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New cinnamic – *N*-benzylpiperidine and cinnamic – *N*,*N*-dibenzyl(*N*-methyl)amine hybrids as Alzheimer-directed multitarget drugs with antioxidant, cholinergic, neuroprotective and neurogenic properties

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Abstract

Here we describe new families of multi-target directed ligands obtained by linking antioxidant cinnamic-related structures with *N*-benzylpiperidine (NBP) or *N*,*N*-dibenzyl(*N*-methyl)amine (DBMA) fragments. Resulting hybrids, in addition to their antioxidant and neuroprotective properties against mitochondrial oxidative stress, are active at relevant molecular targets in Alzheimer's disease, such as cholinesterases (hAChE and hBuChE) and monoamine oxidases (hMAO-A and hMAO-B). Hybrids derived from umbellic – NBP (**8**), caffeic – NBP (**9**), and ferulic – DBMA (**12**) displayed balanced biological profiles, with IC_{50}s in the low-micromolar and submicromolar range for hChEs and hMAOs, and an antioxidant potency comparable to vitamin E. Moreover, the caffeic – NBP hybrid **9** is able to improve the differentiation of adult SGZ-derived neural stem cells into a neuronal phenotype *in vitro*.

Keywords: cinnamic-based hybrids; antioxidants; neuroprotectants; neurogenic agents; human cholinesterases; human monoamine oxidases

Abbreviations: AChE, acetylcholinesterase; AD, Alzheimer's disease; βA, beta-amyloid peptide; BACE-1, beta-site amyloid precursor protein cleaving enzyme 1; BuChE, butyrylcholinesterase; DBMA, *N*,*N*-dibenzyl(*N*-methyl)amine; HMBC, heteronuclear multiple bond correlation; HSQC, heteronuclear single quantum correlation; MAOs, Monoamine oxidases; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NBP, *N*-benzylpiperidine; NMDA, *N*-methyl-D-aspartate receptor; ORAC, oxygen radical absorbance capacity; ROS, reactive oxygen species; SGZ, subgranular zone; SH-SY5Y, a human neuroblastoma cell line; SVZ, subventricular zone.

1. Introduction

The current therapeutic arsenal against Alzheimer's disease (AD) is composed by three acetylcholinesterase (AChE) inhibitors (donepezil, rivastigmine, and galantamine) and one antagonist of the *N*-methyl-D-aspartate (NMDA) receptor, named memantine [1]. These marketed drugs modestly alleviate some symptoms, like memory impairment, but they do not stop neuronal degeneration or repair brain damage [2].

Nowadays it is widely accepted that neuronal loss in AD and other neurodegenerative diseases is the outcome of a wide variety of factors highly interconnected. In the core of such complex scenario, oxidative stress emerges as an important actor that could trigger many pathological cascades [3]. The oxidative stress hypothesis states that toxicity exerted by free radical species is in the basis of many abnormal signalling pathways that finally lead to neuronal death. Reactive entities are able to interact with lipids in cellular membranes, altering their composition, function and permeability. By-products from peroxidation of unsaturated fatty acids include highly reactive and toxic species such as 4-hydroxynonenal, malondialdehyde and acrolein, which seems to be especially increased in AD. Moreover, other vital biomolecules are affected by free radicals. Arachidonic acid and docosahexaenoic acid, two important molecules whose concentrations reach the highest levels in brain, are susceptible to be oxidized under oxidative conditions [4-6].

Despite of the effective system of the human body to counteract the harmful effects of oxidative metabolism (e.g., superoxide dismutase, aldehyde dehydrogenase, and glutathione peroxidase), during aging the brain becomes particularly sensitive to oxidative damage due to its great requirement of oxygen, its high levels of unsaturated fatty acids and its relatively low levels of antioxidant enzymes [7].

Several investigations have demonstrated a correlation between the severity of AD and the levels of free radicals. Moreover, the AD-hallmark beta-amyloid peptide (βA) and subsequent amyloid plaques deposition has been proven to induce the production of hydrogen peroxide by reduction of metals such as iron and copper [8]. Simultaneously, βA is able to stimulate glial cells, enhancing the production of an oxidative environment and pro-inflammatory molecules [9-11]. Pathologic Aβs are the consequence of an abnormal cleavage of the amyloid precursor protein by beta-site amyloid precursor protein cleaving enzyme 1 (BACE-1) and several clinical studies have demonstrated a correlation between low levels of BACE-1 and an effective protection against AD and cognitive decline in the elderly [12]. Thus, in the last years BACE-1 inhibitors have been developed as potential drugs for AD [13].

Monoamine oxidases (MAO) are metabolic enzymes whose levels are increased in neurodegenerative diseases, such as AD and Parkinson disease. These high levels of MAOs correlate with the exacerbated production of reactive oxygen species (ROS), which are responsible of the toxic environment characteristic of neurodegeneration [14]. Consequently, MAO inhibitors have been explored as a complementary alternative in the search for new drugs for AD treatment, both to reduce ROS in the case of MAO-B, and to treat concomitant depression of AD patients, in the case of MAO-A [15].

Recently, neurogenic agents have emerged as innovative arms to combat AD and other neurological diseases [16]. The adult brain preserves a vestigial neurogenic activity in two stem-cells niches: the subventricular zone (SVZ) of the lateral ventricle and the subgranular zone (SGZ) in the hippocampus. Neurogenesis has been related to auto-repair processes in the brain and different molecular targets and signalling pathways involved in such processes have been identified. As a consequence, different drugs have been tested in neuronal plasticity [17]. For instance, it has been

demonstrated that several antioxidants promote the transition of pluripotent stem-cells to a fully reprogrammed phenotype and increase the number of connections between neurons [18,19]

According to the oxidative stress hypothesis, antioxidants could play an important role in prevention and therapy of AD. Among the most investigated natural antioxidants, there are some derivatives of the cinnamic acid (e.g., ferulic, caffeic, *p*-coumaric and umbellic acids) and natural molecules containing its structure, such as curcumin derivatives [20-25] (Figure 1).

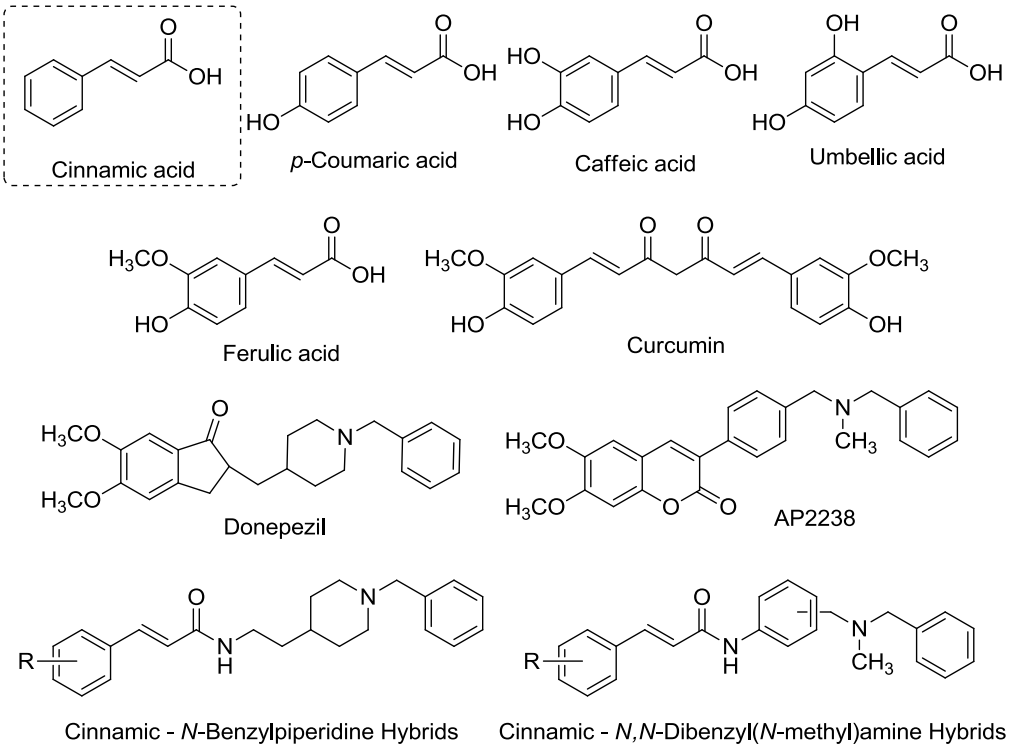


Figure 1. Structures of natural antioxidants containing the cinnamic acid structure (*p*-coumaric, caffeic, umbellic, and ferulic acids, curcumin), donepezil, AP2238, and new cinnamic – *N*-benzylpiperidine (NBP) (**1**-**9**) and cinnamic – *N*,*N*-dibenzyl(*N*-methyl)amine (DBMA) hybrids (**10**-**17**).

However, antioxidant molecules *per se* might not be sufficient to treat such highly complex pathologies like AD, as shown by the ambiguous results obtained from several clinical trials with this kind of compounds [26]. In this sense, multi-target directed ligands (MTLs) that combine antioxidant properties and other activities in additional targets of interest for AD (e.g, AChE, BACE, MAO) may greatly improve the treatment of the disease. In the last years, several MTLs have been developed [27,28], such as ladostigil [(*R*)-3-(prop-2-yn-1-ylamino)-2,3-dihydro-1*H*-inden-5-yl ethyl(methyl)carbamate] [29]. This dual AChE-MAO inhibitor enhances the expression of neurotrophic factors and reverses neuronal damage by promoting endogenous neurogenic processes.

Continuing with our interest in AD-directed drugs, which some of them displayed interesting neurogenic properties [30-35], in this work we planned to obtain new cinnamic-based antioxidants endowed with other fragments with AChE and MAO inhibition. For this purpose, we selected *N*-benzylpiperidine (NBP) and *N*,*N*-dibenzyl(*N*-methyl)amine (DBMA) fragments, which are present in the well-known AChE inhibitors donepezil and 3-(4-((benzyl(methyl)amino)methyl)phenyl)-6,7-dimethoxy-2*H*-chromen-2-one (AP2238), respectively [36-38] (Figure 1).

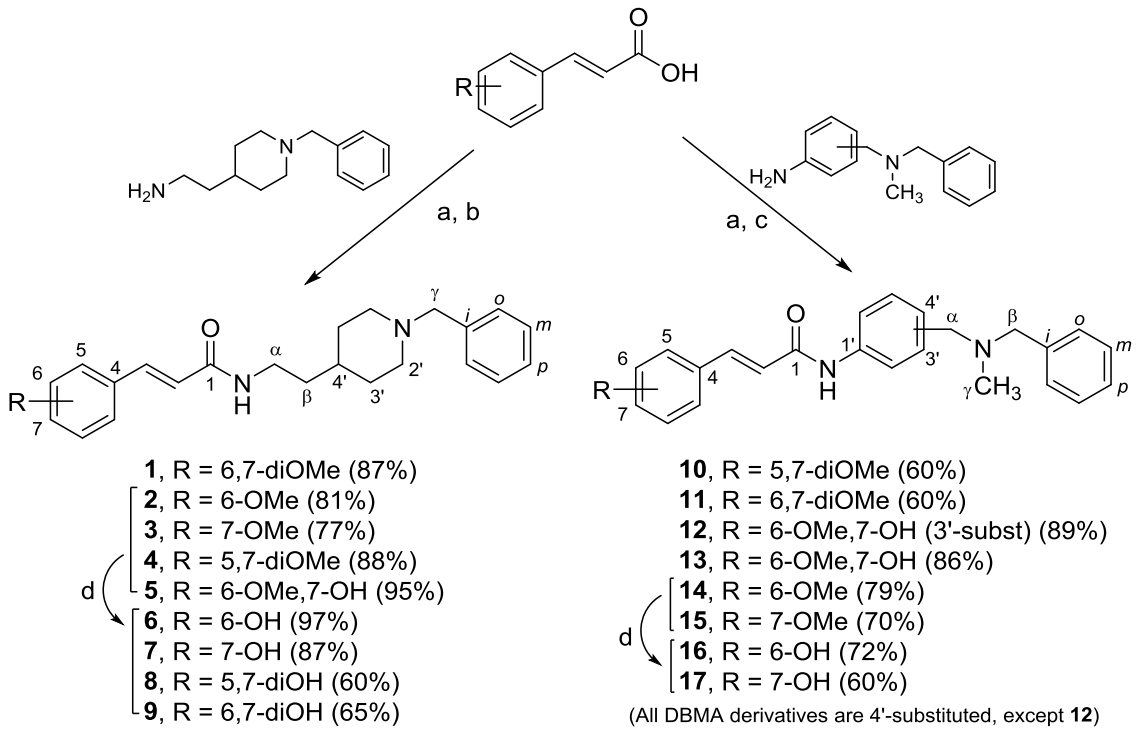
In this work, we describe the synthesis of new cinnamic – *N*-benzylpiperidine (**1**-**9**) and cinnamic – *N*,*N*-dibenzyl(*N*-methyl)amine (**10**-**17**) hybrids and their biological evaluation, which includes inhibition of human cholinesterases (hChEs) and monoamino oxidases (MAOs), a study of their oxygen radical absorbance capacity (ORAC) and their neuroprotective effects against death provoked by mitochondrial oxidative stress in the human neuroblastoma cell line SH-SY5Y. Finally, we explored the neurogenic effects of a selected hybrid using primary SGZ stem-cells from adult mice.

2. Results and discussion

*Synthesis of hybrids **1**-**17**.* Scheme 1 illustrates the synthetic routes to obtain cinnamic-NBP (**1**-**9**) and cinnamic-DBMA hybrids (**10**-**17**) from commercially available cinnamic-derived acids and amines carrying the NBP or the DBMA fragments. 2-(1-Benzylpiperidin-4-yl)ethan-1-amine is commercially available, whereas 3- and 4-((benzyl(methyl)amino)methyl)aniline were obtained as previously described (see Supplementary Information for further details). In a microwave oven operating at 120 ºC, activation of the corresponding acid was performed with 1,1'-carbonyldiimidazole (CDI) in THF during 7 minutes and the subsequent reaction with the proper amine during 10 or 40 minutes depending on its character, aliphatic or aromatic. Derivatives **1**-**5** and **10**-**15** were isolated in yields comprised between 60 and 95% (Scheme 1).

Deprotection of methoxy groups was carried out under mild conditions by overnight treatment with boron tribromide (BBr_3) at room temperature, giving hybrids **6**-**9**,**16**,**17** in moderate to excellent yields (60-97%). It is worth mentioning that increased yields were obtained when we used 1 equiv of BBr_3 per ether group to be cleavage, plus an additional equiv per each heteroatom included in the molecule, due to the well-known complexation tendency of boron with free electron pairs, which decreases the effective amount of reactive BBr_3 [39,40].

All cinnamic-based hybrids (**1**-**17**) were purified using an automatic chromatographic apparatus (IsoleraOne, Biotage) in silica gel cartridges and were characterized by their analytical (HPLC, HRMS) and spectroscopic data (^{1}H NMR, ^{13}C NMR). Complete NMR assignment of their hydrogen and carbon atoms (see Experimental Part for details) were made by ^{1}H – ^{13}C two-dimensional diagrams, mainly HSQC (heteronuclear single quantum correlation) and HMBC (heteronuclear multiple bond correlation).



Scheme 1. Reagents and conditions: (a) CDI, THF, MW, 7 min, 120 °C; (b) 2-(1-benzylpiperidin-4-yl)ethan-1-amine, MW, 10 min, 120 °C; (c) 3- or 4-((benzyl(methyl)amino)methyl)aniline, MW, 40 min, 120 °C; (d) BBr_3, THF, rt, overnight.

Biological Evaluation. The preliminary biological evaluation of new cinnamic-based hybrids comprised: (i) Inhibition of recombinant human cholinesterases (hAChE and hBuChE), using donepezil as reference and following the spectrophotometric method described by Ellman et al. [41]. (ii) Inhibition of recombinant human monoamine oxidases (hMAO-A and hMAO-B), expressed in baculovirus infected BTI insect cells, using three known MAO inhibitors as references (selegiline, iproniazid, and moclobemide) and following a described method [42]. (iii) Assessment of the radical scavenger ability, using trolox (an analogue of vitamin E) as internal standard and

following the oxygen radical absorbance capacity method (ORAC), as previously described [43,44]. Results are gathered in Table 1.

Regarding hAChE and hBuChE, the NBP series displayed a moderate inhibition of both isoforms, with IC_{50} in the micro- and sub-micromolar range, and in general, with a slight preference for hAChE. Methoxy derivatives showed just a little better inhibition than their hydroxy counterparts. The best hChEs inhibitor of the NBP series is **5** that unexpectedly exhibited the inversed preference than the rest of its congeners, with an IC_{50} = 76 nM towards hBuChE. Replacement of the NBP part with a DBMA fragment maintained the hAChE inhibition in the low-micromolar range, but reduced drastically the potency towards hBuChE. Thus, the cinnamic – DBMA hybrids showed a better hAChE-selectivity than the cinnamic – NBP series.

In relation to hMAO-A and hMAO-B inhibition, both series displayed almost the same pattern. Methoxy derivatives were found to be inactive and only hybrids bearing a *p*-hydroxy group in the cinnamic fragment showed a meaningful inhibitory activity in the micromolar range, with a slight preference for the hMAO-A isoform. In contrast, when the hydroxy group was located at position meta- the resulting derivative was almost inactive, as it can see in the couple **17** (7-OH, active) and **16** (6-OH, not active). Maintaining the *p*-hydroxy group, introduction of a second hydroxy functionality in position ortho- or meta- increased the inhibitory potency towards both isoforms. In fact, **8** (5,7-diOH) and **9** (6,7-diOH) were the most potent inhibitors of hMAO-A and hMAO-B described in this work, displaying one-digit-micromolar IC_{50}s values for both enzymes. These inhibitory activities are comparable with iproniazide, a drug that has been clinically used for treating depression in humans [45] and that was employed in our MAO experiments for comparative purposes (Table 1).

Concerning ORAC assay, methoxy derivatives were almost inactive. On the contrary, hydroxy substituted hybrids exhibited good antioxidant capacity in both series with ORAC values between 1.0 and 3.2 trolox equivalents. This means they were up to 3.2-fold more potent than trolox, the aromatic part of vitamin E and the responsible for the radical scavenger capacity. Thus, cinnamic-based hybrids bearing one or two hydroxy groups could be considered as good antioxidant agents. In contrast with hMAOs in which a *p*-methoxy group is essential to find inhibition, for the radical capture activity, cinnamic hybrids bearing one *m*-hydroxy group were superior. Hybrids **6** and **16** (6-OH) exhibited the best results with ORAC values of 2.2 and 3.2 in the NBP and DBMA series, respectively. Simultaneously, they inhibited both hChEs in the low-micromolar range, but were unable to inhibit any isoform of hMAO. Finally, hybrids bearing one hydroxy group in the *p*-cinnamic position or two in *o,p*- or *m,p*-positions showed also good antioxidant activities, ranging from the same than trolox to 1.8-fold more potent (Table 1).

Table 1. Inhibition of human cholinesterases (hAChE and hBuChE) and monoamine oxidases (hMAO-A and hMAO-B) (IC_{50}, μM); oxygen radical absorbance capacity (ORAC, trolox equiv.) [a]

Compd.	**hAChE**	**hBuChE**	**MAO-A**	**MAO-B**	**ORAC**
1	0.26±0.04	0.69±0.10	>100	45-55%[b]	0.4±0.04
2	0.75±0.10	0.98±0.12	45-55%[b]	>100	n.a.
3	0.49±0.06	0.76±0.20	45-55%[b]	45-55%[b]	n.a.
4	0.63±0.08	4.39±0.86	>100	>100	n.a.
5	0.39±0.05	0.076±0.01	11.4±0.7	13.1±0.8	1.8±0.2
6	1.12±0.10	1.02±0.21	45-55%[b]	>100	2.2±0.1
7	1.01±0.08	0.93±0.16	57.5±3.8	>100	1.7±0.1
8	0.99±0.10	0.26±0.08	5.5±0.3	8.3±0.5	1.3±0.6
9	1.75±0.12	0.69±0.12	3.5±0.2	6.0±0.4	1.0±0.1
10	8.73±0.92	>10	>100	>100	0.5±0.1
11	3.98±0.29	>10	n.d.	n.d.	0.4±0.08
12	5.99±0.49	5.89±0.48	4.5±0.3	7.7± 0.5	1.4±0.11
13	33%[c]	45%[c]	n.d.	n.d.	1.5±0.18
14	4.67±0.31	35%[c]	>100	>100	0.6±0.06
15	5.76±0.40	25%[c]	>100	>100	n.a.
16	3.49±0.31	46%[c]	45-55%[c]	45-55%[c]	3.2±0.2
17	46%[c]	30%[c]	29.7 ± 1.9	29.0 ±1.9	1.8±0.14
Donepezil	0.010±0.002	2.50±0.07	n.d.	n.d.	n.d.
AP2238	0.044[d]	48.9[d]	n.d.	n.d.	n.d.
R-(-)-deprenyl	n.d.	n.d.	68.7 ± 4.2	0.017 ± 0.0019	n.d.
Iproniazide	n.d.	n.d.	6.6 ± 0.8	7.5 ± 0.4	n.d.
Moclobemide	n.d.	n.d.	361 ± 19	>1000	n.d.
Trolox	n.d.	n.d.	n.d.	n.d.	1.0

[a]Results are expressed as mean ± SEM (n =5). [b] Inhibition percentage at 100 μM (highest concentration tested). [c]Inhibition percentage at 10 μM. [d]Taken from ref. [38] n.a.: not active at 10 μM. n.d.: not determined

A joint analysis of the above results points out that three hybrids belonging to the NBP series showed interesting multitarget profiles. The ferulic-based compound **5** showed a potent antioxidant capacity of 1.8 trolox equivalents, nanomolar and sub-micromolar inhibition of hBuChE and hAChE (76 nM and 0.39 μM, respectively), and moderate non-selective inhibitory activity towards hMAO-A and hMAO-B in the micromolar range (11 μM and 13 μM, respectively). Interestingly, dihydroxy substituted hybrids derived from umbellic acid **8** (5,7-diOH) and caffeic acid **9** (6,7-diOH) exhibited more balanced profiles, with IC_{50}s in the one-digit-micromolar and sub-micromolar range for enzymatic targets (hChEs and hMAOs) and an antioxidant potency comparable to vitamin E. Similar balanced multifunctional profile was found in the ferulic – DBMA hybrid **12**, which showed one-digit-micromolar IC_{50}'s in all tested targets (hAChE: 6.0 μM; hBuChE: 5.9 μM; hMAO-A: 4.5 μM; hMAO-B: 7.7 μM) and a radical scavenger activity 1.5-fold more potent than vitamin E.

Moreover, cinnamic – DBP and cinnamic – DBMA hybrids were tested as inhibitors of the human recombinant beta-secretase (hBACE-1), using a fluorescence resonance energy transfer (FRET)-based assay as previously described [40,46]. Cinnamic – DBMA hybrids **12-15** were capable to inhibit poorly this enzyme with percentages around 23-33% at 10 μM (data not shown).

To evaluate the behaviour of new hybrids at the cellular level, their neuroprotective capacity against mitochondrial oxidative stress were tested, using the human neuroblastoma cell line SH-SY5Y and a toxic insult composed by the mixture of rotenone and oligomycin A at concentrations of 30 and 10 μM, respectively. The combination of such toxics blocks complexes I and V of the mitochondrial electronic chain, generating a great amount of free radicals that finally provoke mitochondrial dysfunction and cell death [47,48]. Therefore, this assay is considered a good model of

mitochondrial oxidative stress. Compounds were co-incubated at four concentrations (0.1, 0.3, 1, and 3 μM) with a mixture of rotenone and oligomycin A, and maintained for 24 h period in cell culture. Afterwards, the percentage of cell viability was measured as MTT reduction [49]. The best antioxidant hybrid **16** (ORAC = 3.2 trolox equiv), the most potent hChE inhibitor **5** (IC_{50} = 76 nM) and ferulic-DBMA hybrid **12** with a well-balanced target profile in the micromolar range (hAChE: 6.0 μM; hBuChE: 5.9 μM; hMAO-A: 4.5 μM; hMAO-B: 7.7 μM) were chosen for this neuroprotective assay. Melatonin was used as a positive reference [32], and results are shown in Figure 2. Tested compounds protected around 30-35% of cells at a concentration of 0.3 μM, although a dose-dependent relationship was not observed in these experiments. Nevertheless, in any case they did not potentiate the toxicity exerted by rotenone/oligomycin A, which could be assumed as a lack of toxicity of our newly obtained hybrids.

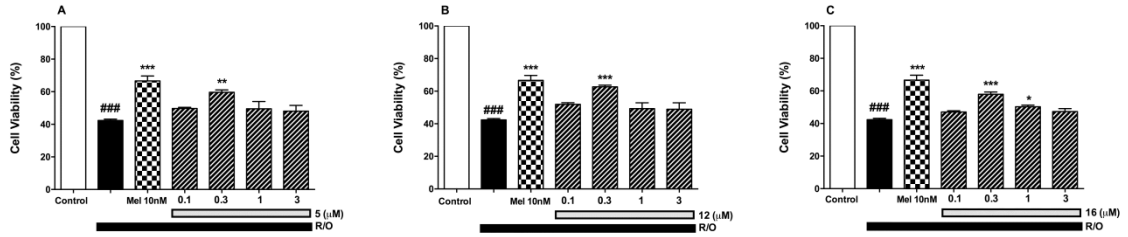


Figure 2. Effect of compounds **5** (A), **12** (B), and **16** (C) at 0.1, 0.3, 1.0, and 3.0 μM on cell death induced by the combination of rotenone (30 μM) and oligomycin A (10 μM). Melatonin (10 nM) is used as positive control in all experiments. Cell viability was measured as MTT reduction and data were normalized as % of control. Data are expressed as the means ± SEM of triplicate of at least three different cultures. All compounds were assayed at increasing concentrations (0.1-3 μM). ***P≤ 0.001, **P≤ 0.01, *P≤0.05, with respect to control group. Comparisons between drugs and control group were performed by one-way ANOVA followed by the Newman–Keuls post-hoc test.

Finally, caffeic – NBP hybrid **9** with a balanced multifunctional profile in hChEs, hMAOs and ORAC (hAChE: 1.75 μM; hBuChE: 0.69 μM; hMAO-A: 3.5 μM; hMAO-B: 6.0 μM; ORAC: 1.0 trolox equiv), was chosen to evaluate the ability of new cinnamic-based hybrids in promoting the differentiation of neural stem-cells towards a neuronal phenotype. To that purpose, neural stem cells (NSC) isolated from one of the main neurogenic niches in the adult, the subgranular zone (SGZ) of the dentate gyrus, were used as described in the experimental section. NSC were grown as neurospheres in the presence of hybrid **9** (10 μM) during 7 days and later on allowed for 3 days to differentiate when adhered on a substrate in the presence of serum and compound. At this point, immunocytochemical analysis was performed to evaluate the ability of tested compound to promote neuronal differentiation. Our results summarized in figure 3 showed that the new caffeic-based hybrid **9** clearly induced the differentiation of adult SGZ-derived NSC into a neuronal phenotype. After treatment with compound **9** a significant increase in the number of β-III-tubulin (early neurogenesis marker) and MAP-2 (microtubule-associated protein expressed in mature neurons) expressing cells in the neurospheres in shown. These results suggest that the new caffeic – NBP hybrid **9** has the ability to induce the differentiation of adult neural stem cells into neurons, promoting its maturation *in vitro* and showing a great neurogenic effect.

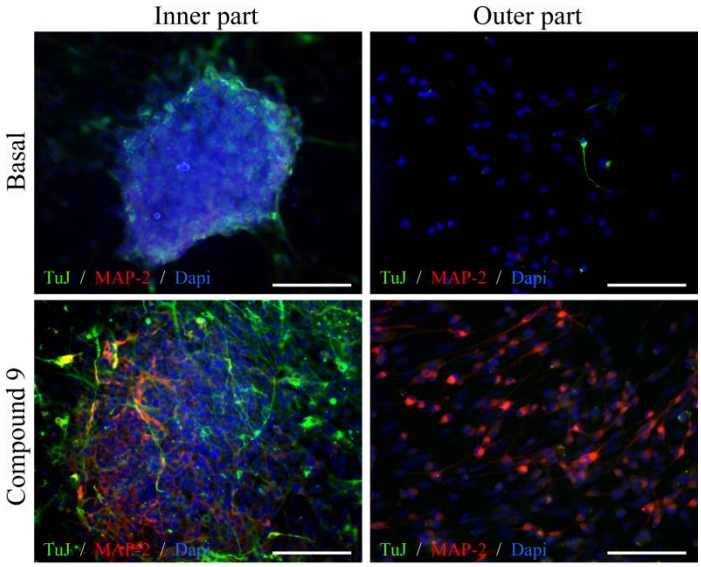


Figure 3. Caffeic-based hybrid **9** promotes neuronal differentiation *in vitro*. Adult murine neural stem cells isolated from the neurogenic niche of the SGZ of the hippocampus were grown as neurospheres (NS) during 7 days in the presence of compound **9** (10 µM). Then, NS were allowed to differentiate on a substrate for another 3 days in the presence of tested compound. Immunocytochemical analysis shows the expression of two well-known neuronal markers: β-III-tubulin (TuJ clone; green) and MAP-2 (red) inside the NS (inner part) and in the distal area (outer part). DAPI was used for nuclear staining. Scale bar, 200µm.

3. Conclusions

The combination of antioxidant cinnamic-related structures with NBP and DBMA fragments gave new hybrids with interesting *in vitro* biological activities. At micro- and sub-micromolar range they inhibited human AChE, BuChE, MAO-A, and MAO-B, and showed antioxidant, neuroprotective, and neurogenic properties. In relation to hChEs inhibition, NBP-based hybrids were more potent than DBMA derivatives and methoxy derivatives showed better inhibition than their hydroxy counterparts. Regarding hMAOs the presence of a *p*-hydroxy group in the cinnamic fragment was essential for getting inhibition, and introduction of a second hydroxy functionality in position ortho- or meta- increased the inhibitory potency towards both hMAO-A and hMAO-B shifting IC_{50}s to one-digit-micromolar values. For the radical capture activity, both NBP and DBMA hybrids bearing one *m*-hydroxy group in the cinnamic fragment displayed better ORAC values than methoxy derivatives and even, better than diphenolic derivatives. In addition, a selection of hybrids protected the human neuroblastoma cell line SH-SY5Y against damage provoked by mitochondrial free radicals.

Among the different molecules investigated, we found that hybrids derived from umbellic – NBP (**8**), caffeic – NBP (**9**), and ferulic – DBMA (**12**) displayed balanced biological profiles, with IC_{50}s in the low-micromolar and submicromolar range for hChEs and hMAOs, and an antioxidant potency comparable to vitamin E. Last but not least, the caffeic – NBP hybrid **9** stimulated the differentiation of adult SGZ-derived neural stem cells into a neuronal phenotype, showing a great neurogenic effect.

Thus, it is expected that these new cinnamic-based hybrids could increase patient memory, decrease free radical levels, protect neurons from mitochondrial oxidative stress, and promote brain auto-repair processes. This biological profile highlights these new cinnamic-based hybrids as useful multi-target prototypes in the search for new

protective and regenerative drugs for the potential treatment of AD and other neurodegenerative diseases.

4. Experimental

4.1. Chemistry. General Methods

Reagents and solvents were purchased from common commercial suppliers, mostly Sigma-Aldrich, and were used without further purification. Analytical thin-layer chromatography (TLC) was carried out using Merck silica gel 60 F254 plates, and the compounds were visualized under UV-light (λ = 254 or 365 nm) and/or stained with phosphomolybdic acid 10% wt. in ethanol. Automatized chromatographic separation was carried out in an IsoleraOne (Biotage) equipment, using different silica Biotage ZIP KP-Sil 50μ cartridges. High-performance liquid chromatography was performed on a Waters analytical HPLC-MS (Alliance Watters 2690) equipped with a SunFire C_{18} 4.6 x 50 mm column, a UV photodiode array detector (λ = 214–274 nm) and quadrupole mass spectrometer (Micromass ZQ). HPLC analyses were used to confirm the purity of all compounds (≥ 95%) and were performed on Waters 6000 equipment, at a flow rate of 1.0 mL/min, with a UV photodiode array detector (λ = 214–274 nm), and using a Delta Pak C_{18} 5 μm, 300 Å column. The elution was performed in a gradient mixture of MeCN/water.

Melting points (uncorrected) were determined in a MP70 apparatus (Mettler Toledo). ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ or CD_3OD solutions using the following NMR spectrometers: Varian INOVA-300, Varian INOVA-400, Varian Mercury-400 or Varian Unity-500. Chemical shifts are reported in δ scale (ppm) relative to internal Me_4Si. *J* values are given in hertz, and spin multiplicities are expressed as s (singlet), broad signal (bs), d (doublet), t (triplet), q (quartet), or m (multiplet). High Resolution Mass Spectra (HRMS) were obtained by using an Agilent 1200 Series LC system (equipped with a binary pump, an autosampler, and a column oven) coupled to a 6520 quadrupole-time of flight (QTOF) mass spectrometer.

Acetonitrile:water (75:25, v:v) was used as mobile phase at 0.2 mL min-1. The ionization source was an ESI interface working in the positive-ion mode. The electrospray voltage was set at 4.5 kV, the fragmentor voltage at 150 V and the drying gas temperature at 300 °C. Nitrogen (99.5% purity) was used as nebulizer (207 kPa) and drying gas (6 L min^{-1}).

*4.2. General procedure for the synthesis of cinnamic-based hybrids **1**-**5** and **10**-**13**.*

The corresponding acid (1 mmol) and CDI (1.15 mmol) were mixed into a microwave tube under nitrogen atmosphere. The tube was sealed up and 3 mL of anhydrous THF were added using a syringe to dissolve the mixture (CO_2↑). This solution was heated into a microwave reactor at 120 ^{0}C during 7 min to complete the acid activation. Afterward, a solution of 1.2 mmol of the corresponding amine in 2 mL of THF was added with a syringe into the tube; this mixture was heated at 120 ^{0}C during 10 minutes for NBP derivatives and 40 min for DBMA hybrids. After solvent evaporation under reduced pressure, the crude was re-dissolved in 25 mL of EtOAc and washed with water (3x5 mL) and brine (3x5 mL), dried over magnesium sulfate and concentrated under reduced pressure. The crude was purified by column chromatography using EtOAc:MeOH (9:1) or hexane/EtOAc (0→70%) as eluent.

*4.2.1. (E)-N-(2-(1-Benzylpiperidin-4-yl)ethyl)-3-(3,4-dimethoxyphenyl)acrylamide (**1**)*

From 3,4-dimethoxy cinnamic acid (0.100 g, 0.48 mmol) and 2-(1-benzylpiperidin-4-yl)ethan-1-amine (0.126 g, 0.58 mmol), hybrid **1** was obtained (0.170 g, 87%) as a light yellow solid of mp: 108.1-110.5 ºC. ^{1}H NMR (500 MHz, MeOD) δ 7.46 (d, *J* = 15.7 Hz, 1H, H_3), 7.32 (s, 2H, H_m), 7.31 (s, 2H, H_o), 7.29 – 7.24 (m, 1H, H_p), 7.14 (d, *J*

= 2.0 Hz, 1H, H_5), 7.12 (ddd, *J* = 8.2, 2.0, 0.5 Hz, 1H, H_9), 6.96 (d, *J* = 8.2 Hz, 1H, H_8), 6.47 (d, *J* = 15.7 Hz, 1H, H_2), 3.86 (s, 3H, H_{71}), 3.85 (s, 3H, H_{61}), 3.51 (s, 2H, H_γ), 3.35 – 3.32 (m, 2H, H_α), 2.90 (dt, *J* = 12.1, 3.5 Hz, 2H, $H_{2'eq}$), 2.05 – 1.99 (m, 2H, $H_{2'ax}$), 1.76 – 1.71 (m, 2H, $H_{3'eq}$), 1.50 (dt, *J* = 7.9, 6.6 Hz, 2H, H_β), 1.37 (dddd, *J* = 16.4, 9.7, 6.4, 3.5 Hz, 1H, $H_{4'}$), 1.28 (qd, *J* = 12.1, 3.5 Hz, 2H, $H_{3'ax}$). ^{13}C NMR (126 MHz, MeOD) δ 168.86 (C_1), 152.20 (C_6), 150.70 (C_7), 141.56 (C_3), 138.19 (C_i), 130.92 (C_o), 129.36 (C_4), 129.26 (C_m), 128.43 (C_p), 123.16 (C_9), 119.67 (C_2), 112.71 (C_8), 111.31 (C_5), 64.34 (C_γ), 56.42 (C_{61}), 56.40 (C_{71}), 54.64 ($C_{2'}$), 38.10 (C_α), 37.10 (C_β), 34.44 ($C_{4'}$), 32.72 ($C_{3'}$). HRMS [ESI+] m/z =408.2407 $[M]^+$, Calcd for $[C_{25}H_{32}N_2O_3]^+$ 408.2413. HPLC purity 99%.

*4.2.2. (E)-N-(2-(1-Benzylpiperidin-4-yl)ethyl)-3-(3-methoxyphenyl)acrylamide (**2**)*

From 3-methoxy cinnamic acid (0.250 g, 1.40 mmol) and 2-(1-benzylpiperidin-4-yl)ethan-1-amine (0.360 g, 1.61 mmol) derivative **2** was obtained (0.430 g, 81%) as a white amorphous solid of mp: 68-70 ºC. ^{1}H NMR (300 MHz, MeOD) δ 7.48 (d, *J* = 15.8 Hz, 1H, H_3), 7.39 – 7.21 (m, 6H, Ph, H_7), 7.18 – 7.06 (m, 2H, $H_{5,9}$), 6.94 (ddd, *J* = 8.2, 2.6, 1.0 Hz, 1H, H_8), 6.58 (d, *J* = 15.8 Hz, 1H, H_2), 3.82 (s, 3H, H_{61}), 3.51 (s, 2H, H_γ), 3.38 – 3.32 (m, 2H, H_α), 2.90 (d, *J* = 11.4 Hz, $2H_{2'eq}$), 2.02 (t, *J* = 11.3 Hz, 2H, $H_{2'ax}$), 1.74 (d, *J* = 12.2 Hz, 2H, $H_{3'eq}$), 1.51 (q, *J* = 6.8 Hz, 2H, H_β), 1.42 – 1.19 (m, 3H, $H_{3'ax,\ 4'}$). ^{13}C NMR (75 MHz, MeOD) δ 168.47 (C_1), 161.53 (C_6), 141.51 (C_3), 138.28 (C_i), 137.68 (C_4), 130.92 (C_7), 130.90 (C_o), 129.25 (C_m), 128.40 (C_p), 122.16 (C_2), 121.32 (C_9), 116.48 (C_8), 113.83 (C_5), 64.37 (C_γ), 55.73 (C_{61}), 54.66 ($C_{2'}$), 38.13 (C_α), 37.06 (C_β), 34.46 ($C_{4'}$), 32.75 ($C_{3'}$). HRMS [ESI+] m/z =378.2304 $[M]^+$, Calcd for $[C_{24}H_{30}N_2O_2]^+$ 378.2307. HPLC purity 100%.

*4.2.3. (E)-N-(2-(1-Benzylpiperidin-4-yl)ethyl)-3-(4-methoxyphenyl)acrylamide (**3**)*

From 4-methoxy cinnamic acid (0.250 g, 1.40 mmol) and 2-(1-benzylpiperidin-4-yl)ethan-1-amine (0.360 g, 1.70 mmol) derivative **3** was obtained (0.424 g, 77%) as a white amorphous solid of mp: 103-105 ºC. ^{1}H NMR (500 MHz, MeOD) δ 7.49 (d, *J* = 8.5 Hz, 2H, H_5), 7.45 (d, *J* = 15.7 Hz, 1H, H_3), 7.34 – 7.28 (m, 4H, $H_{o,\,m}$), 7.28 – 7.23 (m, 1H, H_p), 6.93 (d, *J* = 8.5 Hz, 2H, H_6), 6.44 (d, *J* = 15.7 Hz, 1H, H_2), 3.81 (s, 3H, H_{71}), 3.50 (s, 2H, H_γ), 3.32 – 3.30 (m, 2H, H, H_α), 2.89 (dt, *J* = 12.2, 3.5 Hz, 2H, $H_{2'eq}$), 2.01 (td, *J* = 11.8, 2.4 Hz, 2H, $H_{2'ax}$), 1.73 (d, *J* = 12.3 Hz, 2H, $H_{3'eq}$), 1.50 (q, *J* = 6.9 Hz, 2H, H_β), 1.37 (dtq, *J* = 13.9, 6.9, 3.5 Hz, 1H, $H_{4'}$), 1.27 (qd, *J* = 12.2, 3.5 Hz, 2H, $H_{3'ax}$). ^{13}C NMR (126 MHz, MeOD) δ 168.95 (C_1), 162.56 (C_7), 141.34 (C_5), 138.25 (C_i), 130.92 (C_o), 130.37 (C_5), 129.25 (C_m), 128.87 (C_4), 128.41 (C_p), 119.30 (C_2), 115.33 (C_6), 64.37 (C_γ), 55.82 (C_{71}), 54.65 ($C_{2'}$), 38.10 (C_α), 37.10 (C_β), 34.45 ($C_{4'}$), 32.74 ($C_{3'}$). HRMS [ESI+] m/z =378.2304 $[M]^+$, Calcd for $[C_{24}H_{30}N_2O_2]^+$ 378.2307. HPLC purity 100%.

*4.2.4. (E)-N-(2-(1-Benzylpiperidin-4-yl)ethyl)-3-(2,4-dimethoxyphenyl)acrylamide (**4**)*

From 2,4-dimethoxy cinnamic acid (0.300 g, 1.44 mmol) and 2-(1-benzylpiperidin-4-yl)ethan-1-amine (0.370 g, 1.73 mmol) hybrid **4** was obtained (0.517 g, 88%) as a beige solid of mp: 90-93 ºC. ^{1}H NMR (300 MHz, MeOD) δ 7.74 (d, *J* = 15.8 Hz, 1H, H_3), 7.44 (d, *J* = 8.2 Hz, 1H, H_9), 7.36 – 7.28 (m, 4H, $H_{o,\,m}$), 7.29 – 7.18 (m, 1H, H_p), 6.62 – 6.48 (m, 3H, H_2, H_6, H_8), 3.87 (s, 3H, H_{51}), 3.82 (s, 3H, H_{71}) 3.49 (s, 2H, H_γ), 3.37 – 3.26 (m, 2H, H_α), 2.97 – 2.78 (m, 2H, $H_{2'eq}$), 2.00 (t, *J* = 11.2 Hz, 2H, $H_{2'ax}$), 1.80 – 1.62 (m, 2.5 Hz, 2H, $H_{3'eq}$), 1.49 (q, *J* = 6.9 Hz, 2H, H_β), 1.39 - 1.19 (m, 3H, $H_{4'}$,

$H_{3'ax}$). ^{13}C NMR (75 MHz, MeOD) δ 169.64 (C_1), 163.96 (C_7), 161.03 (C_5), 138.27(C_i), 136.98 (C_3), 130.91 (C_o), 130.83 (C_9), 129.24 (C_m), 128.39 (C_p), 119.53 (C_2), 117.83 (C_4), 106.69 (C_6), 99.23 (C_8), 64.37 (C_γ), 56.01 (C_{51}), 55.91(C_{71}), 54.66 ($C_{2'}$), 38.08 (C_α), 37.13 (C_β), 34.44 ($C_{4'}$), 32.74 ($C_{3'}$). HRMS [ESI+] m/z =408.2416 $[M]^+$, Calcd for $[C_{25}H_{32}N_2O_3]^+$ 408.2413. HPLC purity 99%.

*4.2.5. (E)-N-(2-(1-Benzylpiperidin-4-yl)ethyl)-3-(4-hydroxy-3-methoxyphenyl)acrylamide (**5**)*

From ferulic acid (0.100 g, 0.50 mmol) and 2-(1-benzylpiperidin-4-yl)ethan-1-amine (0.135 g, 0.6 mmol) ferulic – NBP hybrid **5** was obtained (0.193 g, 95%), as a bright yellow solid of mp: 83.1-84.5 ºC. ^1H NMR (500 MHz, MeOD) δ 7.43 (d, *J* = 15.7 Hz, 1H, H_3), 7.35 – 7.31 (m, 4H, $H_{o,\ m}$), 7.30 – 7.24 (m, 1H, H_p), 7.12 (d, *J* = 2.0 Hz, 1H, H_5), 7.03 (dd, *J* = 8.2, 2.0 Hz, 1H, H_9), 6.80 (d, *J* = 8.2 Hz, 1H, H_8), 6.42 (d, *J* = 15.7 Hz, 1H, H_2), 3.89 (s, 3H, H_{61}), 3.54 (s, 2H, H_γ), 3.35 – 3.33 (m, 2H, H_α), 2.92 (dt, *J* = 11.9, 3.5 Hz, 2H, $H_{2'eq}$), 2.05 (td, *J* = 11.9, 2.5 Hz, 2H, $H_{2'ax}$), 1.75 (dt, *J* = 13.0, 2.5 Hz, 2H, $H_{3'eq}$), 1.51 (q, *J* = 7.0 Hz, 2H, H_β), 1.39 (dtt, *J* = 13.5, 6.6, 3.5 Hz, 1H, $H_{4'}$), 1.30 (td, *J* = 12.5, 3.5 Hz, 2H, $H_{3'ax}$). ^{13}C NMR (126 MHz, MeOD) δ 169.11 (C_1), 149.90 (C_7), 149.30 (C_6), 141.97 (C_3), 138.01 (C_i), 130.97 (C_o), 129.29 (C_m), 128.51 (C_p), 128.23 (C_4), 123.16 (C_9), 118.70 (C_2), 116.49 (C_8), 111.52 (C_5), 64.29 (C_γ), 56.36 (C_{61}), 54.63 ($C_{2'}$), 38.06 (C_α), 37.10 (C_β), 34.39 ($C_{4'}$), 32.67 ($C_{3'}$). HRMS [ESI+] m/z =394.2260 $[M]^+$, Calcd for $[C_{24}H_{30}N_2O_3]^+$ 394.2256. HPLC purity 99%.

*4.2.6. (E)-N-(4-((Benzyl(methyl)amino)methyl)phenyl)-3-(2,4-dimethoxyphenyl)acrylamide (**10**)*

Reaction of 2,4-dimethoxy cinnamic acid (0.150 g, 0.72 mmol) and 4-((benzyl(methyl)amino)methyl)aniline (0.195 g, 0.86 mmol) yielded hybrid **10** (0.180 g, 60%) as a white amorphous solid of mp: 105-107 ^{0}C. ^{1}H NMR (300 MHz, MeOD) δ 7.90 (d, *J* = 15.8 Hz, 1H, H_3), 7.65 (d, *J* = 8.2 Hz, 2H, $H_{2'}$), 7.51 (d, *J* = 8.7 Hz, 1H, H_9), 7.35 – 7.23 (m, 7H, Ph, $H_{3'}$), 6.77 (d, *J* = 15.7 Hz, 1H, H_2), 6.62 – 6.49 (m, 2H, $H_{8,6}$), 3.91 (s, 3H, H_{51}), 3.85 (s, 3H, H_{71}), 3.52 (s, 2H, H_β), 3.50 (s, 2H, H_α), 2.18 (s, 3H, H_γ). ^{13}C NMR (75 MHz, MeOD) δ 167.83 (C_1), 164.21 (C_7), 161.26 (C_5), 139.69 (C_i), 139.33 ($C_{1'}$), 138.31 (C_3), 135.31 ($C_{4'}$), 131.19 (C_9), 130.84 ($C_{3'}$), 130.40 (C_o), 129.30 (C_m), 128.25 (C_p), 121.05 ($C_{2'}$), 119.82 (C_2), 117.83 (C_4), 106.81 (C_6), 99.27 (C_8), 62.61 (C_β), 62.21 (C_α), 56.05 (C_{51}), 55.93 (C_{71}), 42.31 (C_γ). HRMS [ESI+] m/z = 416.2099 $[M]^+$, Calcd for $[C_{26}H_{28}N_2O_3]^+$ 416.2100. HPLC purity 100%.

*4.2.7. (E)-N-(4-((Benzyl(methyl)amino)methyl)phenyl)-3-(3,4-dimethoxyphenyl)acrylamide (**11**)*

3,4-Dimethoxy cinnamic acid (0.050 g, 0.24 mmol) and 4-((benzyl(methyl)amino)methyl)aniline (0.065 g, 0.28 mmol) were reacted as described in general procedure to obtain **11** (0.060 g, 60%) as a white amorphous solid of mp: 107-110 ºC. ^{1}H NMR (300 MHz, MeOD) δ 7.68 – 7.57 (m, 3H, $H_{3,2'}$), 7.38 – 7.24 (m, 7H, Ph, $H_{3'}$), 7.22 – 7.13 (m, 2H, $H_{5,9}$), 6.98 (d, *J* = 8.0 Hz, 1H, H_8), 6.67 (d, *J* = 15.5 Hz, 1H, H_2), 3.89 (s, 3H, H_{61}), 3.87 (s, 3H, H_{71}), 3.52 (s, 2H, H_α), 3.50 (s, 2H, H_β), 2.17 (s, 3H, H_γ). ^{13}C NMR (75 MHz, MeOD) δ 166.97 (C_1), 152.44 (C_7), 150.73 (C_6), 142.78 (C_3), 139.66 (C_i), 139.18 ($C_{1'}$), 135.47 ($C_{4'}$), 130.88 ($C_{3'}$), 130.40 (C_o), 129.36 (C_4), 129.31 (C_m), 128.26 (C_p), 123.43 (C_9), 121.03 ($C_{2'}$), 119.98 (C_2), 112.74 (C_8), 111.43

(C_5), 62.62 (C_α), 62.20 (C_β), 56.44 (C_{61}), 56.42 (C_{71}), 42.31 (C_γ). HRMS [ESI+] m/z =416.2112 $[M]^+$, Calcd for $[C_{26}H_{28}N_2O_3]^+$ 416.2100. HPLC purity 100%.

*4.2.8. (E)-N-(3-((Benzyl(methyl)amino)methyl)phenyl)-3-(4-hydroxy-3-methoxyphenyl)acrylamide (**12**)*

From ferulic acid (0.100 g, 0.50 mmol) and 3-((benzyl(methyl)amino)methyl)aniline (0.14 g, 0.60 mmol) ferulic – DBMA hybrid **12** was obtained (0.184 g, 89%), as a bright yellow solid of mp: 72 – 74 ºC. ^{1}H NMR (400 MHz, MeOD) δ 7.64 (s, 1H, $H_{2'}$), 7.56 (s, 1H, $H_{6'}$), 7.56 (d, *J* = 15.7 Hz, 1H, H_3), 7.36 – 7.21 (m, 6H, Ph, $H_{5'}$), 7.15 (d, *J* = 2.0 Hz, 1H, H_5), 7.10 – 7.05 (m, 2H, $H_{9,4'}$), 6.80 (d, *J* = 8.1 Hz, 1H, H_8), 6.60 (d, *J* = 15.6 Hz, 1H, H_2), 3.88 (s, 3H, H_{61}), 3.51 (s, 2H, H_β), 3.49 (s, 2H, H_α), 2.16 (s, 3H, H_γ). ^{13}C NMR (101 MHz, MeOD) δ 167.27 (C_1), 150.16 (C_7), 149.33 (C_4), 143.25 (C_3), 140.66 ($C_{3'}$), 140.18 (C_i), 139.70 ($C_{1'}$), 130.40 (C_o), 129.78 (C_p), 129.32 (C_m), 128.27 ($C_{5'}$), 128.18 (C_4), 126.08 ($C_{4'}$), 123.42 (C_9), 121.97 ($C_{2'}$), 120.06 ($C_{6'}$), 119.04 (C_2), 116.54 (C_8), 111.65 (C_5), 62.74 (C_β), 62.62 (C_α), 56.36 (C_{61}), 42.41 (C_γ). HRMS [ESI+] *m/z* =402.1934 $[M]^+$, Calcd for $[C_{25}H_{26}N_2O_3]^+$ 402.1943. HPLC purity 98%.

*4.2.9. (E)-N-(4-((Benzyl(methyl)amino)methyl)phenyl)-3-(4-hydroxy-3-methoxyphenyl)acrylamide (**13**)*

From ferulic acid (0.100 g, 0.50 mmol) and 4-((benzyl(methyl)amino)methyl)aniline (0.140 g, 0.60 mmol), ferulic – DBMA hybrid **13** was obtained (0.177 g, 86%), as a bright yellow solid of mp: 80 – 83 ºC. ^{1}H NMR (500 MHz, MeOD) δ 7.63 (d, *J* = 8.5 Hz, 2H, $H_{2'}$), 7.57 (d, *J* = 15.6 Hz, 1H, $H_{3'}$), 7.36 – 7.31

(m, 6H, Ph, $H_{3'}$), 7.29 – 7.23 (m, 1H, H_p), 7.17 (d, *J* = 2.1 Hz, 1H, H_5), 7.08 (dd, *J* = 8.2, 2.2 Hz, 1H, H_9), 6.82 (d, *J* = 8.2 Hz, 1H, H_8), 6.61 (d, *J* = 15.6 Hz, 1H, H_2), 3.90 (s, 3H, H_{61}), 3.56 (s, 2H, H_β), 3.55 (s, 2H, H_α), 2.20 (s, 3H, H_γ). ^{13}C NMR (126 MHz, MeOD) δ 167.23 (C_1), 150.16 (C_6), 149.33 (C_7), 143.26 (C_3), 139.44 (C_i), 136.29 ($C_{1'}$), 134.77 ($C_{4'}$), 131.03 ($C_{3'}$), 130.54 (C_o), 129.40 (C_m), 128.48 (C_4), 128.19 (C_p), 123.43 (C_9), 121.05 ($C_{2'}$), 119.00 (C_2), 116.55 (C_8), 111.67 (C_5), 62.50 (C_α), 62.09 (C_β), 56.38 (C_{61}), 42.12 (C_γ). HRMS [ESI+] m/z =402.1902 $[M]^+$, Calcd for $[C_{25}H_{26}N_2O_3]^+$ 402.1943. HPLC purity 97%.

*4.2.10. (E)-N-(4-((Benzyl(methyl)amino)methyl)phenyl)-3-(3-methoxyphenyl)acrylamide (**14**)*

(*E*)-3-(3-Methoxyphenyl)acrylic acid (0.150 g, 1.00 mmol) and 4-((benzyl(methyl)amino)methyl)aniline (0.220 g, 1.00 mmol), were reacted as described in the general procedure b to obtain **14** (0.255 g, 79%) as a white amorphous solid of mp: 90-93 ºC. ^{1}H NMR (300 MHz, MeOD) δ 7.68 – 7.60 (m, 3H, $H_{3,\,2'}$), 7.38 – 7.25 (m, 8H, Ph, $H_{8,\,3'}$), 7.21 – 7.12 (m, 2H, $H_{5,\,9}$), 6.97 (dd, *J* = 8.1, 2.6 Hz, 1H, H_7), 6.79 (d, *J* = 15.7 Hz, 1H, H_2), 3.84 (s, 3H, H_{61}), 3.52 (s, 2H, H_β), 3.50 (s, 2H, H_α), 2.17 (s, 3H, H_γ). ^{13}C NMR (75 MHz, MeOD) δ 166.55 (C_1), 161.56 (C_6), 142.70 (C_3), 139.70 (C_i), 139.07 ($C_{1'}$), 137.60 (C_4), 135.67 ($C_{4'}$), 130.99 (C_8), 130.87 ($C_{3'}$), 130.39 (C_o), 129.30 (C_m), 128.25 (C_p), 122.55 (C_2), 121.48 (C_9), 121.08 ($C_{2'}$), 116.72 (C_7), 113.98 (C_5), 62.64 (C_β), 62.20 (C_α), 55.75 (C_{61}), 42.32 (C_γ). HRMS [ESI+] m/z =386.1998 $[M]^+$, Calcd for $[C_{25}H_{26}N_2O_2]^+$ 386.1994. HPLC purity 99%.

*4.2.11. (E)-N-(4-((Benzyl(methyl)amino)methyl)phenyl)-3-(4-methoxyphenyl)acrylamide (**15**)*

4-Methoxy cinnamic acid (0.150 g, 0.84 mmol) and 4-((benzyl(methyl)amino)methyl)aniline (0.220 g, 1.00 mmol) were reacted as described in the general procedure b to obtain hybrid **15** (0.230 g, 70%) as a white amorphous solid of mp: 131-134 ºC. ^{1}H NMR (300 MHz, MeOD) δ 7.70 – 7.60 (m, 3H, $H_{3,2'}$), 7.57 (d, *J* = 8.6 Hz, 2H, H_5), 7.39 – 7.27 (m, 7H, Ph, $H_{3'}$), 6.99 (d, *J* = 8.6 Hz, 2H, H_6), 6.67 (d, *J* = 15.6 Hz, 1H, H_2), 3.86 (s, 3H, H_{71}), 3.54 (s, 2H, H_β), 3.52 (s, 2H, H_α), 2.19 (s, 3H, H_γ). ^{13}C NMR (75 MHz, MeOD) δ 167.09 (C_1), 162.80(C_7), 142.58(C_3), 139.70(C_i), 139.20($C_{1'}$), 135.51($C_{4'}$), 130.87($C_{3'}$), 130.59(C_o), 130.39(C_5), 129.31(C_m), 128.85(C_4), 128.26(C_p), 121.08($C_{2'}$), 119.65(C_2), 115.42(C_6), 62.64(C_β), 62.21(C_α), 55.85(C_{71}), 42.32(C_γ). HRMS [ESI+] m/z =386.2000 $[M]^+$, Calcd for $[C_{25}H_{26}N_2O_2]^+$ 386.1994. HPLC purity 99%.

4.3. General procedure for deprotection of methoxy substituted hybrids.

To the corresponding methoxy derivative (0.1 mmol) dissolved in 3 mL of anhydrous THF, was added slowly under magnetic stirring, 1 equivalent of BBr_3 per each heteroatom present in the molecule; air was displaced by N_2 and the mixture was allowed to react overnight at room temperature. Reaction was quenched with methanol (MeOH) (dropwise until end of effervescence) and the solvent evaporated under reduced pressure to eliminate the remaining BBr_3, this process was repeated several times depending on the quantity of BBr_3 used, until no fumes were observed when adding MeOH. When necessary, purification was carried out by column chromatography using a gradient of EtOAc/MeOH 0→10% as eluent

*4.3.1. (E)-N-(2-(1-Benzylpiperidin-4-yl)ethyl)-3-(3-hydroxyphenyl)acrylamide (**6**)*

From **2** (0.1 g, 0.26 mmol) and BBr_3 (7 mmol), hybrid **6** was obtained (93 mg, 97%), as a light brown amorphous solid of mp: 190-193 ºC. ^{1}H NMR (500 MHz, MeOD) δ 7.53 – 7.50 (m, 2H, H_o), 7.48 – 7.46 (m, 3H, $H_{m,\ p}$), 7.42 (d, *J* = 15.7 Hz, 1H, H_3), 7.18 (t, *J* = 7.8 Hz, 1H, H_8), 6.99 (dt, *J* = 7.8, 1.2 Hz, 1H, H_9), 6.95 (t, *J* = 2.0 Hz, 1H, H_5), 6.78 (ddd, *J* = 7.9, 2.5, 1.0 Hz, 1H), 6.55 (d, *J* = 15.7 Hz, 1H, H_2), 4.29 (s, 2H, H_γ), 3.49 – 3.45 (m, 2H, $H_{2'eq}$), 3.37 – 3.33 (m, 2H, H_α), 3.01 (td, *J* = 13.0, 3.0 Hz, 2H, $H_{2'ax}$), 2.05 – 1.99 (m, 2H, $H_{3'eq}$), 1.74 – 1.61 (m, 1H, H_4), 1.53 (q, *J* = 6.9 Hz, 2H, H_β), 1.50 – 1.40 (m, 2H, $H_{3'ax}$). ^{13}C NMR (126 MHz, MeOD) δ 168.74 (C_1), 159.01 (C_6), 141.96 (C_3), 137.49 (C_4), 132.43 (C_o), 131.22 (C_p), 130.95 (C_6), 130.38 (C_i), 130.32 (C_m), 121.56 (C_2), 120.37 (C_9), 118.00 (C_7), 115.06 (C_5), 61.78 (C_γ), 53.73 ($C_{2'}$), 37.55 (C_α), 36.36 (C_β), 32.33 ($C_{4'}$), 30.36 ($C_{3'}$). HRMS [ESI+] *m/z* =364.2150 $[M]^+$, Calcd for $[C_{23}H_{28}N_2O_2]^+$ 364.2151. HPLC purity 98%.

*4.3.2. (E)-N-(2-(1-Benzylpiperidin-4-yl)ethyl)-3-(4-hydroxyphenyl)acrylamide (**7**)*

From **3** (0.1 g, 0.26 mmol) and BBr_3 (7 mmol), hybrid **7** was obtained (83 mg, 87%), as a white amorphous solid of mp: 164-166 ºC. ^{1}H NMR (500 MHz, MeOD) δ 7.50 – 7.46 (m, 5H, Ph), 7.44 (d, *J* = 15.7 Hz, 1H, H_3), 7.40 (d, *J* = 8.7 Hz, 2H, H_6), 6.79 (d, *J* = 8.6 Hz, 2H, H_5), 6.40 (d, *J* = 15.7 Hz, 1H, H_2), 4.21 (s, 2H, H_γ), 3.42 – 3.37 (m, 2H, $H_{2'eq}$), 3.34 (t, *J* = 7.0 Hz, 2H, H_α), 2.91 (m, 2H, $H_{2'ax}$), 2.00 (d, *J* = 14.0 Hz, 2H, $H_{3'eq}$), 1.65 (bs, 1H, $H_{4'}$), 1.55 (q, *J* = 7.0 Hz, 2H, H_β), 1.48 – 1.44 (m, 2H, $H_{3'ax}$). ^{13}C NMR (126 MHz, MeOD) δ 167.84 (C_1), 159.20 (C_7), 140.48 (C_3), 130.77 (C_o), 130.74 (C_i), 129.49 (C_p), 129.13 (C_5), 128.80 (C_m), 126.14 (C_4), 116.79 (C_2), 115.30

(C_6), 60.46 (C_γ), 52.23 ($C_{2'}$), 36.13 (C_α), 35.01 (C_β), 31.04 ($C_{4'}$), 29.08 ($C_{3'}$). HRMS [ESI+] *m/z* =364.2158 $[M]^+$, Calcd for $[C_{23}H_{28}N_2O_2]^+$ 364.2151. HPLC purity 98%.

*4.3.3. (E)-N-(2-(1-Benzylpiperidin-4-yl)ethyl)-3-(2,4-dihydroxyphenyl)acrylamide (**8**)*

From **4** (0.1 g, 0.26 mmol) and BBr_3 (7 mmol) the umbellic – NBP hybrid **8** was obtained (60 mg, 60%) as a white amorphous solid of mp: 254-257^{0}C.^{1}H NMR (500 MHz, MeOD) δ 7.43 – 7.42 (m, 5H, Ph), 7.38 (d, *J* = 15.7 Hz, 1H, H_3), 7.00 (d, *J* = 2.1 Hz, 1H, H_6), 6.90 (dd, *J* = 8.2, 2.1 Hz, 1H, H_8), 6.77 (d, *J* = 8.2 Hz, 1H, H_9), 6.34 (dd, *J* = 15.6, 1.3 Hz, 1H, H_2), 3.98 (s, 2H, H_γ), 3.37 – 3.32 (m, 2H, H_α), 3.26 – 3.18 (m, 2H, $H_{2'eq}$), 2.61 (s, 2H $H_{2'ax}$), 1.92 (d, *J* = 13.5 Hz, 2H, $H_{3'eq}$), 1.57 – 1.50 (m, 3H, $H_{\beta,\ 4'}$), 1.45 – 1.32 (m, 2H, $H_{3'ax}$). ^{13}C NMR (126 MHz, MeOD) δ 169.28 (C_1), 148.81 (C_7), 146.76 (C_5), 142.26 (C_3), 131.73 (C_o), 130.08 (C_i), 129.91 (C_m), 128.22 (C_p), 122.08 (C_8), 118.24 (C_2), 116.43 (C_9), 114.99 (C_6), 62.60 (C_γ), 53.96 ($C_{2'}$), 37.73 (C_α), 36.63 (C_β), 33.18 ($C_{4'}$), 31.20 ($C_{3'}$). HRMS [ESI+] *m/z* =380.2094 $[M]^+$, Calcd for $[C_{23}H_{28}N_2O_3]^+$ 380.2100. HPLC purity 99%.

*4.3.4. (E)-N-(2-(1-Benzylpiperidin-4-yl)ethyl)-3-(3,4-dihydroxyphenyl)acrylamide (**9**)*

From **5** (0.1 g, 0.25 mmol) and BBr_3 (7 mmol) the caffeic – NBP hybrid **9** was obtained (60 mg, 65%) as a white amorphous solid of mp: 164-166 ºC. ^{1}H NMR (500 MHz, MeOD) δ 7.48 – 7.40 (m, 5H, Ph), 7.37 (d, *J* = 15.7 Hz, 1H, H_3), 7.01 (d, *J* = 2.1 Hz, 1H, H_5), 6.90 (dd, *J* = 8.2, 2.1 Hz, 1H, H_9), 6.77 (d, *J* = 8.2 Hz, 1H, H_8), 6.39 (d, *J* = 15.7 Hz, 1H, H_2), 4.00 (s, 2H, H_γ), 3.34 (t, *J* = 6.9 Hz, 2H, H_α), 3.24 (d, *J* = 12.3 Hz, 2H, $H_{2'eq}$), 2.64 (t, *J* = 11.6 Hz, 2H, $H_{2'ax}$), 1.95 – 1.82 (m, 2H, $H_{3'eq}$), 1.56 (m, 1H, $H_{4'}$), 1.53 (q, *J* = 6.7 Hz, 2H, H_β), 1.47 – 1.33 (m, 2H, $H_{3'ax}$). ^{13}C NMR (126 MHz, MeOD) δ

169.23 (C_1), 148.77 (C_7), 146.72 (C_6), 142.15 (C_3), 133.40 (C_i), 131.86 (C_o), 130.13 (C_p), 129.90 (C_m), 128.21 (C_4), 122.15 (C_9), 118.33 (C_2), 116.44 (C_8), 114.93 (C_5), 62.49 (C_γ), 53.83 ($C_{2'}$), 37.70 (C_α), 36.52 (C_β), 32.99 ($C_{4'}$), 31.01 ($C_{3'}$). HRMS [ESI+] *m/z* =380.2104 $[M]^+$, Calcd for $[C_{23}H_{28}N_2O_3]^+$ 380.2100. HPLC purity 99%.

*4.3.5. (E)-N-(4-((Benzyl(methyl)amino)methyl)phenyl)-3-(3-hydroxyphenyl)acrylamide (**16**)*

From **14** (0.1 g, 0.26 mmol) and BBr_3 (7 mmol) hybrid **16** was obtained (69 mg, 72%) as a light brown amorphous solid of mp: 156-158 ºC. ^{1}H NMR (300 MHz, MeOD) δ 7.84 (d, *J* = 8.6 Hz, 2H, $H_{2'}$), 7.62 (d, *J* = 15.7 Hz, 1H, H_3), 7.56 – 7.44 (m, 7H, Ph, $H_{3'}$), 7.25 (t, *J* = 7.7 Hz, 1H, H_8), 7.09 (d, *J* = 7.7 Hz, 1H, H_9), 7.04 (t, *J* = 2.1 Hz, 1H, H_5), 6.85 (dd, *J* = 7.8, 2.2 Hz, 1H, H_7), 6.85 (d, *J* = 15.6 Hz, 1H, H_2), 4.50 (dd, *J* = 13.1, 10.3 Hz, 2H, $H_{\alpha,\ \beta}$), 4.28 (d, *J* = 13.2 Hz, 2H, $H_{\alpha,\ \beta}$), 2.73 (s, 3H, H_γ). ^{13}C NMR (75 MHz, MeOD) δ 166.91 (C_1), 159.10 (C_6), 143.60 (C_3), 142.00 ($C_{1'}$), 137.38 (C_4), 133.08 (C_m), 132.29 (C_o), 131.33 (C_p), 131.02 (C_8), 130.77 (C_i), 130.48 ($C_{3'}$), 125.82 ($C_{4'}$), 121.76 (C_2), 121.53 ($C_{2'}$), 120.58 (C_9), 118.33 (C_7), 115.28 (C_5), 60.72 (CH_2), 60.52 (CH_2), 39.49 (C_γ). HRMS [ESI+] m/z =372.1844 $[M]^+$, Calcd for $[C_{24}H_{24}N_2O_2]^+$ 372.1838. HPLC purity 99%.

*4.3.6. (E)-N-(4-((Benzyl(methyl)amino)methyl)phenyl)-3-(4-hydroxyphenyl)acrylamide (**17**)*

From **15** (0.1 g, 0.26 mmol) and BBr_3 (7 mmol) hybrid **16** was obtained (58 mg, 60%) as a white amorphous solid of mp: 160-163 ºC. ^{1}H NMR (300 MHz, MeOD) δ 7.82 (d, *J* = 8.7 Hz, 2H, $H_{2'}$), 7.63 (d, *J* = 15.6 Hz, 1H, H_3), 7.55 – 7.45 (m, 5H, $H_{5,\ 3',\ p}$

), 7.20 (d, *J* = 8.5 Hz, 2H, H_o), 6.84 (d, *J* = 8.5 Hz, 2H, H_6), 6.76 (d, *J* = 8.4 Hz, 1H, H_m), 6.63 (d, *J* = 15.6 Hz, 1H, H_2), 4.35 (bs, 4H, $H_{\alpha,\beta}$), 2.69 (s, 3H, H_γ). ^{13}C NMR (75 MHz, MeOD) δ 167.51 (C_1), 161.00 (C_7), 143.65 (C_3), 142.13 ($C_{1'}$), 133.20 (C_o), 132.21 ($C_{3'}$), 131.28 (C_i), 131.20 ($C_{4'}$), 130.89 ($C_{5'}$), 130.46 (C_p), 127.51 (C_4), 121.46 ($C_{2'}$), 118.33 (C_2), 116.84 (C_6), 116.07 (C_m), 60.96 (CH_2), 60.36 (CH_2), 39.39 (C_γ). HRMS [ESI+] m/z =372.1846 $[M]^+$, Calcd for $[C_{24}H_{24}N_2O_2]^+$ 372.1838. HPLC purity 96%.

4.4. Biochemical Studies

4.4.1. Inhibition of Human AChE and BuChE

Using human recombinant acetylcholinesterase (h-AChE) and butyrylcholinesterase from human serum (h-BuChE), the Ellman method [41] was followed according to the experimental details previously described [50].

4.4.2. Inhibition of human monoamino oxidases (hMAO-A and hMAO-B).

MAO inhibition measurements were evaluated following the general procedure previously described by us [42]. Briefly, test drugs and adequate amounts of recombinant hMAO-A or hMAO-B (Sigma-Aldrich Quımica S.A., Alcobendas, Spain) required and adjusted to oxidize 165 pmol of p-tyramine/min in the control group, were incubated for 15 min at 37 ºC in a flat-black-bottom 96-well microtest plate (BD Biosciences, Franklin Lakes, NJ) placed in the dark fluorimeter chamber. The reaction was started by adding 200 mM Amplex Red reagent (Molecular Probes, Inc., Eugene, OR), 1 U/mL horseradish peroxidase, and 1 mM *p*-tyramine and the production of resorufin, was quantified at 37 ºC in a multidetection microplate fluorescence reader

(FLX800, Bio-Tek Instruments, Inc., Winooski, VT) based on the fluorescence generated (excitation, 545 nm; emission, 590 nm). The specific fluorescence emission was calculated after subtraction of the background activity, which was determined from wells containing all components except the hMAO isoforms, which were replaced by a sodium phosphate buffer solution.

4.4.3. Oxygen Radical Absorbance Capacity Assay

The ORAC method was followed, using a Polarstar Galaxy plate reader (BMG Labtechnologies GmbH, Offenburg, Germany) with 485-P excitation and 520-P emission filters [43].

4.4.4. Human BACE1 Inhibition Assay.

This experiment was carried out using fluorescence resonance energy transfer (FRET), according to the protocol described by the manufacturer (Invitrogen) [51].

*4.4.5. Effect of compounds **5**, **12** and **16** on R/O-induced oxidative cell injury in SH-SY5Y cells*

Human dopaminergic neuroblastoma SH-SY5Y cells were maintained in a 1:1 mixture of nutrient mixture F-12 and Eagle´s minimum essential medium (EMEM) supplemented with 15 nonessential amino acids, sodium pyruvate (1 mM), 10% heat-inactivated FBS, 100 units/ml penicillin, and 100 µg/ml streptomycin. Cultures were seeded into flasks containing supplemented medium and maintained at 37 ºC in a humidified atmosphere of 5% CO_2 and 95% air. For assays, SH-SY5Y cells were

subcultured in 96-well plates at a seeding density of $8x10^4$ cells per well for 2 days. Cells were co-incubated with rotenone (30 µM) / oligomycin A (10 µM) (R/O) for 24 h to induce oxidative stress at concentrations of 0.1, 0.3, 1 and 3 µM in F-12/EMEM with 1% FBS. A vehicle group containing 0.1% dimethyl sulfoxide (DMSO) was employed in parallel for each experiment. All SH-SY5Y cells used in this study were used at a low passage number (<13).

4.4.6. Measurement of cell viability

MTT reduction was performed as described [52]. Briefly, 50 µL of the MTT labeling reagent, at a final concentration of 0.5 mg/ml, was added. After incubation for 2 h, in a humidified incubator at 37 ºC with 5% CO_2 and 95% air (v/v), the supernatant was removed, the obtained purple formazan product was re-suspended in 100 µL of Dimethyl Sulfoxide (DMSO). Colorimetric determination of MTT reduction was measured in an ELISA microplate reader at 540 nm. Control cells treated with EMEM were taken as 100% viability.

4.4.7. Neurogenic Assays

Adult (3 months old) male C57BL/6 mice were used following the animal experimental procedures previously approved by the Ethics Committee for Animal Experimentation of the CSIC in accordance with the European Communities Council, directive 2010/63/EEC and National regulations, normative 53/2013. Special care was taken to minimize animal suffering. Neural stem cells were isolated from the SGZ of the dentate gyrus of the hippocampus of adult mice and cultured as NS according to previously published protocols [53,54]. Neural stem cells grown as NS were treated for

7 days in culture with compound **9** (10 μM). Now, NS were adhered onto 100 μg/mL poly-L-lysine-coated coverslips and treated for 3 additional days in the presence of serum but in the absence of exogenous growth factors to induce differentiation [55]. Finally, the expression of neuronal markers was analyzed by immunocytochemistry using antibodies linked to neurogenesis: β-III-tubulin polyclonal antibody (TuJ clone; Abcam), a protein expressed at early stages of neurogenesis and a monoclonal microtubule-associated protein type 2 (MAP-2) antibody, a classical marker of late neuronal maturation. To visualize primary antibodies Alexa-fluor-labeled secondary antibodies (Molecular probes) were used. Nuclei were stained with DAPI. Fluorescent representative images were acquired with a Nikon fluorescence microscope 90i coupled to a digital camera Qi. The microscope configuration was adjusted to produce the optimum signal-to-noise ratio.

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Figure 1. Structures of natural antioxidants containing the cinnamic acid structure (*p*-coumaric, caffeic, umbellic, and ferulic acids, curcumin), donepezil, AP2238, and new cinnamic – *N*-benzylpiperidine (NBP) (**1**-**9**) and cinnamic – *N*,*N*-dibenzyl(*N*-methyl)amine (DBMA) hybrids (**10**-**17**).

Scheme 1. Reagents and conditions: (a) CDI, THF, MW, 7 min, 120 °C; (b) 2-(1-benzylpiperidin-4-yl)ethan-1-amine, MW, 10 min, 120 °C; (c) 3- or 4-((benzyl(methyl)amino)methyl)aniline, MW, 40 min, 120 °C; (d) BBr_3, THF, rt, overnight.

Table 1. Inhibition of human cholinesterases (hAChE and hBuChE) and monoamine oxidases (hMAO-A and hMAO-B) (IC_{50}, μM); oxygen radical absorbance capacity (ORAC, trolox equiv.) [a]

Figure 2. Effect of compounds **5** (A), **12** (B), and **16** (C) at 0.1, 0.3, 1.0, and 3.0 μM on cell death induced by the combination of rotenone (30 μM) and oligomycin A (10 μM). Melatonin (10 nM) is used as positive control in all experiments. Cell viability was measured as MTT reduction and data were normalized as % of control. Data are expressed as the means ± SEM of triplicate of at least three different cultures. All compounds were assayed at increasing concentrations (0.1-3 μM). ***P≤ 0.001, **P≤ 0.01, *P≤0.05, with respect to control group. Comparisons between drugs and control group were performed by one-way ANOVA followed by the Newman–Keuls post-hoc test.

Figure 3. Caffeic-based hybrid **9** promotes neuronal differentiation *in vitro*. Adult murine neural stem cells isolated from the neurogenic niche of the SGZ of the hippocampus were grown as neurospheres (NS) during 7 days in the presence of compound **9** (10 μM). Then, NS were allowed to differentiate on a substrate for another 3 days in the presence of tested compound. Immunocytochemical analysis shows the expression of two well-known neuronal markers: β-III-tubulin (TuJ clone; green) and MAP-2 (red) inside the NS (inner part) and in the distal area (outer part). DAPI was used for nuclear staining. Scale bar, 200μm.