Han, J. Chen, Discovery of novel rivastigmine-hydroxycinnamic acid hybrids as multi-targeted agents for Alzheimer's disease, European Journal of Medicinal Chemistry, 125 (2017) 784-792.

[20] A. Kulshreshtha, P. Piplani, Current pharmacotherapy and putative disease-modifying therapy for Alzheimer's disease, Neurological sciences : official journal of the Italian Neurological Society and of the Italian Society of Clinical Neurophysiology, 37 (2016) 1403-1435.

[21] A. Nordberg, C. Ballard, R. Bullock, T. Darreh-Shori, M. Somogyi, A Review of Butyrylcholinesterase as a Therapeutic Target in the Treatment of Alzheimer's Disease, The Primary Care Companion for CNS Disorders, 15 (2013) PCC.12r01412.

[22] A. Venneri, W.J. McGeown, M.F. Shanks, Empirical evidence of neuroprotection by dual cholinesterase inhibition in Alzheimer's disease, Neuroreport, 16 (2005) 107-110.

[23] W. Luo, Y.-P. Li, Y. He, S.-L. Huang, J.-H. Tan, T.-M. Ou, D. Li, L.-Q. Gu, Z.-S. Huang, Design, synthesis and evaluation of novel tacrine-multialkoxybenzene hybrids as dual inhibitors for cholinesterases and amyloid beta aggregation, Bioorganic & medicinal chemistry, 19 (2011) 763-770.

[24] Z. Wang, Y. Wang, B. Wang, W. Li, L. Huang, X. Li, Design, Synthesis, and Evaluation of Orally Available Clioquinol-Moracin M Hybrids as Multitarget-Directed Ligands for Cognitive Improvement in a Rat Model of Neurodegeneration in Alzheimer's Disease, Journal of Medicinal Chemistry, 58 (2015) 8616-8637.

ACCEPTED MANUSCRIPT

[25] K. Ono, K. Hasegawa, H. Naiki, M. Yamada, Curcumin has potent anti-amyloidogenic effects for Alzheimer's β-amyloid fibrils in vitro, Journal of neuroscience research, 75 (2004) 742-750.

[26] L. Di, E.H. Kerns, K. Fan, O.J. McConnell, G.T. Carter, High throughput artificial membrane permeability assay for blood–brain barrier, European journal of medicinal chemistry, 38 (2003) 223-232.

[27] R.S. Keri, C. Quintanova, S. Chaves, D.F. Silva, S.M. Cardoso, M.A. Santos, New Tacrine Hybrids with Natural-Based Cysteine Derivatives as Multitargeted Drugs for Potential Treatment of Alzheimer's Disease, Chemical biology & drug design, 87 (2016) 101-111.

[28] N. Guzior, A. Wieckowska, D. Panek, B. Malawska, Recent development of multifunctional agents as potential drug candidates for the treatment of Alzheimer's disease, Current medicinal chemistry, 22 (2015) 373-404.

[29] A. Kulshreshtha, P. Piplani, Ameliorative effects of amide derivatives of 1, 3, 4-thiadiazoles on scopolamine induced cognitive dysfunction, European journal of medicinal chemistry, 122 (2016) 557-573.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Highlights

1. A symbiotic combination of rivastigmine skeleton with natural antioxidant agents has been performed
2. The ChEs inhibitory activity as well as the % inhibition of Ab-aggregation were evaluated
3. LA2 and CA2 showed high scavenger properties and a good BBB permeability in PAMPA assay.
5. GA1 and CA2 produced a significant neuroprotection at 10 μM

ACCEPTED MANUSCRIPT