Xanthone and Flavone Derivatives as Dual Agents with Acetylcholinesterase Inhibition and Antioxidant Activity as Potential Anti-Alzheimer Agents

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Alzheimer's disease (AD) is a multifactorial neurodegenerative disorder that is associated with the elderly. The current therapy that is used to treat AD is based mainly on the administration of acetylcholinesterase (AChE) inhibitors. Due to their low efficacy there is a considerable need for other therapeutic strategies. Considering that the malfunctions of different, but interconnected, biochemical complex pathways play an important role in the pathogenesis of this disease, a promising therapy may consist in administration of drugs that act on more than a target on biochemical scenery of AD. In this work, the synthesis and evaluation of xanthone and flavone derivatives as antioxidants with AChE inhibitory activity were accomplished. Among the obtained compounds, Mannich bases 3 and 14 showed capacity to inhibit AChE and antioxidant property, exerting dual activity. Moreover, for the most promising AChE inhibitors, docking studies on the target have been performed aiming to predict the binding mechanism. The results presented here may help to identify new xanthone and flavone derivatives as dual anti-Alzheimer agents with AChE inhibitory and antioxidant activities.

1. Introduction

AD is a neurodegenerative disorder that is characterized by progressive memory loss and cognitive deficits, being the most common form of dementia observed in the elderly [1, 2]. AD is usually designated as a multifactorial disease, because its genesis is associated with a diversity of factors, including genetic, environmental, and endogenous factors. Although the pathogenesis of AD is not yet fully elucidated, the formation of senile plaques, the neurofibrillary tangles, loss of cholinergic neural transmission, and oxidative stress have been identified as the major characteristic hallmarks of this disease [1, 3, 4].

The current therapy for AD is based on the administration of AChE inhibitors, such as tacrine, rivastigmine, donepezil, and galantamine [1]. However, this therapeutic strategy only is capable of treating the symptoms of AD resulting in favoring of behavioral and cognitive improvements on AD patients [2]. Concerning this and that the number of cases of AD is increasing, it is urgent to find new effective therapeutic strategies for the treatment of AD [2, 5].

Currently, one of the most interesting approaches for the treatment of complex diseases, like AD, is based on the “one molecule, multiple targets” paradigm [6, 7]. Among the multipotent approaches, the association of AChE inhibition and antioxidant activity has been considered as an attractive strategy for the treatment of AD [2, 6] and several multipotent natural and synthetic compounds have been developed as potential drug candidates to treat this neurodegenerative disease [2].
Xanthones and flavones frequently found in nature [8–13] correspond to "privileged structures," showing a wide variety of biological activities [9, 11, 14–16]. Some xanthones and flavones have been shown to inhibit AChE and MAO enzymes [20–27], and the aggregation of Aβ peptides [28–31], as well as to act as antioxidants [21, 32–37].

Herein we describe the synthesis and biological evaluation of xanthone and flavone derivatives with concomitant AChE inhibitory and antioxidant activities. The planning focused on the development of aminated derivatives of xanthones/flavones polyphenols, since FDA-approved acetylcholinesterase inhibitors for AD consist of amine drugs [1]. Moreover, for the most promising AChE inhibitors, docking studies on the target have been performed aiming to predict their binding mechanism.

2. Materials and Methods

2.1. Materials. All the reagents and solvents were purchased from Sigma Aldrich and were used without further purification. MW reactions were performed using a glassware setup for atmospheric-pressure reactions and a 100 mL reactor (internal reaction temperature measurements with a fiberoptic probe sensor) and were carried out using an Ethos MicroSYNTH 1600 Microwave Labstation from Milestone. All reactions were monitored by TLC carried out on pre-coated plates (silica gel, 60 F254 Merck) with 0.2 mm of thickness. The purifications of compounds were performed by flash column chromatography using Macherey-Nagel silica gel 60 (0.04–0.063 mm). IR spectra were measured on a KBr microplate in a FTIR spectrometer Nicolet iS10 from Thermo Scientific with Smart OMNI-Transmission Accessory (Software OMNIC 8.3). 1H and 13C NMR spectra were taken in CDCl3 (Deutero GmbH) at r.t. or DMSO-d6 (Deutero GmbH) at r.t., on Bruker Avance 300 instruments (300.13 MHz for 1H and 75.47 MHz for 13C). ESI-HRMS experiments were performed at CACTI, University of Vigo (Spain), on an APEXqe FT-ICR MS (Bruker Daltonics, USA), equipped with a 7T actively shielded magnet. Ions were generated using a Combi MALDI-electrospray ionization (ESI) source. Ionization was achieved by electrospray, using a voltage of 4500 V applied to the needle and a counter voltage of 300 V applied to the capillary. Samples were prepared by adding a spray solution of 70 : 29.9 : 0.1 (v/v/v) CH3OH/water/formic acid or 70 : 29.9 : 0.1 (v/v/v) CH3CN/water/formic acid to a solution of the sample at a v/v ratio of 1 to 5% to give the best signal-to-noise ratio. Data acquisition was performed using the ApexControl software version 3.0.0, and data processing was performed using the DataAnalysis software, version 4.0, both from Bruker Daltonics. Melting points were obtained in a Köfler microscope and are uncorrected. The purity of each compound was determined by analytical HPLC-DAD performed on a SpectraSYS-TEM (Thermo Fisher Scientific, Inc., USA) equipped with a P4000 pump, an AS3000 autosampler, and a diode array detector UV8000. The separation was carried out on a 250 × 4.6 mm i.d. FortisBIO C18 (5 μm) (Fortis™ Technologies Ltd., Cheshire, UK). ChromQuest 5.0 (version 3.2.1) software (Thermo Fisher Scientific Inc.) managed chromatographic data. Methanol (HPLC grade) was obtained from Carlo Erba Reagents (Val de Reuil, Italy), acetic acid (HPLC grade) was obtained from Romil Pure Chemistry (Cambridge, UK), and HPLC grade water was obtained from Simplicity® UV Ultrapure Water System, Millipore Corporation (USA). Prior to use, mobile phase solvents were degassed in an ultrasonic bath for 15 min. All synthesized compounds possessed a purity of at least 95%.

3,6-Dihydroxy-9-oxo-9H-xanthene-9-one (4) and 4-hydroxy-3-methoxy-9-oxo-9H-xanthene-1-carbaldehyde (10) were obtained according to described procedures [38, 39].

2.2. Chemistry

2.2.1. 1,3,8-Trihydroxy-9H-xanthene-9-one (1)

Method A (Eaton’s Reaction). A mixture of phosphorus pentoxide (1.00 g, 3.50 mmol) and methanesulfonic acid (15 mL) was heated on a steam bath (90°C) until a clear solution was obtained (30 min). Then, a mixture of phloroglucinol (0.38 g, 3.3 mmol) and 2,6-dihydroxybenzoic acid (0.46 g, 3.3 mmol) was added to the reaction mixture. This mixture was stirred at reflux (80°C) for 1 h and the progress of the reaction was monitored by TLC. Then, the resulting mixture was slowly poured into crushed ice and allowed to stand overnight in the fridge. The solid was collected by filtration, washed with water, and dried in oven (60°C). The crude product was purified by flash column chromatography (SiO2, petroleum ether: EtOAc (9:1 v/v)) to afford 1 (yield: 2.07%).

Method B (GSS Reaction). A mixture of zinc chloride (13.25 g, 97.2 mmol) and phosphorus oxychloride (55 mL) was submitted to microwave irradiation at 400 W of potency during 10 min at 60°C. Then, the 2,6-dihydroxybenzoic acid (5.00 g, 32.40 mmol) was added and the reaction mixture was subjected to microwave irradiation at 400 W of power for 10 min at 60°C. Finally, phloroglucinol (4.10 g, 32.40 mmol) was added and the mixture was submitted to microwave irradiation at 400 W of power during 60 min at 60°C. After cooling, the resulting mixture was poured into crushed ice and extracted successively with CH2Cl2 (9 × 200 mL) and a solution of 5% NaHCO3 (3 × 250 mL). The organic layers were joined and dried over anhydrous Na2SO4 and evaporated under reduced pressure. The crude product was purified by flash column chromatography (SiO2, n-hexane: EtOAc (9.5 : 0.5 v/v)) to give compound 1 as yellow solid (yield: 2.38%). mp 219-220°C; IR (KBr, ν (cm−1)) = 3500−3400 (OH); 1663 (C=O); 1601, 1565, 1483 (aromatic C=C); 1293 (C-O); 1 H NMR (300 MHz, DMSO-d6), δ (ppm): 11.89 (1 H, s, OH-1), 11.81 (1 H, s, OH-8), 7.68 (1 H, brt, J = 8.4 Hz, H-6), 7.00 (1 H, dd, J = 8.4, 0.9 Hz, H-5), 6.79 (1 H, dd, J = 8.4, 0.9 Hz, H-7), 6.38 (1 H, d, J = 2.1 Hz, H-4), 6.21 (1 H, d, J = 2.1 Hz, H-2); 13C NMR (75.47 MHz, DMSO-d6), δ (ppm): 183.26 (C-9), 176.07 (C-3), 162.16 (C-1), 160.27 (C-8), 157.51 (C-4a), 155.49 (C-10a), 137.18 (C-6), 110.60 (C-7), 107.10 (C-5), 106.88 (C-8a), 101.00 (C-9a), 98.70 (C-2), 94.48 (C-4).
2.2.2. 1,8-Dihydroxy-3-methoxy-9H-xanthone-9-one (2). A mixture of 1,3,8-trihydroxyxanthon (1) (0.30 g, 1.23 mmol), methyl iodide (0.803 mL, 12.30 mmol), and anhydrous K₂CO₃ (0.21 g, 1.24 mmol) in anhydrous acetone (40 mL) was stirred at reflux (60°C) for 3 h. Then, the solids was filtered, the solvent was removed under reduced pressure, and the crude product was purified by crystallization with acetone and by flash column chromatography (SiO₂, n-hexane) to yield compound 2 as yellow needle solid (yield: 74%).

**Mp 170–172°C; IR (KBr, v (cm⁻¹)): 3500–3400 (OH); 1662 (C=O); 1603, 1568, 1505, 1470 (aromatic C=C); 2959, 2925, 2852 (aliphatic C-H); 1294 (C-O); 1194 (C-CH₃); 1 C NMR (75.47 MHz, CDCl₃); δ (ppm): 114.29 (C); 129.04 (C=O); 160.36 (C=O); 110.89 (C-7), 106.96 (C-5), 106.96 (C-8), 102.70 (C-9), 101.46 (C-10a), 98.92 (C-4), 73.64 (C-3), 50.02 (C-CH₃).**

2.2.2.3. 1,8-Dihydroxy-3-methoxy-9H-xanthone-9-one (3). Dimethylinine (0.420 mL, 0.8395 mmol, 4.35 equiv.) was cooled in the ice bath for about 5 min and 37% formaldehyde solution (0.104 mL, 1.28 mmol) and acetic acid (0.965 mL, 16.85 mmol) were added dropwise. The reaction mixture was stirred at room temperature for 1 h and then 2 was added (0.050 g, 0.193 mmol). The mixture was stirred at room temperature for 6 days and then treated with water (5 mL) and stirring was continued for about 12 h. The resulting solid was filtered and the filtrate was treated with 10% NaOH until the pH was 9–10. Finally, the precipitate was also filtered, washed with water, dried, and purified by flash column chromatography (SiO₂, petroleum ether: chloroform : ammonium hydroxide (95: 5: 1 v/v/v)) to get 3 as yellow solid (yield: 6.29%). **Mp 170–172°C; IR (KBr, v (cm⁻¹)): 3600–3400 (OH); 1653 (C=O); 1605, 1558, 1599, 1490 (aromatic C=C); 2923, 2853 (aliphatic C-H); 1296 (C-O); 1230 (C-N); 1 H NMR (300 MHz, CDCl₃); δ (ppm): 12.09 (1 H, s, OH-1), 12.09 (1 H, s, OH-8), 7.55 (1 H, br, J = 8.3 Hz, H-6), 6.86 (1 H, brd, J = 8.3 Hz, H-5), 6.78 (1 H, brd, J = 8.3, Hz, H-7), 6.44 (1 H, H, H-4), 3.94 (3 H, s, 3-OCH₃), 3.64 (2 H, CH₂, J = 10 Hz, H; 2.37 (6 H, s, N(CH₃)₂); 13 C NMR (75.47 MHz, CDCl₃); δ (ppm): 184.33 (C-9), 165.79 (C-3), 161.39 (C-1), 157.69 (C-2), 151.95 (C-4a), 136.58 (C-6), 110.90 (C-7), 107.85 (C-5), 106.79 (C-8a), 102.70 (C-9a), 103.5 (C-2), 90.05 (C-4), 56.34 (3-OCH₃), 49.97 (CH₃), 44.96 (N(CH₃)₂). ESI-HRMS (+) m/z: Anal. Calcld for C₁₁H₁₅O₃ (M+H)⁺: 285.0396; found: 285.0392.**

2.25. General Procedure for the Reductive Amination. The carbaldhexe xanthone (5 or 10, 0.1 mmol) was dissolved in methanol (5 mL) and 2.4 equivol of the appropriate amine (0.24 mmol) was added to the solution under N₂ gas. A 4.0 equimolar quantity of NaBH(OAc)₃ (0.4 mmol) was added to the mixture solution. The reaction mixture was stirred at room temperature for 30 min. Then, 2.0 equimolar quantity of AcOH was added to the reaction mixture. The reaction mixture was stirred at room temperature under N₂ gas for overnight. After the solvent was evaporated, the crude product was further purified by flash column chromatography (SiO₂, chloroform : methanol: ammonium hydroxide (90: 10: 2 v/v/v)) to afford compounds 6–9, 11, and 12 in 32–51% yield.

(1) 4.5 Bis(((2- (diethylamino)ethyl)ethyl)amino)methyl)-3,6-dihydroxy-9H-xanthone-9-one (6). Pale yellow solid (yield: 51%). mp > 220°C; IR (KBr, v (cm⁻¹): 3446 (OH, broad spectrum); 1602 (C=O); 1431 (aromatic C=C); 1283 (C-O); 1201 (C-N); 1083 (C-O-C). 1 H NMR (300 MHz, DMSO-d₆), δ (ppm): 8.63 (2 H, d, J = 8.8 Hz, H-7, H-8), 8.13 (2 H, d, J = 8.8 Hz, H-2, H-3), 2.45 (4 H, s, 4-CH₂-5-CH₂), 2.84 (4 H, t, J = 6.2 Hz, CH₂), 2.67 (4 H, t, J = 6.8 Hz, CH₂), 2.58 (8 H, m, CH₂), 0.98 (12 H, t, J = 6.8 Hz, CH₂); 13 C NMR (75.47 MHz, DMSO-d₆), δ (ppm): 166.80 (C-5, C-6), 157.69 (C-3, C-4), 151.95 (C-4a), 136.58 (C-6), 110.90 (C-7), 107.85 (C-5), 106.79 (C-8a), 102.70 (C-9a), 103.5 (C-2), 90.05 (C-4), 56.34 (3-OCH₃), 49.97 (CH₃), 44.96 (N(CH₃)₂). ESI-HRMS (+) m/z: Anal. Calcld for C₁₁H₁₇O₄ (M+H)⁺: 485.31223; found: 485.3119.

(2) 3,6-Dihydroxy-4,5-bis(piperidin-1-ylmethyl)-9H-xanthen-9-one (7). Pale yellow solid (yield: 37%). mp > 220°C; IR (KBr, v (cm⁻¹): 3424 (OH, broad spectrum); 2955 (aliphatic C-H); 1616 (C=C); 1431, 1438 (aromatic C=C); 1289 (C-O); 1200 (C-N); 1087 (C-O-C). 1 H NMR (300 MHz, DMSO-d₆), δ (ppm): 791.2 (2 H, dd, J = 8.8, 1.4 Hz, H-1, H-8), 6.91 (1 H, d, J = 8.7 Hz, H-2, H-3), 6.78 (1 H, d, J = 8.7 Hz, H-7/H-2), 4.78 (4 H, s, 4-CH₂-5-CH₂), 2.61(8 H, m, CH₂), 1.52 (12 H, m, CH₂); 13 C NMR (75.47 MHz, DMSO-d₆), δ (ppm): 174.18 (C-9) 164.56 (C-3, C-6), 154.81 (C-4a, C-10a), 126.09 (C-1, C-8), 113.86
(3.6-Dihydroxy-4,5-bis((4-(2-hydroxyethyl)piperazin-1-yl)methyl)-9H-xanthene-9-one (8)). Pale yellow solid (yield: 32%). mp >220°C (KBr, v (cm⁻¹)): 3454 (OH, broad spectrum); 2925 (aliphatic C-H); 1644 (C=O); 1604, 1525, 1469, 1416 (aromatic C=C); 1323 (C-O); 1221 (C-N); 1075 (C-O-C).

1H NMR (300 MHz, CDCl₃), δ (ppm): 8.14 (2 H, d, J = 8.8 Hz, H-1, H-8), 6.83 (2 H, d, J = 8.8 Hz, H-2, H-7), 4.06 (4 H, s, 4-CH₂, 5-CH₂), 3.67 (4 H, t, J = 5.3 Hz, CH₂OH), 2.25 (20 H, m, CH₂), 13C NMR (75.47 MHz, CDCl₃), δ (ppm): 170.79 (C-9), 159.30 (C-3, C-6), 149.4 (C-4a, C-10a), 122.64 (C-1, C-8), 105.69 (C-8a, C-9a), 100.97 (C-2, C-7), 101.95 (C-4, C-5), 54.41 (CH₂OH), 53.20 (4-CH₂, 5-CH₂), 49.26 (C-CH₂N), 48.15 (CH₂N), 47.80 (C-CH₂N).

(4) 11'-(((3,6-Dihydroxy-9-oxo-9H-xanthen-4,5-diyldi)bis(methylenedioxy)bis(piperazin-4,1-diyl))bis(ethan-1-one) (9). Pale yellow solid (yield: 42%). mp >220°C; IR (KBr, v (cm⁻¹)): 3448 (OH, broad spectrum); 2921, 2851 (aliphatic C-H); 1624, 1602 (C=O); 1474, 1430, 1400 (aromatic C=C); 1265 (C-O); 1210 (C-N); 1073 (C-O-C).

1H NMR (300 MHz, DMSO-d₆), δ (ppm): 7.96 (2 H, d, J = 8.7 Hz, H-1, H-8), 6.91 (2 H, d, J = 8.8 Hz, H-2, H-7), 4.05 (4 H, s, 4-CH₂, 5-CH₂), 2.50 (16 H, m, CH₂), 1.81 (6 H, s, CH₃), 13C NMR (75.47 MHz, DMSO-d₆), δ (ppm): 168.30 (C-9, COCH₃), 162.40 (C-3, C-6), 155.40 (C-4a, C-10a), 126.40 (C-1, C-8), 113.60 (C-2, C-7), 113.40 (C-8a, C-9a), 109.40 (C-4, C-5), 52.70 (4-CH₂, 5-CH₂), 45.50 (C-CH₂N), 21.20 (C-CH₂).

ESI-HRMS (+) m/z: Anal. Calcd for C₃₂H₃₃N₅O₆ (M+H)⁺: 509.23946; found: 509.23910.

(5) 1-(((Diethylamino)methyl)methyl)-4-hydroxy-3-methoxy-9H-xanthene-9-one (II). Pale yellow solid (yield: 35%). mp >220°C; IR (KBr, v (cm⁻¹)): 3448 (OH, broad spectrum); 2921, 2851 (aliphatic C-H); 1624 (C=O); 1576, 1467 (aromatic C=C); 1320 (C=O); 1203 (C-N); 1071 (C-O-C).

1H NMR (300 MHz, DMSO-d₆), δ (ppm): 8.00 (1 H, d, J = 8.5 Hz, H-5), 7.58 (1 H, d, J = 8.0 Hz, H-6), 7.34 (1 H, dt, J = 1.7, 8.5 Hz, H-7), 7.05 (1 H, s, H-2), 6.73 (1 H, d, J = 8.8 Hz, H-5), 4.20 (2 H, s, 1-CH₂), 3.86 (3 H, s, 3-OCH₃), 2.71 (2 H, t, J = 6.09 Hz, CH₂), 2.50 (6 H, m, CH₂), 0.94 (6 H, t, J = 7.1 Hz, CH₃), 13C NMR (75.47 MHz, DMSO-d₆), δ (ppm): 177.26 (C-9), 161.11 (C-8b), 154.60 (C-3a), 151.50 (C-10a), 147.16 (C-4), 134.82 (C-6), 125.83 (C-8), 123.71 (C-7), 121.67 (C-1), 121.28 (C-8a), 117.59 (C-9a), 113.50 (C-10a), 110.92 (C-2), 55.82 (C-CH₂), 52.14 (C-CH₃), 31.75 (1-CH₂), 46.20 (3-OCH₃), 45.51 (C-CH₂N), 11.70 (C-CH₂N).

ESI-HRMS (+) m/z: Anal. Calcd for C₂₇H₂₇N₂O₄ (M+H)⁺: 517.19653; found: 517.19573.

(6) 4-Hydroxy-1-((4-(2-hydroxyethyl)piperazin-1-yl)methyl)-3-methoxy-9H-xanthene-9-one (I2). Pale yellow solid (yield: 39%). mp >220°C; IR (KBr, v (cm⁻¹)): 3422 (OH, broad spectrum); 2950, 2834 (aliphatic C-H); 1603 (C=O); 1460, 1412 (aromatic C=C); 1274 (C-O); 1235 (C-N); 1079 (C-O-C).

1H NMR (300 MHz, DMSO-d₆), δ (ppm): 8.10 (1 H, d, d, J = 1.5, 8.0 Hz, H-7), 7.80 (1 H, dt, J = 1.7, 8.6 Hz, H-8), 7.60 (1 H, d, J = 7.8 Hz, H-5), 7.41 (1 H, dt, J = 1.0, 8.0 Hz, H-6), 7.33 (1 H, s, H-2), 4.15 (2 H, s, 1-CH₃), 3.02 (2 H, s, CH₂), 2.93 (2 H, m, CH₂), 2.41–2.69 (8 H, m, CH₂), 15C NMR (75.47 MHz, DMSO-d₆), δ (ppm): 174.19 (C-9), 171.25 (C-10a), 154.67 (C-3), 153.11 (C-4a), 146.71 (C-4), 134.88 (C-6), 132.72 (C-8), 126.11 (C-1), 123.89 (C-8a), 121.72 (C-7), 117.60 (C-9a), 113.68 (C-5), 108.70 (C-2), 58.33 (C-CH₂OH), 56.06 (3-OCH₃), 52.77 (C-1CH₂), 48.67 (C-CH₂N), 45.65 (C-CH₂N), 43.22 (C-CH₂N). ESI-HRMS (+) m/z: Anal. Calcd for C₂₅H₂₅N₂O₅ (M+H)⁺: 385.17580; found: 385.17425.

2.3. Biological Activity

2.3.1. DPPH Radical Scavenging Assay. Scavenging activity for stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was evaluated according to a method previously described [40–42] with some modifications. A solution of DPPH radical in methanol was prepared daily and protected from light. Reaction mixtures containing 100 μL of methanol or DMSO solutions of tested compounds (100–1.56 μM) and 100 μL of DPPH (0.03 mg/mL) methanolic solution were shaken in a 96-well microtiter plate and incubated for 30 min in darkness at room temperature (until stable absorbance values were obtained). Controls containing 100 μL of methanol instead of compounds, blanks containing 200 μL of methanol, and sample’s blanks containing 100 μL of methanol instead of DPPH methanolic solution were also made. The absorbances were measured at 520 nm in a microplate reader (BioTek Instruments, Vermont, US). The percentage of inhibition activity was calculated according to the following formula: DPPH radical scavenging effect (%) = [1 – (Absample − Absblank)]/Abscontrol × 100. Ascorbic acid was used as positive control. All the tests were performed in triplicate. The scavenging activities of the tested compounds towards DPPH radical were expressed as the effective concentration at which DPPH radical was scavenged by 50% (IC₅₀).
The IC$_{50}$ value was obtained by interpolation from linear regression analysis.

2.3.2. **Ferrox Ion Chelating Assay.** The chelation of ferrous ions (Fe$^{2+}$) was assessed by the methodology described by Dinis et al. with modifications [43]. This assay was performed in a 96-well plate and the absorbance was measured at 562 nm. Briefly, 10 $\mu$L of FeCl$_2$ (50 $\mu$M) was added to 50 $\mu$L of methanolic solution of compounds in different concentrations (100–50 $\mu$M) and 120 $\mu$L of methanol. The mixture was allowed to stand 5 min and the reaction was initiated by the addition of 20 $\mu$L of 3-(2-pyridyl)-5,6-bis(4-phenylsulphonic acid)-1,2,4-triazine (ferrozine, 100 $\mu$M) and the mixture was shaken at room temperature for 10 min. Then, the absorbance of the solution was measured in a microplate reader (BioTek Instruments, Vermont, US). Controls containing 50 $\mu$L of solvent instead of compounds and blanks containing 50 $\mu$L of solvent instead of compounds and 20 $\mu$L of milliQ$^+$ purified water instead of ferrozine were made. All tests and analyses were carried out in triplicate and EDTA was used as positive control. The percentage of inhibition of Fe$^{2+}$–ferrozine complex formation was given below: % inhibition = [(Abs$_{control}$ – Abs$_{blank}$) – (Abs$_{sample}$ – Abs$_{sample/blank}$)]/Abs$_{sample}$blank × 100. The chelating activities of the purified compounds towards ferrous ions were expressed as the effective concentration at which Fe$^{2+}$–ferrozine complex formation was inhibited in 50% (IC$_{50}$). The IC$_{50}$ value was obtained by interpolation from linear regression analysis.

2.3.3. **Copper Chelating Activity.** The chelation of copper ions was assessed by the methodology described by Brown et al. [44] and adapted to a 96-well plate [45]. Stock solutions of each compound (1 mM) were prepared in methanol or DMSO. Then 50 $\mu$L of these solutions was prepared with PBS (10 mM) pH 7.4 and 50 $\mu$L of each was taken to 96-well UV plate (well A–D). Afterward, 100 $\mu$L of ultrapure water (well A), or 50 $\mu$L of ultrapure water and 50 $\mu$L CuSO$_4$ (200 $\mu$M) (well B), or 50 $\mu$L of ultrapure water and 50 $\mu$L of CuSO$_4$ (100 $\mu$M) (well C), or 50 $\mu$L of EDTA (500 $\mu$M) and 50 $\mu$L CuSO$_4$ (200 $\mu$M) (well D) were added. The absorption spectra were recorded between 200 and 800 nm in a microplate reader (BioTek Instruments, Vermont, US), and the scans in the presence of 1:1 (green spectrum) and 2:1 (blue spectrum) of copper-to-compound ratios were compared with the scan with compound alone (red spectrum). All tests and analyses were carried out in triplicate.

2.3.4. **AChE Inhibitory Assay.** AChE inhibitory activity was measured according to the method of Ellman et al. [46] with some modifications using a 96-well plate reader. Briefly, in the performed assay 20 $\mu$L of 0.22 U/mL AChE from Electrophorus electricus was added to the wells containing 20 $\mu$L of tested compounds (100 and 75 $\mu$M in methanol or DMSO), 100 $\mu$L of 3 mM 5,5$'$/2-thiodiobis(2-nitrobenzoic acid) (DTNB), and 20 $\mu$L of 15 mM acetylthiocholine iodide. Absorbance of the colored end product was measured at 412 nm for 10 minutes in a microplate reader (BioTek Instruments, Vermont, US). Controls containing 20 $\mu$L of compound vehicle (methanol or DMSO) instead of compounds and blanks containing 20 $\mu$L of buffer (0.1% (w/v) bovine serum albumin in 50 mM Tris-HCl) instead of enzyme and 20 $\mu$L of compound vehicle (methanol or DMSO) instead of compounds were made. In this assay, sample’s blanks containing 20 $\mu$L of buffer (0.1% (w/v) bovine serum albumin in 50 mM Tris-HCl) instead of AChE were also performed. Percentage of enzymatic inhibition was calculated as percentage of inhibition = 100 – ([Abs$_{sample}$ – Abs$_{sample/blank}$]/(Abs$_{control}$ – Abs$_{control/blank}$)) × 100. Every experiment was done in triplicate and galantamine was used as positive control. The inhibitory activities of the purified compounds towards AChE were expressed as the effective concentration at which AChE was inhibited by 50% (IC$_{50}$). The IC$_{50}$ value was obtained by interpolation from linear regression analysis.

2.4. **Statistical Analyses.** Data were reported as means ± standard error of the mean (SEM) of at least three independent experiments. Statistical analysis of the results was performed with GraphPad Prism (GraphPad Software, San Diego, CA). Unpaired t-test was carried out to test for any significant differences between the means. Differences at the 5% confidence level were considered significant.

2.5. **Docking Studies.** Crystal structure of AChE (PDB code: 4EY7) [47], downloaded from the protein databank (PDB) [48], was used for the study. Structure files of 14 known AChE inhibitors and compounds 2, 3, 6–9, and 14 were created and minimized using the chemical structure drawing tool Hyperchem 7.5 (Hypercube, FL, USA) [49]. Structure-based docking was carried out using AutoDock Vina (Molecular Graphics Lab, CA, USA) [50]. The active site was defined as the region of AChE that comes within 12 Å from the crystallographic ligand. Default settings for small molecule–protein docking were used throughout the simulations. Top 9 poses were collected for each molecule and the lowest docking score value was associated with the more favorable binding conformation. PyMol3.3 (Schrödinger, NY, USA) [51] and MOE (Chemical Computing Group, Montreal, Canada) [52] were used for visual inspection of results and graphical representations.

3. **Results and Discussion**

3.1. **Chemistry.** The synthesis of xanthones 1, 2, and 3 was achieved according to the general reaction pathway outlined in Scheme 1. 1,3,8-Trihydroxyxanthone (I) was obtained by one-step synthesis using two different approaches: Eaton’s reaction and Grover Shah and Shah (GSS) reaction. In Eaton’s reaction, I was obtained by the condensation of 2,6-dihydroxybenzoic acid with phenylglycinol in the presence of a mixture of phosphorus pentoxide-methanesulfonic acid (Eaton’s reagent) as condensing agent (Scheme 1) [53]. In the GSS reaction xanthone I was synthesized by the condensation of the same building blocks in the presence of zinc chloride in phosphorus oxychloride, instead of Eaton’s reagent [54] (Scheme 1). Although this condensation agent has been
shown to be efficient for the synthesis of hydroxylated xanthones, this synthetic methodology is associated with low yields [9, 55, 56]. Taking these into account, some experimental modifications to the classic GSS reaction were performed including the stepwise addition of reactants as described before and the use of microwave assisted organic synthesis (MAOS), instead of conventional thermal heating (classical synthesis). Particularly, in the original GSS methodology, a mixture of a hydroxybenzoic acid, a phenol, fused zinc chloride, and phosphorus oxychloride was heated together on a water bath. Instead, in the GSS reaction here reported, firstly, a mixture of fused zinc chloride and phosphorus oxychloride was irradiated by MW at 400 W for 10 min at 60°C; then the hydroxybenzoic acid was added and the mixture was subjected to MW irradiation at 400 W for 10 min at 60°C and after the appropriated phenol was added and the mixture was once more submitted to MW at 400 W for 60 min at 60°C to afford xanthone 1 (Scheme 1). In general, both approaches showed short reaction times (Eaton’s reaction: 1 h 30 min; modified GSS reaction by MW: 1 h 20 min) and low yields. Although purification of 1 in both reactions was time consuming, this process was more complex in the GSS reaction. In fact, in this methodology, three different procedures had been performed: extraction with CH₂Cl₂ in order to remove a brown slimy material; extraction with 5% NaHCO₃ to eliminate traces of benzoic acid; and a flash column chromatography to get 1. In contrast, in Eaton’s reaction, xanthone 1 was obtained using merely flash chromatography as purification method. In conclusion, according to these results, Eaton’s reaction seemed to be more suitable than GSS for the synthesis of 1, because it was a cleaner approach, requiring less purification procedures.

The hydroxylated xanthone 1 was then methylated with methyl iodide under basic conditions to afford compound 2 (Scheme 1). Following the synthetic route as shown in Scheme 1, this methylated derivative 2 reacted with formaldehyde and dimethylamine through Mannich reaction to afford the respective Mannich base derivative 3.

The aminated xanthones 6–9, 11, and 12 were obtained by reductive amination from the corresponding carbaldehydes 5 and 10 (Scheme 2). Both precursors were obtained from oxygenated xanthones by a Duff formylation according to the described procedures [38, 39].

8-((Dimethylamino)methyl)-5,6,7-trihydroxy-2-phenyl-4H-chromen-4-one (14) was obtained according to the method previously described by Zhang et al. [57]. Concerning this synthetic approach, aminoflavone 14 was achieved from the reaction of baicalein (13) with formaldehyde and dimethylamine by Mannich reaction (Scheme 3).

3.2. Biological Activity

3.2.1. DPPH Radical Scavenging Activity. The DPPH assay has been widely used to assess the antioxidant potential of compounds. Using this assay, the capacity of compounds 1–3, 6–9, and 11–14 to transfer labile H-atoms to this free radical was measured (Table I).

While no promising DPPH radical scavenging activity was observed for xanthones 1–3 and 6–9 at 100 μM, the aminated derivatives 11 and 12 exhibited some scavenging activity, suggesting that the introduction of amino side chains at C-1, instead of C-4,5, is favorable for this activity. Among the compounds tested the baicalein derivative 14 was the most active, showing an IC₅₀ value of 30.40 ± 1.28 μM, very similar to the precursor baicalein (13) (IC₅₀ = 35.09 ± 4.13 μM), suggesting that the presence of 8-(dimethylamino)methyl group on the flavone skeleton did not significantly alter its ability to scavenge free radicals.

3.2.2. Ferrous Ion Chelating Activity. Among the transition metals, iron is well known as playing a crucial role in
Scheme 2: Reagents and conditions for the synthesis of carbaldehyde xanthone 5 and aminated xanthenes 6–9 and 11–12. (a) (1) HMTA/AcOH, reflux, 3 h, (2) 15% HCl, reflux, 30 min; (b) amine (2.4 eq), NaBH₃(OAc)₃ (4 eq), AcOH (2 eq), MeOH, room temperature, overnight.

Scheme 3: Reagents and conditions for the synthesized flavone 14. (a) Dimethylamine/HCHO/MeOH, room temperature, 1 h 30 min. The numbering used concerns the NMR assignments.
potent, showing an IC and a hydroxyl group instead of a methoxy group at C-3 but
chelating effect, the presence of this group at C-1 (derivatives ferrozine-based colorimetric assay. Only compounds for xanthones groups at position C-2 in derivative influence this effect. In fact, although the presence of amino side chains in the xanthone scaffold may suggests that not only the presence but also the position 11 – found in AD [58]. The ability of compounds reaction, which contribute to the oxidative stress commonly performed in triplicate; n.a.: not active; n.d.: not determined. EDTA (positive control): IC value of ∗∗∗𝑝<0.001.

Table 1: DPPH radical scavenging activity of tested compounds.

| Compounds | % of scavenging DPPH radical at 100 μM | IC_{50} (μM) |
|-----------|----------------------------------------|--------------|
| 1         | n.a.                                   | n.d.         |
| 2         | n.a.                                   | n.d.         |
| 3         | n.a.                                   | n.d.         |
| 6         | 5.92 ± 0.84**                          | n.d.         |
| 7         | 2.32 ± 0.25*                           | n.d.         |
| 8         | 5.74 ± 0.83*                           | n.d.         |
| 9         | n.a.                                   | n.d.         |
| 11        | 69.26 ± 2.11***                        | 61.62 ± 0.72*** |
| 12        | 47.09 ± 1.35***                        | 108.99 ± 0.87*** |
| 13        | 96.03 ± 0.42***                        | 35.09 ± 4.13*** |
| 14        | 93.93 ± 0.20***                        | 30.40 ± 1.28*** |

Results are given as mean ± SEM of three independent experiments performed in triplicate; n.a.: not active; n.d.: not determined. Ascorbic acid 35.09±4.13 was associated with an improvement of the ferrous ion chelating activity.

3.2.3. Copper Chelating Activity. As iron, copper (II) ions also promote oxidative damage in AD by the generation of ROS via the Fenton reaction. In addition, this transition metal still has the ability to interfere with protein aggregation processes implicated in the pathogenesis of this disease [1, 59, 60]. The copper chelating activity of compounds 1–3, 6–9, and 11–14 was assessed by UV-Vis spectroscopy, mainly through the detection of bathochromic shifts in the spectra of the tested compounds in the presence of Cu^{2+} ions at pH 7.4. For this, the UV-Vis spectra of each tested compound in PBS at pH 7.4 alone (red spectra, Figure 1) and in the presence of a solution of 50 μM CuSO_{4} (blue spectra, Figure 1) or 25 μM CuSO_{4} (green spectra, Figure 1) were recorded. In addition, the UV-Vis spectrum of each compound in the presence of 25μM CuSO_{4} after addition of 25 μM EDTA (pink spectra, Figure 1) was also registered.

The changes observed in the spectrum of xanthone derivative 1 after the addition of Cu^{2+} (blue and green spectra, Figure 1(a)) in comparison with the spectrum in the absence of this metal ion (red spectrum, Figure 1(a)) and the regeneration of the original spectrum (pink spectrum, Figure 1(a)) after the addition of EDTA suggest that 1 has Cu^{2+} chelating effect. Nevertheless, no chelating activity was observed for the 1 methylated derivative (2) since all spectra are overlapped (Figure 1(b)).

For xanthone 2 Mannich base derivative (3) some chelating activity was observed. In fact, interactions of 3 with Cu^{2+} ions at 25 μM (green spectrum, Figure 1(c)) and 50 μM (blue spectrum, Figure 1(c)) produced bathochromic shift in bands at 250 nm and 370 nm. The comparison of results obtained for 2 and 3 indicates that the aminomethylation at C-2 of xanthone nucleus potentiates its chelating activity. For derivatives 6–9, 11, and 12 no chelating activity was detected since the spectrum of each compound in the presence of copper was overlapped with the spectrum of the compound alone (data not shown).

The chelation of Cu^{2+} by baicalein (13) and its Mannich base derivative 14 is also evidenced by the changes in the UV-Vis spectra. For baicalein (13) a hypochromic shift in band at 266 nm (blue and green spectra, Figure 1(d)) and a slight bathochromic shift in band at 359 nm after the addition of Cu^{2+} were detected. Moreover, the addition of EDTA (pink spectrum, Figure 1(d)) regenerated the original spectrum. For baicalein Mannich base derivative 14 the chelation of this ion

production of reactive oxygen species (ROS) by Fenton reaction, which contribute to the oxidative stress commonly found in AD [58]. The ability of compounds 1–3, 6–9, and 11–14 to chelate ferrous ions (Fe^{2+}) was evaluated by the ferrozine-based colorimetric assay. Only compounds 1, 3, 13, and 14 had ferrous ion chelating activity at 100 μM (Table 2).

Among the tested xanthones, compound 3 was the most potent, showing an IC_{50} value of 65.94 ± 1.57 μM. The comparison of the ferrous ion chelating effects of tested xanthones suggests that not only the presence but also the position of the amino side chains in the xanthone scaffold may influence this effect. In fact, although the presence of amino groups at position C-2 in derivative 3 is associated with a chelating effect, the presence of this group at C-1 (derivatives 11 and 12) or C-4,5 (derivatives 6–9) is not associated with any effect. Moreover, when comparing the results obtained for xanthones 1–3 it is verified that not only the presence of a hydroxyl group instead of a methoxy group at C-3 but also the presence of a 2-(dimethylamino)methyl group on the xanthonic scaffold can favor the chelating activity.

Also, the presence of ortho-OH groups offers appropriate geometric and electronic environments to bind metal ions [6, 21]. Moreover, considering the chelating activity of 13 (% of chelation at 100 μM = 18.24 ± 3.46) and 14 (% of chelation at 100 μM = 49.90 ± 0.23), it is possible to infer that the presence of 8-(dimethylamino)methyl group in the baicalein derivative 14 was associated with an improvement of the ferrous ion chelating activity.

Table 2: Iron chelating activity of tested compounds.

| Compounds | % of chelation at 100 μM | IC_{50} (μM) |
|-----------|-------------------------|--------------|
| 1         | 12.19 ± 2.55*           | n.d.         |
| 2         | n.a.                    | n.d.         |
| 3         | 88.40 ± 0.56*           | 65.94 ± 1.57* |
| 6         | n.a.                    | n.d.         |
| 7         | n.a.                    | n.d.         |
| 8         | n.a.                    | n.d.         |
| 9         | n.a.                    | n.d.         |
| 11        | n.a.                    | n.d.         |
| 12        | n.a.                    | n.d.         |
| 13        | 18.24 ± 3.46*           | n.d.         |
| 14        | 49.90 ± 0.23**          | 100 ± 0.21** |

Results are given as mean ± SEM of three independent experiments performed in triplicate; n.a.: not active; n.d.: not determined. EDTA (positive control): IC_{50}: 30.66 ± 0.17 μM**. ∗∗∗𝑝<0.001; **∗𝑝<0.01; ∗∗𝑝<0.05; ∗𝑝<0.001.
Figure 1: UV-Vis spectra of 25 μM of compounds 1 (a), 2 (b), 3 (c), 13 (d), and 14 (e) in PBS at pH 7.4 (red spectra), after the addition of 25 μM CuSO₄ (green spectra), 50 μM CuSO₄ (blue spectra), and 25 μM of CuSO₄ plus 125 μM of EDTA (pink spectra).
induced a bathochromic shift in band at 268 nm for both Cu$^{2+}$ concentrations (50 μM and 25 μM, blue and green spectra, Figure 1(e)) and the original spectrum was recovered after the addition of EDTA (pink spectrum, Figure 1(e)).

### 3.2.4. AChE Inhibitory Activity

The AChE inhibitory activity of compounds 1–3, 6–9, and 11–14 was screened by the Ellman spectrophotometric assay with some modifications (Table 3) [46].

Although no effect was observed for 1,3,8-trihydroxyxanthone (1) and xanthones 11 and 12 with an amino group at C-1, xanthone derivatives possessing amino groups at C-4,5 (compounds 6–9) or C-2 (compound 3) revealed AChE inhibitory activity at 100 μM, being derivative 3 the most potent. Overall, these results indicate that the introduction of an amino side chain at C-2 or 4,5 seems to favor the AChE inhibitory activity relatively to position 1.

Regarding the tested flavones, in opposition to baicalein (13) which did not possess AChE inhibitory activity, its derivative 14 was revealed to be a moderate AChE inhibitor, with an IC$_{50}$ value of 81.99 ± 1.16 μM. As for xanthone derivatives 1–3, the introduction of 8-(dimethylamino)methyl group on the flavone skeleton is also associated with the increase of AChE inhibition.

In summary, concerning the results obtained for antioxidant activity (DPPH radical scavenging and ferrous and copper ions chelating activities) and the AChE inhibitory property for all studied xanthones and flavones, it can be seen that some of them showed both activities. Particularly, Mannich base analogues 3 and 14 exhibited interesting AChE inhibitory effect and antioxidant activity through different ways. While aminoxanthone 3 exerted its antioxidant activity by chelating ferrous and copper ions, aminoflavone 14 was an antioxidant agent through radical scavenging and ferrous and copper ions chelating effects.

### 3.3. Docking Studies

In this work, compounds 2, 3, 6–9, and 14 have been discovered as AChE inhibitors. Therefore, docking studies were performed for this series along with compounds already described in the literature as AChE inhibitors [61], being these used as positive controls. For controls, docking scores values ranging from −5.7 to −12.1 kcal·mol$^{-1}$ were obtained (Table 4). The most stable complex (14: AChE) originated a free binding energy within that range (−10 kcal·mol$^{-1}$) in accordance with in vitro studies (Table 3). Compounds 2 and 3 are also predicted as forming stable complexes with AChE, with docking scores of −9.1 and −9.4 kcal·mol$^{-1}$, respectively. Amines 6, 7, and 9 presented docking scores of −7.8, −7.6, and −7.9 kcal·mol$^{-1}$, respectively, whereas less active amine 8 presented a docking score of −5.7 kcal·mol$^{-1}$ (Table 4).

In order to further understand the binding mode of the most potent inhibitors (2, 3, and 14) to AChE, a careful inspection of the most stable docking pose of these small molecules was performed (Figure 2). AChE is a serine hydrolase possessing a narrow, long, and hydrophobic cavity composed by two subsites: the esteratic and anionic subsites [61]. The esteratic subsite, where AChE is hydrolyzed to acetate and choline, contains the catalytic triad of three amino acids: Ser203, His447, and Glu334, the lining of which contains mostly aromatic residues that form a narrow entrance to the catalytic Ser203 [62]. The activation of Ser203 allows the acylation between hydroxyl group of that residue and AChE oxygen. In the anionic site, the indole side chain of the conserved residue Trp86 makes a cation–π interaction with the quaternary amino group of AChE. A peripheral site is composed of several aromatic residues, lining a hydrophobic region that traps AChE and transfers it to the deep catalytic site [63].

### Table 3: AChE inhibitory activity of tested compounds.

| Compounds | % of inhibition at 100 μM | IC$_{50}$ (μM) |
|-----------|--------------------------|----------------|
| 1         | n.a.                     | n.d.           |
| 2         | 34.04 ± 3.12*            | n.d.           |
| 3         | 49.08 ± 2.78**           | 100.00 ± 2.76**|
| 6         | 29.28 ± 5.79**           | n.d.           |
| 7         | 17.34 ± 2.45*            | n.d.           |
| 8         | 21.50 ± 4.02*            | n.d.           |
| 9         | 29.74 ± 3.70**           | n.d.           |
| 11        | n.a.                     | n.d.           |
| 12        | n.a.                     | n.d.           |
| 13        | n.a.                     | n.d.           |
| 14        | 69.34 ± 0.72**           | 81.99 ± 1.16** |

Results are given as mean ± SEM of three independent experiments performed in triplicate; n.a.: not active; n.d.: not determined. Galantamine (positive control): IC$_{50}$: 6.59 ± 0.15 μM**, *p < 0.05; **p < 0.01.

### Table 4: Docking scores of known AChE inhibitors and compounds 2, 3, 6–9, and 14.

| Compound       | Docking scores (kcal·mol$^{-1}$) |
|---------------|----------------------------------|
| Trichlorfon    | −5.7                             |
| Echotoxiphate  | −5.9                             |
| Isoulophosphate| −5.9                             |
| Pyridostigmine | −6.3                             |
| Neostigmine    | −7.5                             |
| Parathion      | −7.6                             |
| Rivastigmine   | −7.9                             |
| Tacrine        | −8.8                             |
| Ladostigil     | −9.1                             |
| Physostigmine  | −9.1                             |
| Huperzine A    | −9.7                             |
| Ungeremine     | −10                               |
| Galantamine    | −10.2                            |
| Donepezil      | −12.1                            |
| 2              | −9.1                             |
| 3              | −9.4                             |
| 6              | −7.8                             |
| 7              | −5.7                             |
| 8              | −7.6                             |
| 9              | −7.9                             |
| 14             | −10                              |
The interactions between inhibitors such as donepezil, galantamine, huperzine A, and the enzyme, as observed from their crystallographic structures (Figure 3), are characterized by hydrogen interactions and π-π stacking and cation-π interactions involving aromatic residues of AChE [47]. Most ligands are located at the bottom of the binding groove that forms a hydrophobic pocket base, although larger ligands as donepezil extend to the periphery of the pocket.

The narrower gorge in AChE results in a conformation where the phenyl ring of donepezil packs against the aromatic hydrophobic portions of the side chain of Phe338. Packing in this region is quite tight, and only the smallest substituents might be accommodated. In contrast, the chromenone ring system of the flavone molecule faces a larger, negatively charged, and hydrophilic cavity, which includes hydroxyl and carbonyl groups of Gly120, Ser125, Tyr133, and Glu202. Upon analysis of the interaction of donepezil in AChE binding site domains, it is observed that the residues Gly120, Ser125, Tyr133, and Glu202 participate in H-interactions. These residues are present in the buried region of the groove. π stacking interactions are established between the phenyl ring of donepezil and Phe338. Although donepezil has a protonable amine group, and cation-π interactions between protonated nitrogen and AChE aromatic residues are described as being important for the activity of the highly potent inhibitors, such as donepezil (Figures 3(a) and 3(b)), this interaction is not predicted to occur between donepezil and AChE binding site (Figures 2(b) and 2(c)). The binding affinity of donepezil allows not only the establishment of several polar interactions, but also stacking interactions with Phe338 favored by the torsion angle between the chromenone and the benzene ring (39.41°). Although 8-(dimethylamino)methyl group of compound donepezil is not predicted to be involved in polar interactions by the docking algorithm used, it has steric complementarity with a small lateral groove, establishing several van der Waals, hydrophobic, and permanent dipole-induced dipole interactions with residues such as Asp74, Trp86, Asn87, Tyr124, and Ser125.

Compound 3 establishes one polar interaction with residue Tyr124 and one cation-π interaction with Trp86, whereas compound 2 establishes polar interactions with Tyr-337 and His-447 and stacking interactions with Trp86 (Figure 4).

In conclusion, molecular modifications on the flavone scaffold placing the amine group on different positions on A ring will be further explored in order to facilitate a cation-π interaction. Moreover, molecular modifications that would allow the establishment of hydrogen interactions with the catalytic residue Ser203 (described for galantamine; Figures 3(c) and 3(d)) and elongation of the molecule that would favor the establishment of additional π-π or cation-π interactions with residues such as Trp86, Trp286, or Tyr337 (described for donepezil and huperzine A; Figures 3(a), 3(b), 3(e), and 3(f)) are foreseen as being important for the AChE inhibitory ability of flavones.

4. Conclusions

AD is the most prevalent form of dementia and it is known that the malfunctions of different, but interconnected, biochemical complex pathways are related to this pathogenesis. Although inhibition of AChE is one of the most accepted therapy strategies for AD, the AChE inhibitors that have been approved for commercial use show lack of selectivity for AChE and AD patients suffer from side-effects. Therefore,
Figure 3: AChE active site (surface) bound to crystallographic donepezil (pdb ID: 4EY7) (a), galantamine (pdb ID: 4EY6) (c), and huperzine A (pdb ID: 4EY5) (e) (blue sticks). Residues involved in hydrogen interactions (yellow broken line), π stacking interactions (yellow double arrow), and π-cation interactions (orange double arrow) are displayed using a stick model. AChE carbon, oxygen, nitrogen, and hydrogen are represented in green, red, blue, and grey, respectively. 2D depiction view of crystallographic donepezil (b), galantamine (d), and huperzine A (f) in AChE active site. Hydrogen and π stacking are represented with the same color scheme. Receptor residues that are close to the ligand, but whose interactions with the ligand are weak or diffuse, such as collective hydrophobic or electrostatic interactions, are also represented (all the ones that have no indication for hydrogen-bonding). Solvent accessible surface area of the ligand is plotted directly onto the atoms in the form of a blue smudge. Solvent accessible surface area for the receptor residues is plotted as a blue halo.

this multifactorial disease requires new therapeutic strategies. Considering that concept, the present work focused on obtaining new AChE inhibitors with antioxidant activity based on the xanthonic and flavonic scaffolds. From this study, the Mannich base derivatives 3 and 14 emerged as dual agents with AChE inhibition and antioxidant activity. The synthesis of salt form of these amines to obtain bioactive water-soluble compounds will allow further investigating their potential as dual AChE inhibitors and antioxidants [64].

Conflicts of Interest
The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors’ Contributions
Inês Cruz and Ploenthip Puthongking performed the synthesis, purification, and the evaluation of biological activity
Figure 4: AChE active site (surface) bound to 2 (pink sticks) and 3 (purple sticks) (pdb ID: 4EY7) (a); residues involved in hydrogen interactions (yellow broken line), π stacking interactions (yellow double arrow), and π-cation interactions (orange double arrow) are displayed using a stick model. AChE carbon, oxygen, nitrogen, and hydrogen are represented in green, red, blue, and grey, respectively; 2D depiction view of 2 (b) and 3 (c) docked into AChE active site. Hydrogen, π stacking, and π-cation are represented with the same color scheme. Receptor residues that are close to the ligand, but whose interactions with the ligand are weak or diffuse, such as collective hydrophobic or electrostatic interactions, are also represented (all the ones that have no indication for hydrogen-bonding). Solvent accessible surface area of the ligand is plotted directly onto the atoms in the form of a blue smudge. Solvent accessible surface area for the receptor residues is plotted as a blue halo.

(both contributed equally to this work); Sara Cravo contributed to the structure elucidation of compounds; Andreia Palmeira performed the docking studies; Honorina Cidade and Madalena Pinto conceived and designed the work; Honorina Cidade, Madalena Pinto, and Emília Sousa contributed to acquisition, analysis, and interpretation of data and wrote the paper. All authors read, critically revised, and approved the final manuscript.

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