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Synthesis of novel vanillin derivatives: novel multi-targeted scaffold ligands against Alzheimer's disease

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Abstract

Alzheimer's Disease (AD) is the most common cause of dementia worldwide, normally affecting people aged over 65. Due to the multifactorial nature of this disease, a "multi-target-directed ligands" (MTDLs) approach for the treatment of this illness has generated intense research interest in the past few years. Vanillin is a natural antioxidant and it provides a good starting point for the synthesis of new compounds with enhanced antioxidant properties, together with many biological activities, including β-amyloid peptide aggregating and acetylcholinesterase inhibiting properties. Here we report novel vanillin derivatives, bearing a tacrine or a naphthalimido moiety. All compounds exhibited improved antioxidant properties using DPPH assay, with IC_{50} as low as 19.5 μM, FRAP and ORAC assays, with activities up to 1.54 and 6.4 Trolox equivalents, respectively. In addition, all compounds synthesized showed inhibitory activity toward acetylcholinesterase enzyme at μmolar concentrations using the Ellman assay. Computational docking studies of selected compounds showed interactions with both the catalytic anionic site and the peripheral anionic site of the enzyme. Furthermore, these compounds inhibited $A\beta_{(1-42)}$ amyloid aggregation using the fluorometric ThT assay, with compound **4** showing comparable inhibitory activity to the positive control, curcumin. At cellular level compound **4** (1 μM) showed significant protective effects of neuroblastoma SH-SY5Y cell line when treated with hydrogen peroxide (400 μM). In our opinion, vanillin derivatives could provide a viable platform for future development of multi-targeted ligands against AD.

Keywords: Alzheimer's Disease, Synthetic antioxidants, AChE inhibitors, Multitarget-directed ligands, Vanillin derivatives.

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Introduction

Amongst all neurodegenerative diseases, Alzheimer's disease (AD) is the most common cause of dementia, mostly affecting people over the age of 65 [1]. In developed countries, AD is the fifth cause of death [2]. AD is characterized by memory loss, confusion with space and time, speaking problems, poor judgment and changes in mood and personality. Due to increased life expectancy, 1 million new cases per year are expected by 2050 [3]. For this reason, there is an urgent need for effective therapies.

AD is a multifactorial disease normally characterized by neuronal loss and β-amyloid deposition that leads to extracellular amyloid plaques in the cerebral cortex [4]. Although the cause of these events is not fully understood, the "amyloid hypothesis" is the most recognized for development of AD [2]. More recently, oxidative injury to macromolecules, including proteins, lipids and nucleic acids has been identified as a key feature in the development of AD, thus leading to the "oxidative stress hypothesis" [5]. Finally, a decrease in acetylcholine availability at neuronal synapses is a common hallmark in AD patients [2], making this a possible therapeutic target.

Due to the many contributing factors involved in the development and progression of AD, the current trend of drug development based on a "one target-one molecule" point of view is no longer favored. A multi-targeted approach, aimed at targeting different steps of the neurotoxic cascade, has started to attract much interest among the research community [6]. In particular, the approach of designing multi-target agents against neurodegenerative diseases has shown an increase [7–9]. For example, recent reports include multifunctional agent chromone-2-carboxyamidoalkylbenzylamines [10] and tacrine-8-hydroxyquinoline hybrids [11]. Both groups of compounds target the cholinesterase enzymes acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE), act as chelating agent with copper ion and inhibit $A\beta_{(1-42)}$ aggregation. All these events play a crucial role in the onset and progression of AD.

Multi-target compounds acting as antioxidants and acetylcholinesterase inhibitors including tacrine, a well-known acetylcholinesterase inhibitor [12] and resveratrol, a strong natural antioxidant [13] have been recently reported [14]. The compounds described in this work were strong inhibitors of acetylcholinesterase (with IC_{50} at nanomolar concentrations) and $A\beta_{(1-42)}$ aggregation, while showing antioxidant activities in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay comparable to Trolox [14]. Furthermore several hybrids between tacrine and Trolox, a common synthetic antioxidant, were previously reported; the compounds showed inhibitory activity toward AChE comparable to tacrine, with a two-fold decrease in the antioxidant activities in DPPH assay when compared to Trolox standard [15].

Previously, we reported novel vanillin derivatives with strong antioxidant properties; in particular, compound **2c** (4,4',4'',4'''-((1,4-phenylenebis(azanetriyl))tetrakis(methylene))tetrakis(2-methoxyphenol)) exhibited remarkable scavenging activity toward DPPH free radical, as well as reducing activity in FRAP assay. In addition, the latter compound protected the SH-SY5Y neuroblastoma cell line against hydrogen peroxide (400 μM) treatment, increasing the cell viability by 30% at compound concentrations as low as 10 μM [16].

Here we report the design and synthesis of four novel vanillin derivatives with multi-target functionalities that would be expected to exhibit antioxidant properties, as well as inhibitory AChE and amyloid Aβ$_{(1-42)}$ aggregation activities (Figure 1). Among the vanillin derivatives synthesized, three contained a naphthalimido moiety and one has a tacrine group. It is noteworthy to mention previous work reporting a naphthalimido moieties linked with ranitidine to produce potential multi-targeted AD agents [17]. The antioxidant properties, inhibitory AChE activity and β-amyloid aggregation inhibitory properties and their protective effect on peroxide-treated neuroblastoma SH-SY5Y cells of all the compounds synthesized in this work will be discussed.
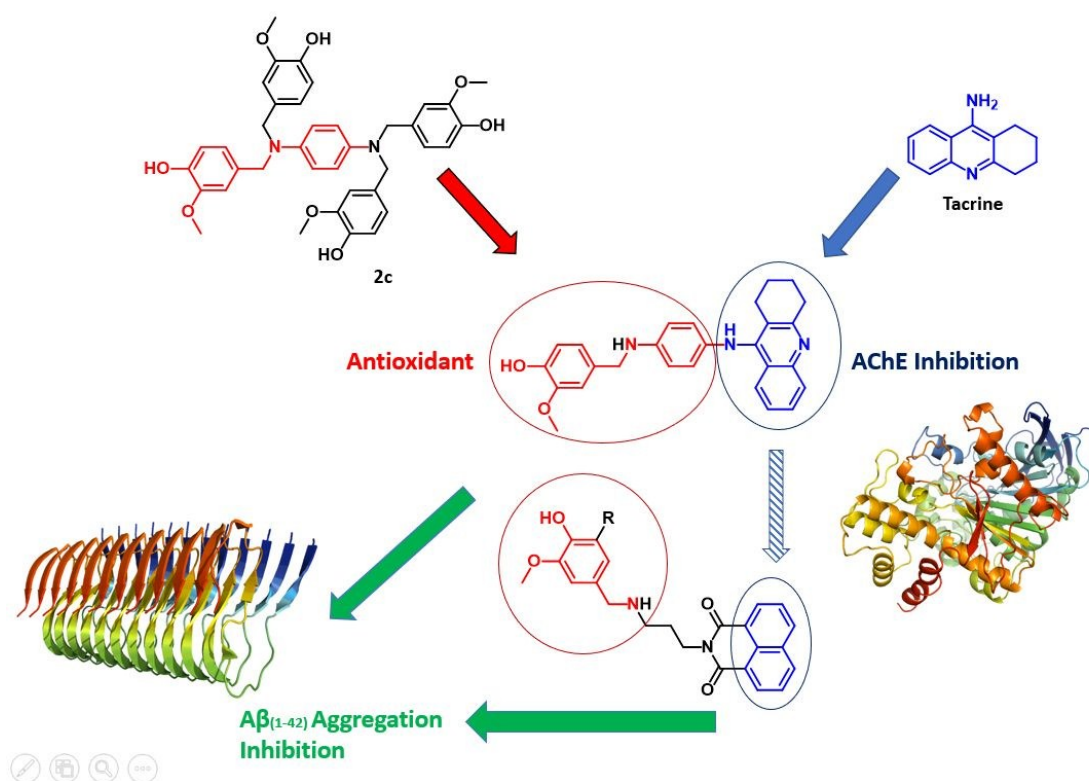

Figure 1. Rationale for the synthesis of vanillin derivatives [18,19].

The phenolic moiety, important for the antioxidant activity, was linked to the tacrine moiety, a well-known AChE inhibitor. Tacrine was substituted with a naphthalimido structure to determine the impact of the latter on AChE and β-amyloid inhibitory activity. In addition, the aromatic linker was substituted with a propyl moiety, which shows similar distance between the two nitrogens (5.2 and 4.9 Å, respectively, calculated using PyMOL software) and more flexibility for the evaluation of its impact on AChE and amyloid Aβ$_{(1-42)}$ peptide inhibitory activities.

Results and discussion

Chemical Synthesis

The synthesis of novel naphthalimido vanillin derivatives **1**, **2**, and **3** was achieved as depicted in Scheme 1 using 2-(3-aminopropyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (**I-1)** as a precursor. The latter was synthesised from the reaction between naphthalic anhydride and 1,3-diamino propane in ethanol according to previous work [20]. Vanillin derivatives (**1**, **2**, and **3**) were then prepared by reacting vanillin or syringaldehyde with **I-1** to afford the corresponding imines. The latter were then reduced in the presence of sodium borohydride in methanol (**2**, 86%) or propan-2-ol (**3**, 38%).
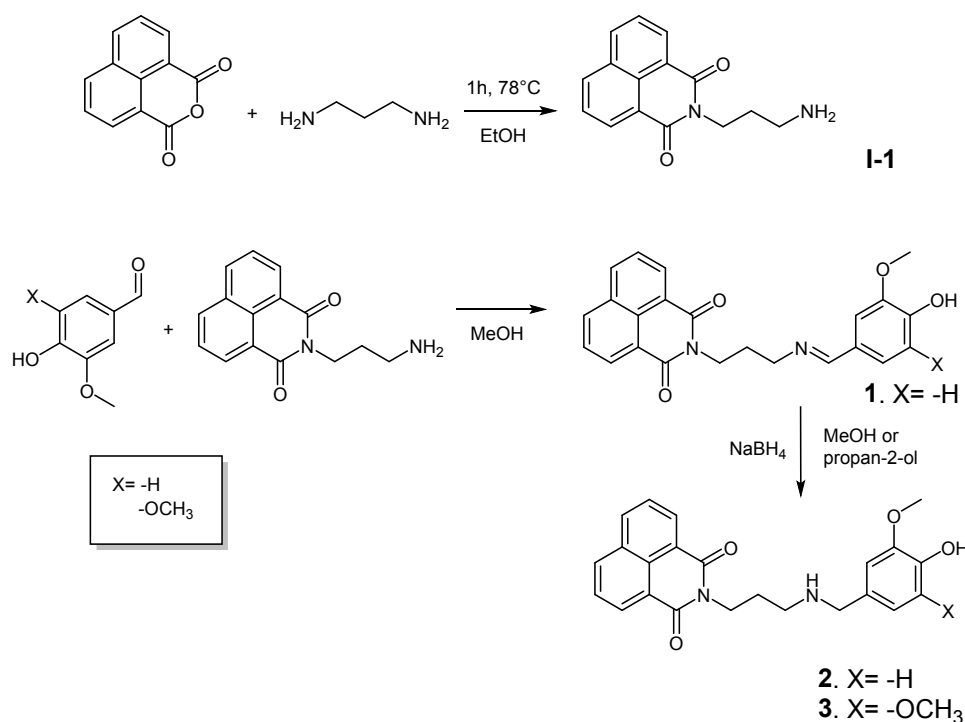
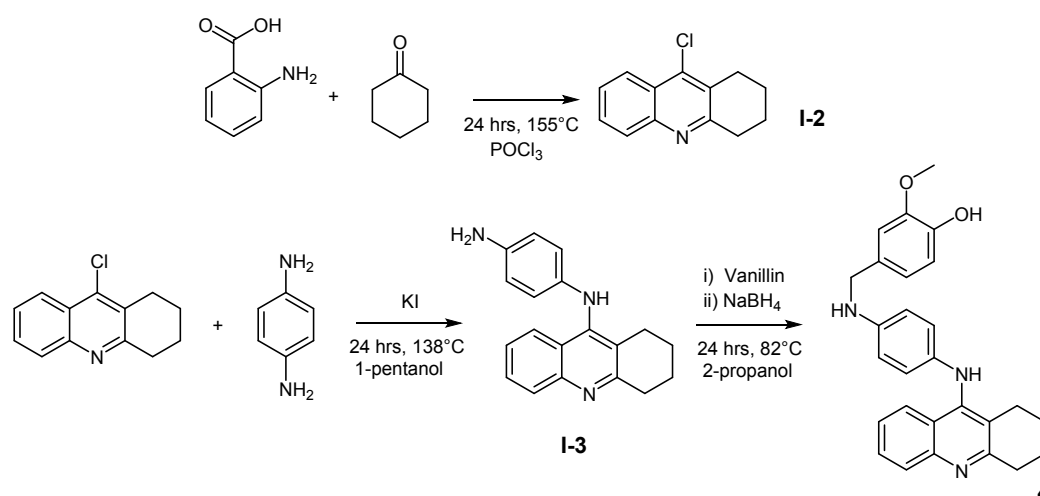*Scheme 1. Chemical strategy for the synthesis of derivatives **1-3**.*

The naphthalimido compounds (**1**, **2**, **3**) showed characteristic features in their ^{1}H NMR spectra. The latter showed three sets of peaks between 8.65 and 7.74 ppm due to the benzo[de]isoquinoline-1,3-dione (naphthalimido) moiety along with two sets of peaks or a singlet between 7.01 and 6.50 ppm attributed to the vanillin or the syringaldehyde moieties, respectively. A singlet between 3.9 and 3.7 ppm is due to the methoxy group (in both vanillin and syringaldehyde structures) and the methylene (Ar-CH$_2$-N) group respectively. The propyl groups in both compounds were identified by the presence of two triplets and a multiplet between 4.4 and 1.9 ppm.

The reduction of compound **1**, to generate compound **2**, was confirmed by the disappearance of the singlet at 8.2 ppm with the appearance of new singlet (CH_2) at 3.76 ppm.

The presence of an extra methoxy group in compound **3** compared to compound **2** was also confirmed by the integration of the peak at 3.79 ppm (6 protons instead of 3).

The synthesis of compound **4** (Scheme 2**)** involved intermediates **I-2** and **I-3**. Compound **I-2** (67% yield) was prepared following the procedure described by Szymański, Zurek and Mikiciuk-Olasik, by reacting anthranilic acid with 2 equivalents of cyclohexanone in $POCl_3$ (7.5mL) [21]. Intermediate **I-3** (39 % yield) was synthesized by the N-alkylation reaction between **I-2** and of 2.5 equivalents of p-phenylenediamine in 1-pentanol in the presence of catalytic amount of potassium iodide (KI).

Scheme 2. Chemical strategy for the synthesis of derivative 4.

The ^{1}H NMR spectrum of compound **4** showed seven sets of peaks in the region between 7.6 and 6.5 ppm attributed to the aromatic protons of the tacrine, *p*-phenylenediamine and phenolic rings. Two multiplets and two triplets between 2.6 and 1.8 ppm of the tetrahydroacridine motif from the tacrine ring system and the singlet at 3.8 ppm were due to the methoxy group from the phenolic moiety. (See experimental section and **Supplementary Information, SI**).

Antioxidant Activity

All compounds were evaluated for their antioxidant properties using three different assays (DPPH, FRAP and ORAC) with differing oxidative potential evaluating mechanisms (Table 1). DPPH assay was employed to evaluate the scavenging activity [22] of the novel vanillin derivatives whereas FRAP assay was selected in order to measure the electron transfer properties [23] of the latter. Finally, the

ORAC assay was utilized for the evaluation of the hydrogen atom transfer (HAT) abilities of our novel compounds emulating a more relevant environment to biological system [24].

Table 1. Antioxidant properties of vanillin derivatives.

Compound	DPPH (IC_{50} μM)	FRAP (TE)	ORAC (TE)
1	> 250 μM	0.07 ± 0.002	2.1 ± 0.5
2	50.7 ± 0.8	0.26 ± 0.04	3.9 ± 1.3
3	19.5 ± 0.3	1.45 ± 0.02	2.0 ± 0.5
4	20.5 ± 0.3	1.54 ± 0.15	6.4 ± 1.6
Trolox	24.4 ± 0.9	1	1
Tacrine	Inactive [a]	Inactive [a]	<0.01[b]

Results from each assay are expressed as a mean ± SD of three independent experiments.
[a] Compounds were tested up to 250 μM. Trolox was used as a positive control
[b] Reported by [25].

2,2-diphenyl-1-picrylhydrazyl (DPPH) Assay

Diphenyl-1-picrylhydrazyl (DPPH) is a stable organic nitrogen radical with an absorption maximum at 515 nm. Its reduction in the presence of antioxidants, acting as free radical scavengers, was monitored spectrophotometrically [22]. The IC_{50} value of each compound was determined (see Table 1). The imine compound **1** showed no IC_{50} up to concentration of 250 μM whereas its corresponding reduced amine showed an IC_{50} of 50.7 μM, confirming our previous finding that an imine exhibits lower activity in free radical scavenging compared with the corresponding amine [16]. In addition, compound **3**, bearing an extra methoxy group in the phenolic moiety compared to compound **2**, turned out to be ~two-fold more active (19.5 μM), confirming the important role of an extra methoxy group for the antioxidant activity. Interestingly, compound **4** showed similar activity to compound **3,** although bearing only one methoxy group in its phenolic moiety (20.5 and 19.5 μM, respectively). This confirms our previous finding regarding the increase of antioxidant activity due to the electronic conjugation between the two nitrogens and an aromatic ring.

The compounds showed greater antioxidant activities compared to the tacrine-resveratrol hybrids reported by Jeřabek *et al*. which showed weak DPPH scavenging abilities due to the absence of phenolic moieties in their chemical structures [14].

Interestingly, tacrine turned out to be completely inactive at concentrations as high as 250 μM, despite the fact it has a nitrogen atom linked with an aromatic moiety. The lack of activity can be explained by the absence of a phenolic moiety in the aromatic structure.

Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay is based on the reduction of the ferric-tripyridyltriazine complex by antioxidants and it was performed at pH 3.5. The ferrous-tripyridyltriazine complex that is formed after the reduction of the iron core leads to a measurable blue color (593 nm) [23]. The results are expressed as Trolox Equivalent (TE) after comparison with the standard Trolox calibration curve. Again, compound **1** showed the lowest activity (0.07 TE) (see table 1), almost 15 times less active than the standard Trolox. Its reduced derivative, compound **2**, was almost 4 times less active than Trolox (0.26 TE), showing better performance compared to the corresponding imine (compound **1**). In contrast, compound **3** showed a 5-fold increase in activity (1.45 TE) compared to compound **2** confirming the importance of the extra methoxy group in the phenolic moiety for improved activity in the FRAP assay. Compound **4** turned out to be the most active among the four vanillin derivatives (1.54 TE), confirming the role of the electronic delocalisation of the nitrogen electron in the antioxidant activity [16]. Again, tacrine on its own showed to be completely inactive in this assay at concentrations as high as 250 μM.

Oxygen Radical Absorbance Capacity (ORAC) Assay

ORAC assay is based on hydrogen atom transfer (HAT) mechanism and measures the ability of antioxidants to inhibit the oxidation of the fluorescent probe fluorescein caused by peroxyl free radical generated by thermal decomposition of the 2,2'-azobis(2-amidinopropane) (AAPH) [26–28]. The use of peroxyl free radical, which is commonly found in the body, makes this assay more relevant to biological systems [24].

All the novel vanillin derivatives showed improved antioxidant activities in this assay compared to the standard Trolox. Unlike the previous assays, the vanillin derivative **2** showed better activity compared to its corresponding syringaldehyde derivative **3** (3.9 and 2.0 TE, respectively) highlighting the deleterious impact of the extra methoxy moiety in the phenolic ring on ORAC assay. It is worth mentioning that vanillin itself is more active than syringaldehyde in this assay (2.2 and 1.5 TE, respectively) [16].

The imine **1** showed weaker scavenging activity compared to the amine **2** (2.1 and 3.9 TE, respectively) in this assay, confirming the role of the nitrogen's electron availability in the antioxidant activity.

Finally, compound **4** showed the highest ORAC value (6.4 TE) highlighting the predominant role of electronic delocalisation in the peroxyl free radical scavenging activity.

The latter showed higher activities compared to the series of melatonin-tacrine hybrid reported by Rodriguez-Franco *et al*., which ORAC values were ranging from 1.7 - 4.0 TE [25].

Cellular Protection

MTT Assay

The production of reactive oxygen species is linked with cell death and it is a peculiar hallmark of many age-related diseases [29]. Based on all the above results, derivative **4** was found be the most active compound with regard to antioxidant properties. Therefore, the latter was chosen to study its ability to protect stressed cells. Neuroblastoma SH-SY5Y cell line was used for this purpose and the cell viability was determined spectrophotometrically using 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay. Hydrogen peroxide was used as a stressor to evaluate the protective effect of compound **4** against oxidative damage; cells were exposed for 24 hours with hydrogen peroxide (400 μM) after pre-treatment (24 hours) with compound **4** at different concentrations, ranging from 0.01 to 5 μM.

The choice of the working concentration in the protective effect of compound **4** was based on its toxicity, after 24 hours, exposure towards SH-SY5Y neuroblastoma cell line. Compound **4** exhibited an IC_{50} of 45.1 ± 3.5 μM, although some toxic effect was observed at concentration of 25 μM. No significant toxicity was however found at 12.5 μM, hence the decision to work with 5 μM which is significantly below the toxic concentrations (see Figure 2). Compound **4** showed strong protective effect against oxidative insult and the results are reported in Figure 2.
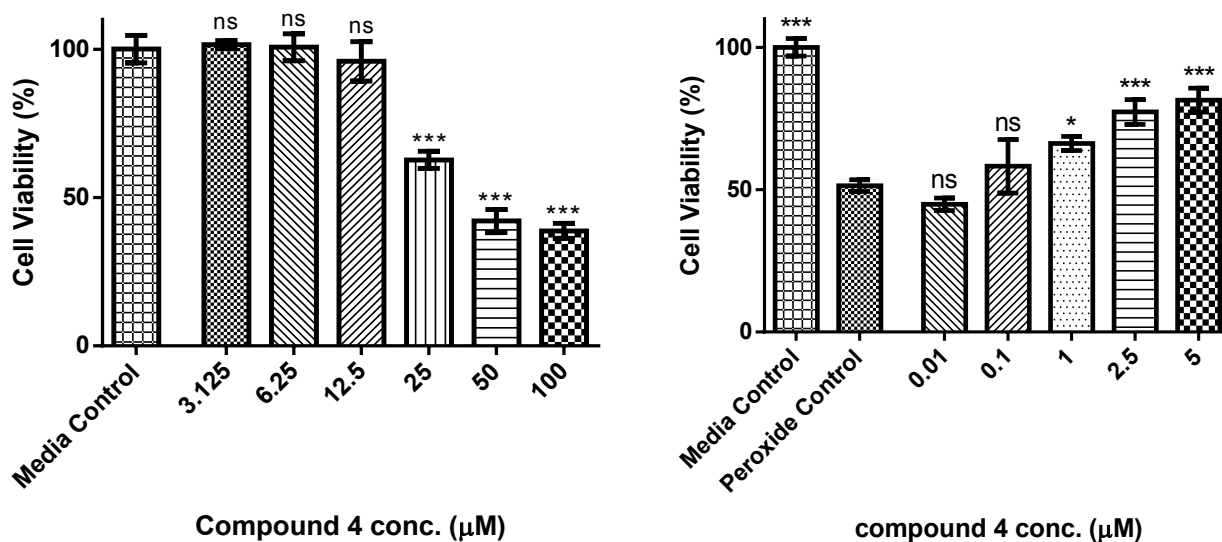

*Figure 2. (Left) Toxicity of Compound 4 toward SH-SY5Y cells after 24 hours exposure. (Right) Protective effects of Compound 4 in hydrogen peroxide (400 μM)- stressed cells. Cells were incubated for 24 hours with compound 4 at different concentrations before the addition of the stressor. After 24 hours, cell viability was measured through MTT assay. Values are expressed as the percentage of the untreated control and represented as mean ± SD of three independent experiments in each group. ***p < 0.001, **p < 0.01, *p < 0.05, ns no significantly different compared to the control.*

Compound **4** almost completely reversed peroxide-induced cell death at the highest concentration tested (5 µM); significant protection was seen at concentrations as low as 1 µM.

No statistical protection was observed by the compound at the lowest concentrations tested (0.01 and 0.1 µM).

It is worth noting that the protective effects of compound **4** are comparable to our previously reported compound **2c,** which showed an increase in peroxide-treated cell viability by 26% at 5 µM, although **2c** showed better antioxidant properties in both the DPPH, FRAP and ORAC assays [16].

AChE Inhibitory Activity

All the compounds were evaluated for their AChE inhibition properties according to Ellman's method with minor modifications [30]. Tacrine was used as reference standard and the results are reported in table 2.

Table 2. Cholinesterase AChE inhibitory activity of vanillin derivatives.

Compound	AChE inhibition (IC$_{50}$ µM)
1	128.3 ± 6.1
2	10.1 ± 1.15
3	24.25 ± 1.35
4	2.13 ± 0.1
Tacrine	0.93 ± 0.09

Results are expressed as a mean ± SD of three independent experiments.

All compounds tested showed weak to good inhibitory activity, with compound **4** the most active, with an IC$_{50}$ value ~2 times higher than the standard tacrine (IC$_{50}$ of 2.13 and 0.93 µM, respectively). The results obtained here for the positive control tacrine agree with the data previously reported (IC$_{50}$ value of 0.9 µM for tacrine) [31]. However, compound **4** showed lower activity compared to related tacrine derivatives reported by Luo *et a*l. which turned out to be several fold-times more active than tacrine [32]. This could be explained by the presence of long alkyl chains in the compounds reported by the authors, which show great flexibility and adaptability within the narrow gorge of AChE compared to compound **4** that shows lower flexibility, on account of the aromatic ring linked to the tacrine moiety. The imine derivative (compound **1**) was found to

be the weakest inhibitor, with an IC_{50} of 128.3 μM. By contrast, its reduced form (compound **2**) showed an increase in activity of 13-fold (IC_{50} of 10.1 μM). This could be explained by the fact that compound **1**, which bears an imino group, will show less flexibility compared to compound **2** (amine) due to the rigidity of the double bond, thus showing reduced flexibility in the narrow gorge of the AChE enzyme. Finally, the presence of an extra methoxy group in the phenolic moiety, as in the case of compound **3,** caused a two times fold reduction in the AChE inhibition when compared with compound **2** (24.25 and 10.1 μM, respectively).

Molecular Modelling

To understand better the molecular elements that contribute to the AChE inhibitory activities of these vanillin derivatives, molecular binding studies of compounds **2** and **4** with TcAChE (PDB code: 2CMF) [33] were performed. Several studies reported the crystal structure of AChE from Torpedo Californica. The active site lies at the bottom of a 20 Å deep gorge and it is characterized by the presence of three main residues (SER-200, GLU-327, and HIS-440), which form the esteratic site involved in the hydrolysis of acetylcholine, and a catalytic anionic site (CAS), characterized by the presence of TRP-84, which plays a fundamental role in binding the acetylcholine molecule through a cation-π interaction with its positive quaternary nitrogen [34], and PHE-330 [35]. Finally, the peripheral anionic site (PAS) lies at the top of the gorge, approximately 20 Å above the active site. The PAS is involved in binding acetylcholine at the first step of the catalytic pathway. It is composed of residues TYR-70, ASP-72, TYR-121, TRP-279, and TYR-334 [36].

Ligand optimization was performed using Chemdraw 16.0 (Cambridgesoft, Waltham, MA) and Chem3D Ultra 16 version (Cambridgesoft, Waltham, MA) using a MM2 force field energy minimization tool. Protein optimization was performed using Autodock Vina 1.1.2 [37]. The results were then visualized using PyMOL (the PyMOL Molecular Graphics System, Version 2.0.7 Schrödinger, LLC).

For the validation of the docking model, the original ligand in the 2CMF structure was redocked. The binding energy obtained was -14.6 kcal/mol, indicating high affinity of the latter for the AChE molecule. In addition, the comparison of the positions of the original and redocked ligands resulted in a RMSD score of 0.598 (see Figure 3), confirming the effectiveness of the Autodock Vina software.
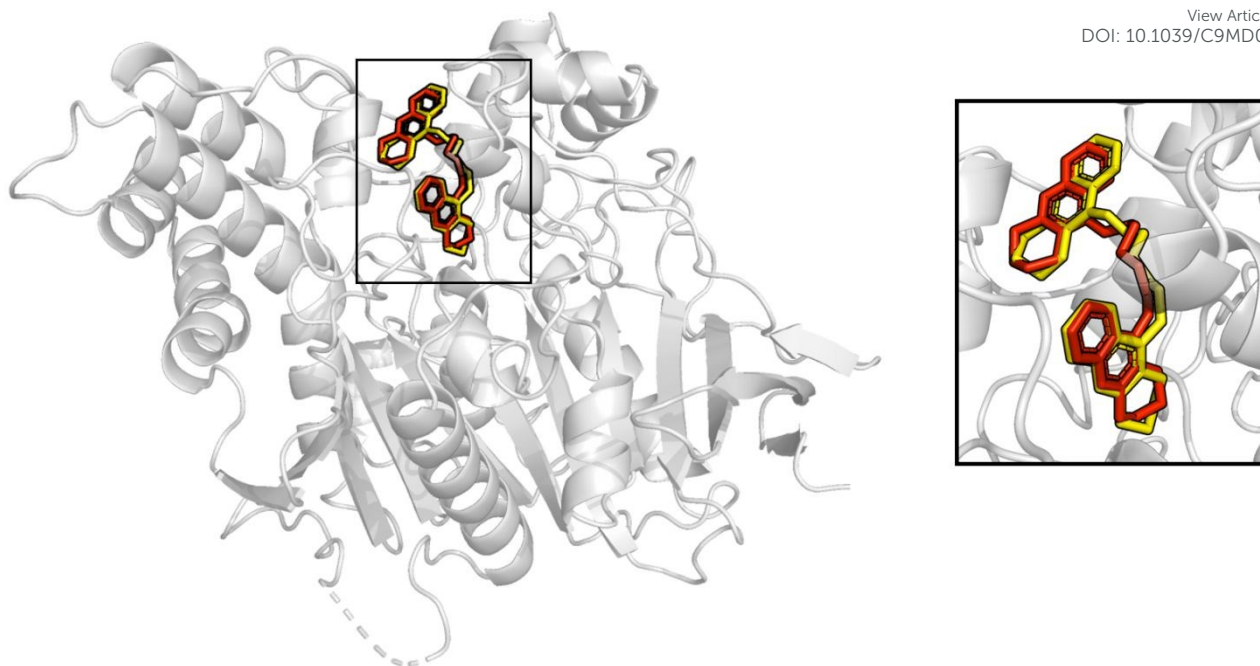
Figure 3. Redocking of the bis-tacrine derivative in Torpedo Californica AChE (2CMF) for the docking validation; in yellow, the ligand from the original crystal and in red, the ligand generated by redocking.

The binding scores for compounds **2** and **4** were -12.0 and -13.3 kcal/mol, respectively, matching the *in vitro* results obtained in the Ellman assay with the highest AChE inhibitory activity displayed by compound **4**. Figure 4 shows the interactions of compound **2** and **4** within the gorge of the enzyme.
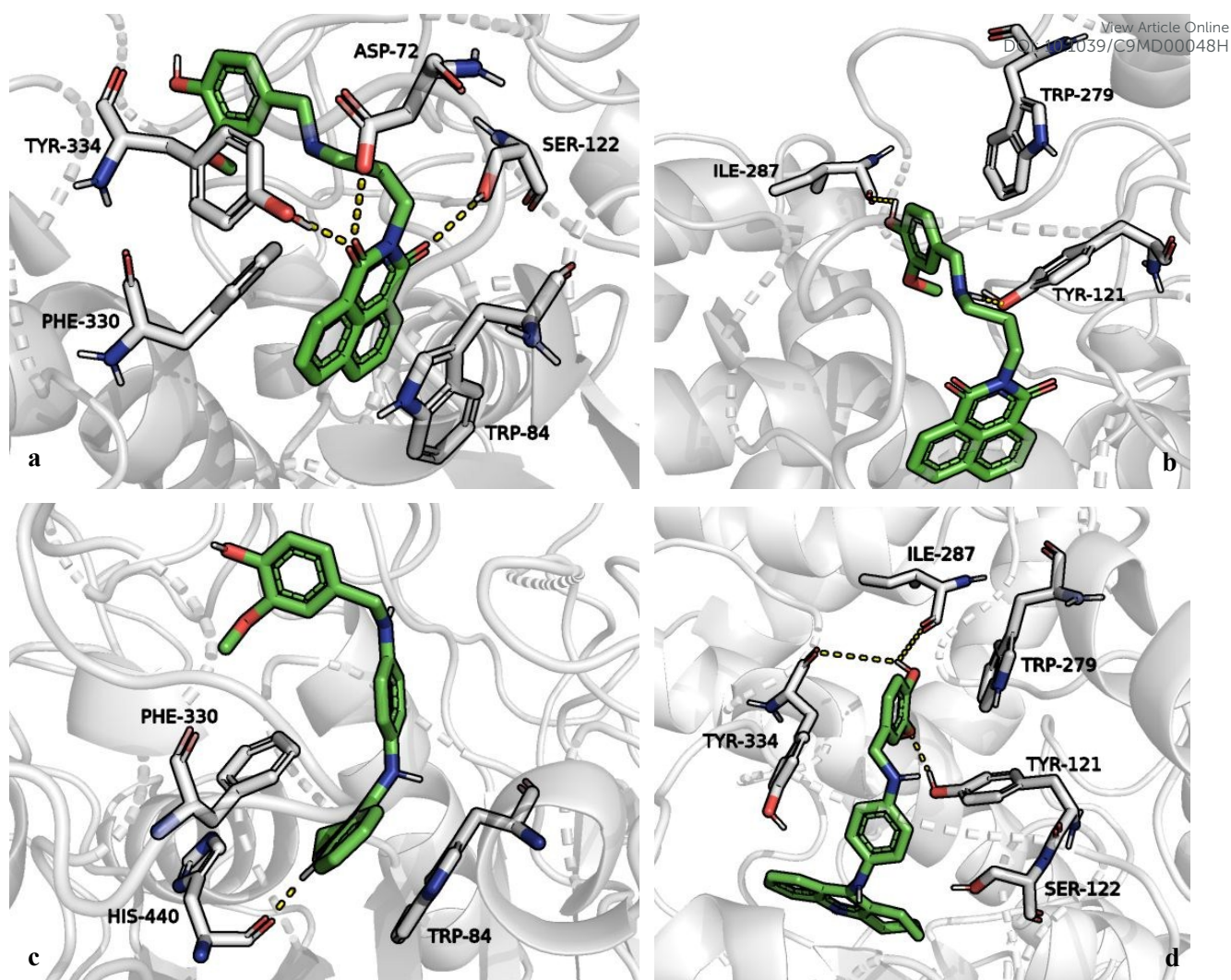
Figure 4. Predicted binding model of compounds 2 (a and b) and 4 (c and d) within the Acetylcholinesterase gorge. Compounds 2 and 4 are in green sticks whereas the amino acids involved in the interactions are in grey. Hydrogen bonds are shown as yellow dashed lines. In both models, the aromatic moieties of the compounds are stacked between the residues PHE-330 and TRP-84 (a and c). The two carbonyl groups from compound 2 for hydrogen bonds with the residues ASP-72, TYR-334, and SER-122 whereas the protonated nitrogen in the quinoline moiety of compound 4 forms hydrogen bond with the oxygen of the carbonyl group in HIS-440. In addition, the two OH-π interactions occur between the hydroxy groups of TYR-334 and SER-122 and the aromatic linker of compound 4. Finally, both compounds bind to TYR-121 and TRP-279 in the PAS to ILE-287 (b and d).

Interestingly, the napthalimido moiety of compound **2** is situated between amino acids PHE-330 and TRP-84 through π-π stacking, whereas the two carbonyl groups are involved in hydrogen bonds with hydroxy groups of residues amino acids ASP-72, TYR-334 (3.2 and 1.9 Å, respectively) and SER-122 (2.5 Å) (Figure 4a). In contrast, the molecular modelling studies reported by Gao *et al.* on their ranitidine derivatives bearing naphtalimido moieties demonstrated strong interactions of the latter structure with the residue TRP-286 of mouse AChE, equivalent to *tc*AChE, TRP-279 at the entrance of the gorge, in the PAS of the AChE [17,38]. Furthermore, hydrogen bonds are established between the phenolic group of the phenolic moiety and the carbonyl group of ILE-287 (2.7 Å) and between the nitrogen and the hydroxy group of TYR-121 (2.1 Å).

Finally, hydrophobic interactions between the phenolic moiety and TRP-279 are apparent (Figure 4b). Similarly, the tacrine moiety of compound **4** is stacked between PHE-330 and TRP-84. However, the charged nitrogen of tacrine provides a cation-π interaction with TRP-84, whereas the protonated nitrogen in the quinoline ring establishes a hydrogen bond (2.0 Å) with the carbonyl group of the main chain of HIS-440 (Figure 4c). Two OH-π interactions are established between the hydroxy group of SER-122 and TYR-334 and the aromatic linker of compound **4**. In addition, the phenolic moiety is involved in hydrogen bonding with two amino acids of the PAS (TYR-121, 2.2 Å and TYR-334, 3.8 Å), along with another hydrogen bond with ILE-287 (3.9 Å) and hydrophobic interactions with TRP-279 (Figure 4d).

Inhibition of Self-Mediated A$\beta_{(1-42)}$ Aggregation

A$\beta_{(1-42)}$ amyloid aggregation is a hallmark of AD, contributing to the deposition of extracellular amyloid plaques in the cerebral cortex [2]. The ability of the compounds to inhibit the self-mediated aggregation of amyloid A$\beta_{(1-42)}$ peptide was tested using Thioflavin T (ThT) fluorescence assay [32] with some modifications. The assay is based on the enhanced fluorescence of ThT upon binding to amyloid fibrils [39]. All the compounds were tested at concentration of 10 μM using a final concentration of A$\beta_{(1-42)}$ peptide of 10 μM as well. Curcumin was used a positive control. The results are shown in Figure 5.
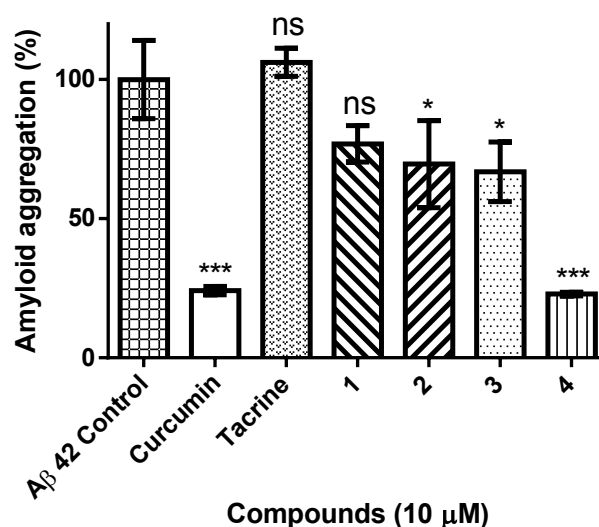
*Figure 5. Aggregation inhibitory effects of vanillin derivatives towards amyloid Aβ(1-42) peptide. All the compounds were tested at concentration of 10 μM. Values are expressed as the percentage of the control and represented as mean ± SD of three independent experiments in each group. ***p < 0.001, **p < 0.01, *p < 0.05, ns no significantly different compared to the control.*

All compounds showed good to excellent inhibitory properties toward peptide aggregation, with the exception of compound **1,** which showed no significant inhibition compared with the amyloid control. Compounds **2** and **3** showed similar activities, inhibiting A$\beta_{(1-42)}$ peptide aggregation by 30.4 and 33.2%,

respectively. Compound **4** inhibited the $A\beta_{(1-42)}$ peptide aggregation by 77.1% showing similar activity to the positive control curcumin (75.9%) and related tacrine-based derivatives reported by Luo et *al*. [32]. Interestingly, tacrine alone did not show significant inhibition toward $A\beta_{(1-42)}$ peptide aggregation. The $A\beta_{(1-42)}$ peptide aggregation inhibitory activities of compounds **2**, **3**, and **4** could be explained by the work of Reinke and Gestwicki, which suggested the importance of two aromatic end groups capable of taking part of hydrogen bonding [40]. All of the compounds described in this work bear a hydroxy group in the phenolic moiety that can be involved in hydrogen bonding. However, compound **1** did not show significant $A\beta_{(1-42)}$ peptide aggregation inhibition compared to the control, despite bearing two aromatic end groups and the hydroxy substituent. This may be explained by the length of the linker between the two aromatic end groups, which is shorter in compound **1** compared to compounds **2** and **3** due to the imino double bond. In fact, Reinke and Gestwicki reported that the optimal length of the linker lies between 8 and 16 Å. The length of the linker in compound **1** is 6.9 Å compared to compounds **2**, **3**, **4**, and positive control curcumin (7.3, 7.3, 8.7, and 9.2 Å respectively). Finally, the remarkable activity of compound **4** in this assay could be explained by the presence of the aromatic linker, which confers low flexibility important for the aggregation inhibitory activity [40]. We would like note that all the biological activities observed with our most active compounds are within the same range of activities reported by others working in similar field [41–43].

Conclusions

A series of vanillin derivatives was synthesized and found to have similar antioxidant activity to the reference antioxidant, Trolox, except for compound **1**. The results obtained in antioxidant assays agree with our previous study regarding the structure activity relationship for this class of antioxidants [16]. Compound **4** displayed the highest antioxidant properties among the vanillin derivatives, showing similar activity to Trolox in DPPH and FRAP assays. However, the latter vanillin derivative turned out to be six times more active than Trolox in the ORAC assay, which is more relevant to the biological systems, involving the use of peroxyl free radicals. To date, there has been limited evidence of multi-target-directed ligands with antioxidant activity in ORAC assay as strong as this vanillin derivative for example. [44]. Furthermore, compound **4** exhibited protection of SH-SY5Y cell line against hydrogen peroxide; at concentrations of 5 μM, which turned out to be completely safe in this cell line, the compound reversed peroxide-induced death by 30% after treatment with hydrogen peroxide (400 μM) for 24 hours and showed significant protection at concentration as low as 1 μM. In addition, the compounds showed inhibitory properties toward eel AChE, with IC_{50} ranging from 2.13 to 128.3 μM with the most active compound (**4**) 2-fold less active than the reference agent, tacrine. Computational docking studies suggested that compounds **2** and **4** could bind both the CAS and the PAS of the AChE enzyme through interaction with different

amino acids residues. Although a reduced activity of AChE inhibitory activity was observed with compounds **4**, the overall activity is well compensated by its enhanced antioxidant properties (due to the p-phenylenediamine linker). All the derivatives, except compound **1**, showed inhibitory activities against the self-mediated aggregation of amyloid peptide, with compound **4** being the most active, reducing $A\beta_{(1-42)}$ peptide aggregation by 77.1% and showing similar activity to curcumin (75.9%). Taken together, the vanillin derivatives reported in this work could provide a viable platform in the development of multi targeted AD therapy.

Experimental section

All the reagents were purchased from Sigma-Aldrich and Fisher Scientific unless otherwise stated. MTT was purchased from ACROS organics. Amyloid $A\beta_{(1-42)}$ was purchased from Calbiochem. All ^1H and ^{13}C spectra were collected using a Magnet Ultrashield Bruker 400 MHz spectrometer. Low-resolution mass spectrometry was performed using Agilent Technologies 1200 series. High-resolution mass spectrometry was performed at EPSRC National Mass Spectrometry Service Centre at Swansea University, Swansea, using Thermo Scientific LTQ Orbitrap XL or Waters Xevo G2-S spectrometers. The progress of each reaction was monitored by thin layer chromatography (TLC aluminium foil silica gel 60 with fluorescence indicator 254 nm, Sigma-Aldrich) through UV light (254-265 nm). Column chromatography was carried out using silica gel (Alfa Aesar 70-230 mesh) as the stationary phase and chloroform/methanol as the mobile phase. The chemical drawing and nomenclature of the compounds were applied according to ChemBioDraw Ultra version 16.0 (CambridgeSoft). Neuroblastoma SH-SY5Y cells were from the European Collection of Authenticated Cell Cultures (ECACC). Cells were maintained at 37 °C (5% CO_2) in DMEM medium (containing GlutaMAX-1 with 25 mM HEPES), supplemented with 10% (v/v) Foetal Bovine Serum (FBS) and 1% Penicillin/Streptomycin (10 mg/mL).

*Synthesis of 2-(3-aminopropyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione – **I-1***

1,8-Naphthalic anhydride (2 g, 10 mmol) was dissolved in ethanol (100 mL); 1,3-Diaminopropane (1.48 g, 20 mmol) was carefully added to the solution and stirred under reflux for 1 hour. The resulting precipitate was filtered and the filtrate concentrated using a rotary evaporator. The solid obtained was washed several times with diethyl ether to give a pale-yellow solid as the product (yield: 56%).

^1HNMR: (CDCl$_3$ solvent peak δ:7.27), 8.63-7.76 (m, Ar-H, 6H), 4.32-4.29 (t, N-CH$_2$-CH$_2$, J = 6.8 Hz, 2H), 2.80-2.77 (t, CH$_2$-NH$_2$, J = 5.6 Hz, 2H), 1.95-1.89 (m, CH$_2$-CH$_2$-CH$_2$, J = 13.6 Hz, 2 H), 1.52 (b, NH$_2$, 2H). *$^{13}CNMR$:* (CDCl$_3$ solvent peak δ: 77.4-76.8) 164.4, 134.0-122.65, 39.48, 37.77, 32.19. LRMS calcd for $C_{15}H_{15}N_2O_2$ [M+H]$^+$ 255.1, *m/z* found 255.0.

Synthesis of 2-(3-((4-hydroxy-3-methoxybenzylidene)amino)propyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione – **1**

Vanillin (0.5 g, 3.3 mmol) was dissolved in methanol followed by the addition of 2-(3-aminopropyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (0.83 g, 3.3 mmol). The reaction was left to stir overnight at RT. The solution obtained was concentrated through rotary evaporation and the solid obtained was dissolved in DCM (25 mL) and extracted 3 times with saturated $NaHCO_3$ (20 mL). The organic layer was collected and dried with anhydrous sodium sulfate and concentrated through rotary evaporator to afford an orange solid (yield: 73%).

^1HNMR: (CDCl$_3$ solvent peak δ:7.30), 8.61-6.85 (m, Ar-$\underline{H}$, 9H), 8.22 (s, Ar-C$\underline{H}$=N, 1H), 4.39-4.35 (t, N-C$\underline{H_2}$-CH$_2$, J = 6.8 Hz, 2H), 3.81 (s, -OC$\underline{H_3}$, 3H), 3.79-3.77 (t, C$\underline{H_2}$-N=CH, J = 6.8 Hz, 2H), 3.77-3.75 (t, N-C$\underline{H_2}$-CH$_2$-, J = 6.8 Hz, 2H), 2.26-2.23 (m, CH$_2$-C$\underline{H_2}$-CH$_2$, J = 14.0 Hz, 2H). *$^{13}CNMR$:* (CDCl$_3$ solvent peak δ: 77.4-76.7) 164.3, 161-107.8, 59.47, 55.87, 38.95, 29.17. HRMS calcd for $C_{23}H_{21}N_2O_4$ [M+H]$^+$ 389.1497, *m/z* found 389.1497.

Synthesis of 2-(3-((4-hydroxy-3-methoxybenzyl)amino)propyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione - **2**

0.4 g of 2-(3-((4-hydroxy-3-methoxybenzylidene)amino)propyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (1 mmol) were dissolved in methanol, then 0.057 g of NaBH$_4$ (1.5 mmol) added. The reaction was stirred for 2 hour and concentrated through rotary evaporation. The solid obtained was dissolved in DCM and extracted 3 times with NaHCO$_3$ (20 mL). The organic layer was collected and dried with anhydrous sodium sulfate and concentrated through rotary evaporation to afford a pale-yellow solid (yield: 86%). *^1HNMR:* (CDCl$_3$ solvent peak δ:7.31), 8.65-6.82 (m, Ar-$\underline{H}$, 9H), 4.34-4.31 (t, N-C$\underline{H_2}$-CH$_2$, J = 7.2 Hz, 2H), 3.91 (s, -OC$\underline{H_3}$, 3H), 3.76 (s, Ar-C$\underline{H_2}$-N, 2H), 2.78-2.74 (t, CH$_2$-C$\underline{H_2}$-N, J = 6.8 Hz, 2H), 2.05-2.01 (t, CH$_2$-C$\underline{H_2}$-CH$_2$, J = 6.8 Hz, 2H). *$^{13}CNMR$:* (CDCl$_3$ solvent peak δ: 77.4-76.7) 164.3, 146.61-110.9, 55.9, 53.8, 46.4, 38.3, 28.3. HRMS calcd for $C_{23}H_{23}N_2O_4$ [M+H]$^+$ 391.1652, *m/z* found 391.1651.

Synthesis of 2-(3-((4-hydroxy-3,5-dimethoxybenzyl)amino)propyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione - **3**

Syringaldehyde (0.29g, 1.57 mmol) was mixed with methanol (8 mL) followed by the addition of 2-(3-aminopropyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (0.40g, 1.57 mmol). The solution was refluxed for 2 hours and left stirring overnight at RT to yield a red solution. The solvent was evaporated under reduced pressure to yield a red solid which was suspended in propan-2-ol followed by the addition of NaBH$_4$ (3.0 mmol). The solution was refluxed for 48 hours. At the completion of the reaction, the solvent was removed under *vacuo* to afford a solid. The latter was collected by filtration, washed thoroughly with water and methanol to yield the final product (38%) . *^1HNMR:* (CDCl$_3$ solvent peak δ:7.20), 8.53-6.50 (m, Ar-$\underline{H}$, 8H),

4.23-4.20 (t, N-C$\underline{H}_2$-CH$_2$, J = 6.8 Hz, 2H), 3.80 (s, -OC$\underline{H}_3$, 6H), 3.64 (s, Ar-C$\underline{H}_2$-N, 2H), 2.68-2.64 (t, C$\underline{H}_2$-CH$_2$-N, J = 6.8 Hz, 2H), 1.95-1.91 (t, CH$_2$-C$\underline{H}_2$-CH$_2$, J = 6.8 Hz, 2H). *13CNMR:* (CDCl$_3$ solvent peak δ: 77.4-76.7) 164.3, 147.1-104.9, 56.2, 54.2, 46.5, 38.3, 28.3. HRMS calcd for C$_{24}$H$_{25}$N$_2$O$_5$ [M+H]$^+$ 421.1758, *m/z* found 421.1756.

Synthesis of 9-chloro-1,2,3,4-tetrahydroacridine - I-2

Intermediate **I-2** was prepared following the procedure reported in literature [21]. To a mixture of anthranilic acid (1.85 g, 1.3 mmol) and cyclohexanone (2.6 mL, 2.6 mmol), 15 mL of POCl$_3$ (0.16 mol) was added in an ice bath. The mixture was heated under reflux and stirred for 24 hours, then cooled and concentrated under reduced pressure. The residue was diluted with ethyl acetate (50 mL), neutralized with saturated Na$_2$CO$_3$ (30 mL) and washed 3 times with brine (30 mL). The organic layer was dried and the product was recrystallized from acetone (yield: 67%).

1HNMR: (CDCl$_3$ solvent peak δ:7.20), 8.11-7.45 (m, Ar-$\underline{H}$, 4H), 3.07-3.04 (t, Ar-C$\underline{H}_2$-CH$_2$, J = 5.2 Hz, 2H), 2.97- 2.94 (t, Ar-C$\underline{H}_2$-CH$_2$, J = 6.4 Hz, 2H), 1.92- 1.84 (m, C$\underline{H}_2$-CH$_2$, 4H). *13CNMR:* (CDCl$_3$ solvent peak δ: 77.8-76.7) 159.6- 123.7, 34.3, 27.6, 22.72, 22.68. LRMS calcd for C$_{13}$H$_{13}$ClN [M+H]$^+$ 218.7, *m/z* found 218.1.

Synthesis of N1-(1,2,3,4-tetrahydroacridin-9-yl)benzene-1,4-diamin - I-3

9-chloro-1,2,3,4-tetrahydroacridine (107 mg, 0.5 mmol) was dissolved in 1-pentanol (5 mL) followed by the addition of KI (80 mg, 0.5 mmol) and heated under reflux. Then, p-phenylenediamine (135 mg, 1.25 mmol) was added and the mixture stirred for 24 hours. The solution was concentrated under pressure and the solid obtained dissolved in 50 mL of DCM and extracted 3 times with Na$_2$CO$_3$ (50 mL). The organic layer was dried through rotary evaporation and the crude was purified through column chromatography (DCM/MeOH 99:1) to afford a brown solid (yield 39%).

1HNMR: (CDCl$_3$ solvent peak δ:7.30), 8.04-6.61 (m, Ar-$\underline{H}$, 8H), 5.95 (s, Ar-N$\underline{H}$-Ar, 1H), 3.59 (s, Ar-N$\underline{H}_2$, 2H), 3.20-3.17 (t, Ar-C$\underline{H}_2$-CH$_2$, J = 6 Hz, 2H), 2.72-2.69 (t, Ar-C$\underline{H}_2$-CH$_2$, J = 6.4 Hz, 2H), 1.98- 1.95 (m, -C$\underline{H}_2$-CH$_2$-, J = 5.6 Hz, 2H), 1.95- 1.89 (m, -CH$_2$-C$\underline{H}_2$-, J = 5.6 Hz, 2H). *13CNMR:* (CDCl$_3$ solvent peak δ: 77.4-76.7) 141.7- 116.1, 33.8, 25.2, 24.9, 22.8, 22.7. LRMS calcd for C$_{19}$H$_{20}$N$_3$ [M+H]$^+$ 290.2, *m/z* found 290.2.

Synthesis of 2-methoxy-4-(((4-((1,2,3,4-tetrahydroacridin-9-yl)amino)phenyl)amino)methyl)phenol - 4

I-3 (50 mg, 0.17 mmol) was dissolved in 2-propanol (10 mL) followed by the addition of vanillin (20 mg, 0.13 mmol). The reaction was stirred under reflux and monitored through TLC; when the vanillin spot had disappeared, the solution was cooled down and NaBH$_4$ (25mg, 0.7 mmol) was added. The solvent was dried

and the solid obtained was purified through column chromatography (DCM/MeOH 99:1) to afford an orange solid (yield 46%).

¹HNMR: (CDCl₃ solvent peak δ:7.19), 8.05-6.50 (m, Ar-H, 11H), 4.14 (s, Ar-CH₂-N, 2H), 3.81 (s, -OCH₃, 3H), 3.13-3.11 (t, Ar-CH₂-CH₂, J = 5.6 Hz, 2H), 2.95-2.93 (t, Ar-CH₂-CH₂, J = 7.2 Hz, 2H), 2.60- 2.56 (m, -CH₂-CH₂-, J = 6.4 Hz, 2H), 1.86- 1.80 (m, -CH₂-CH₂-, J = 12.4 Hz, 2H). *¹³CNMR:* (CDCl₃ solvent peak δ: 77.4-76.7) 146.8- 110.3, 56.0, 48.8, 45.82, 25.0, 22.7, 22.3. HRMS calcd for $C_{27}H_{28}N_3O_2$ [M+H]⁺ 426.2176, *m/z* found 426.2172.

DPPH Assay

The ability of the compounds to scavenge DPPH free radical was determined following the procedure reported by Payet, Sing and Smadja with some modifications in a 96-well set-up [22]. A dilution series of antioxidants ranging from 1.5 to 750 µM was made in Eppendorf® tubes, then 50 µL were transferred to the corresponding well, while 50 µL of vehicle (methanol) were used as negative control wells. 100 µL of DPPH solution (0.1 mM) were added to each well followed by incubation in the dark for 30 min. Absorbance was then measured at 517 nm using a Bio-Rad iMark microplate reader.

FRAP Assay

The reducing properties of the compounds was tested through the FRAP assay following the method described by Firuzi *et al*. with minor modifications in a 96-well plate [23]. FRAP reagent was prepared by mixing 2.5 mL of 10 mM TPTZ (in 40 mM HCl) with 2.5 mL of 20 mM FeCl₃ (in deionized water) and the volume brought to 30 mL with 300 mM sodium acetate buffer (pH 3.6). A dilution series of antioxidants and Trolox ranging from 500 to 5000 µM was made in Eppendorf® tubes, then 10 µL of each was pipetted in the corresponding well in a 96 well-plate along with 190 µL of FRAP reagent. The plate was stored in the dark for 30 minutes before the absorbance was measured at 593 nm using a Bio-Rad iMark microplate reader.

ORAC Assay

The ability of the compounds to prevent oxidative degradation of fluorescein was measured using ORAC assay following the previously protocols reported with minor modifications on a black-walled 96-well plate [16,22,45].

A dilution series of antioxidants and Trolox was made in phosphate buffer (75 mM, pH 7.4) in Eppendorf® tubes and 25 µL of each was transferred into the corresponding well whereas 25 µL of phosphate buffer was added in the control wells.

150 µL of 25 nM sodium fluorescein solution was added in each well and the plate was incubated at 37°C for 30 minutes.

Finally, 25 µL of 0.15 M AAPH solution was added in the positive control and in the antioxidant wells whereas 25 µL of phosphate buffer was added in the fluorescein control.

The fluorescence was measured every 2 minutes over a period of 2 hours (485/20 nm excitation, 525/20 nm emission) using a BioTek Synergy HT microplate reader.

MTT Assay

Cell survival after treatment with H_2O_2 (400 µM) was assessed using the MTT cell assay, using a procedure similar to previous publications [16,46]. SH-SY5Y cells were seeded (7000 cells/well) in a 96-well plate. After adhesion (24 h), cells were treated with different concentrations of compound **4**, ranging from 0.01 to 5 µM, and incubated for 24 h.

Subsequently H_2O_2 solution was added to the positive control and the test drug wells, followed by incubation for another 24 h. After incubation, all the solutions were removed from all the wells and 100 µL of MTT solution (1 mg/mL) was added in each well. The plate was wrapped in aluminum foil and incubated for 4 h at 37 °C.

The solutions in each well were removed by pipette and 100 µL of DMSO added to each well to dissolve the formazan crystals. The plates were gently shaken for 20 minutes and the absorbance measured at 490 nm with a Bio-Rad iMark microplate reader. Cell viability was expressed as a percentage of the absorbance from control cells.

AChE Inhibition Assay

The inhibitory properties of compounds toward AChE was determined through Ellman method with some modifications [30]. A 22 U/mL stock solution of AChE from *Electrophorus electricus* was prepared in 20 mM tris HCl pH 7.5 and diluted 1/100 before use. A 3 mM (5,5'-dithiobis-(2-nitrobenzoic acid)) solution was prepared by dissolving 0.1189 g of DTNB in 0.05 M phosphate/ 0.09 M hepes buffer (pH 7.5). A 15 mM acetylthiocholine iodide solution was prepared by dissolving 0.1084 g of compound in deionized water. A dilution series of compounds (in methanol) was made in Eppendorf® tubes and 25 µL of the latter was pipetted in the corresponding well in a 96-well plate along with 125 µL of DTNB solution and 25 µL of diluted AChE solution. 25 µL of methanol were added in the control wells. The plate was incubated for 10 minutes at 37°C then 25 µL of acetylthiocholine iodide solution were added to each well and incubated for another 10 minutes. The absorbance was measured at 415 nm using a Bio-Rad iMark microplate reader.

Molecular Modelling

For the docking procedure, the pdb structure of 2CMF (*Torpedo californica* AChE in complex with a bis-tacrine linked by a five-carbon spacer) was taken from Protein Data Base (http://www.rcsb.org). The choice of the *Tc*AChE instead of an *electrophoresus electricus* model of AChE (which was employed for the

Ellman assay) was based on the good resolution of the structure (2.5 Å), the similarity of the ligand in the structure with compound **4** and the unavailability of high resolution eeAChE structures [47]. It is worth noting the high degree of identity between *Tc*AChE and *Ee*AChE [48]. Water molecules and the original ligand were removed from the protein structure and polar hydrogens and charges were added. The protein was saved as pdbqt file. The 3D structures of compounds **2** and **4** were built using ChemDraw 16.0 (Cambridgesoft, Waltham, MA) and Chem3D Ultra 16 version (Cambridgesoft, Waltham, MA) and its MM2 force field energy minimization tool. The structures were saved as pdb file. The ligands and the macromolecule were then loaded into AutoDock Vina 1.1.2 (Molecular graphics laboratory, The Scripps research group, La Jolla, CA) and prepared for docking. Docking was performed on a grid box (40x40x40 Å) centered on the active site of AChE (residue TRP-84). The lowest energy conformation of each ligand enzyme complex was selected for analyzing the interactions between AChE and the inhibitor and the results were visualized using PyMOL (the PyMOL Molecular Graphics System, Version 2.0.7 Schrödinger, LLC).

ThT Assay

The ability of the compounds to inhibit self-mediated amyloid $A\beta_{(1-42)}$ aggregation was determined through the fluorometric ThT assay following the method described by Luo *et al*. with some modifications [32]. A 500 μM stock solution of amyloid $A\beta_{(1-42)}$ peptide was prepared by dissolving 0.25 mg of the peptide in 110 μL of DMSO. The solution was aliquoted in Eppendorf tubes and stored at -20°C.

Briefly, 2 μL of peptide solution was pipetted in 96 μL of 10 mM phosphate/ 10 mM NaCl buffer (pH 8) along with 2 μL of inhibitor solution (dissolved in DMSO) or 2 μL of DMSO (for the control). The final concentrations of both the amyloid peptide and inhibitors were 10 μM. The solutions were incubated for 24 hours at 37°C. Then, 300 μL of 50 mM glycine/ NaOH buffer (pH 8.5) containing 5 μM of ThT was added to all the samples.

Each solution was transferred into a cuvette and the fluorescence was measured using a Perkin Elmer LS55 luminescence spectrometer (excitation 446 nm, emission 490 nm).

Statistical Analysis

Data are shown as mean ± standard deviation (SD) and all the experiments were conducted on at least 3 separate occasions. Statistical analysis was performed using GRAPH PAD prism (6.00 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com) using ONE WAY ANOVA and Bonferroni's multiple comparison test. Significant differences are labelled accordingly (ns - not significant, $p < 0.05*$, $p < 0.01**$, $p < 0.001***$).

Conflicts of interest

There are no conflicts of interest to declare

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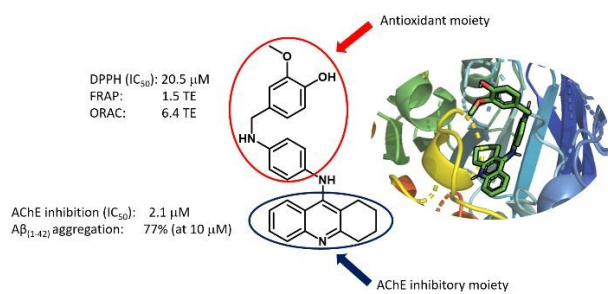
Novel vanillin-tacrine hybrid acting as AChE and $A\beta_{(1-42)}$ amyloid aggregation inhibitor with strong antioxidant properties enhanced by the *p*-phenylenediamine linker.

Supporting Information (SI)

Synthesis of novel vanillin derivatives: novel multi-targeted scaffold ligands against Alzheimer's disease

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[1]School of Pharmacy and Life sciences, Robert Gordon University, Aberdeen, UK

[2]Institute of Health Research & Innovation, University of the Highlands and Islands, Inverness, UK

Compound I-1

Compound 1

MS29

MS29

NAPH-I
(DCM)/MeOH + NH4OAc
C23H20N2O4
SM: 7G

EPSRC National Facility Swansea
LTQ Orbitrap XL

ROBKON
12/12/2016 16:49:01

Observed Data

NL:
1.17E6
ROBKON_0188W_34#22-28
RT: 0.75-1.03 AV: 6 T: FTMS
+ p NSI Full ms
[120.00-1935.00]

Theoretical Isotope Model: [M + H]+

NL:
1.80E4
C 23 H 20 N 2 O 4 H:
C 23 H 21 N 2 O 4
p (gss, s /p:40) Chrg 1
R: 100000 Res .Pwr . @FWHM

Compound 2

Compound 3

NS
(DCM)/MeOH + NH4OAc
C24H24N2O5
SM: 7G

EPSRC National Facility Swansea
LTQ Orbitrap XL

ROBKON
14/11/2017 16:04:00

NL:
4.79E6
ROBKON_93CTM_38304#41-
54 RT: 0.72-1.04 AV: 12 T:
FTMS + p NSI Full ms
[120.00-1935.00]

Observed Data

NL:
1.77E4

$C_{24} H_{24} N_2 O_5$ H:
$C_{24} H_{25} N_2 O_5$
p (gss, s /p:40) Chrg 1
R: 100000 Res .Pwr . @FWHM

Theoretical Isotope Model: [M + H]+

Compound I-2

Compound I-3

TACPPD COLUMN
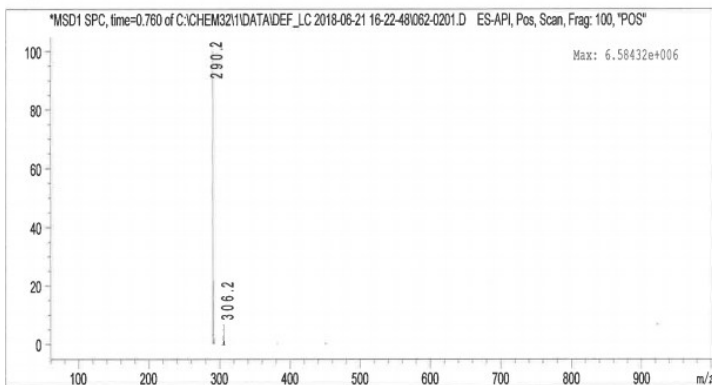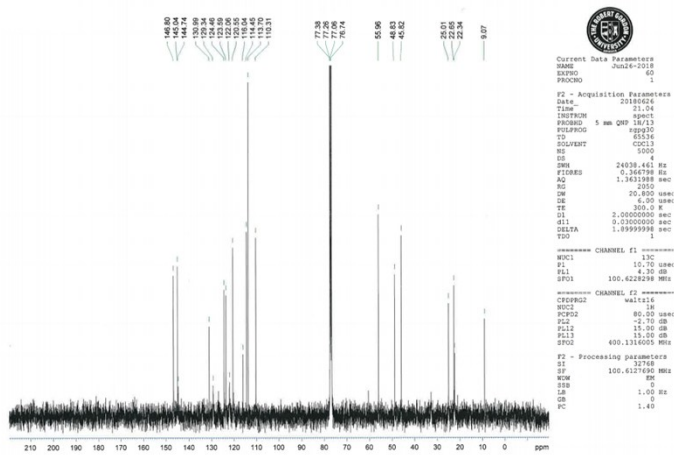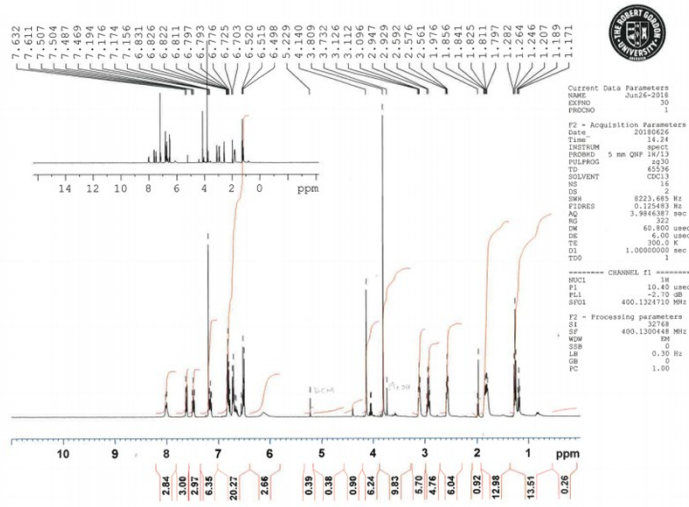
TACPPD COLUMN

Tacppd

*MSD1 SPC, time=0.760 of C:\CHEM32\1\DATA\DEF_LC 2018-06-21 16-22-48\062-0201.D   ES-API, Pos, Scan, Frag: 100, "POS"

Max: 6.58432e+006

290.2

306.2

Compound 4

SM: 7G
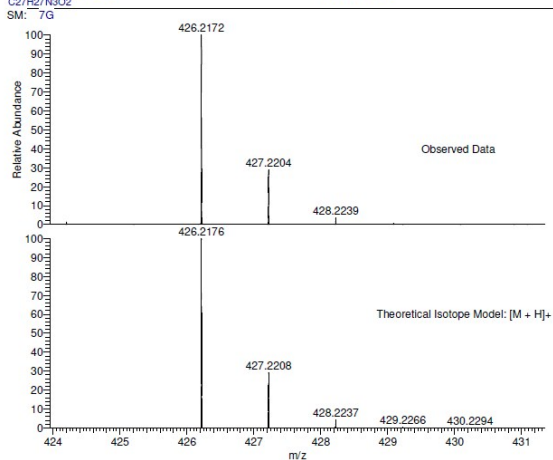
NL:
7.32E6
ROBKON_93KP9_48091#42-
56 RT: 0.74-1.02 AV: 11 T:
FTMS + p NSI Full ms
[120.00-1935.00]

Observed Data

NL:
1.72E4

$C_{27} H_{27} N_3 O_2$ H:
$C_{27} H_{28} N_3 O_2$
p (gss, s /p:40) Chrg 1
R: 100000 Res .Pwr . @FWHM

Theoretical Isotope Model: [M + H]+