Synthesis, antioxidant and Aβ anti-aggregation properties of new ferulic, caffeic and lipoic acid derivatives obtained by the Ugi four-component reaction

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ARTICLE INFO

Keywords:

Aβ1–40 self-aggregation inhibition
Antioxidants
Benzylamine
Benzyl isocyanide
Caffeic acid
Ferulic acid
(R)-Lipoic acid
Multicomponent reactions
Ugi four-component reaction

ABSTRACT

We report herein the synthesis antioxidant and Aβ anti-aggregation capacity of (E)-N-benzyl-N-[2-(benzylamino)-2-oxoethyl]-3-(aryl)acrylamides and related (R)-N-benzyl-N-(2-(benzylamino)-2-oxoethyl)-5-(1,2-dithiolan-3-yl)pentanamides 1–12. These compounds have been obtained, via Ugi four-component reaction, from modest to good yields. Their antioxidant analysis, using the DPPH and ORAC assays, allowed us to identify compounds 8 and 9, as potent antioxidant agents, showing also strong Aβ1–40 self-aggregation inhibition, two biological properties of interest in pathologies linked to the oxidative stress, such as Alzheimer’s disease.

1. Introduction

Over the last decade, we have been actively working in the design, synthesis and pharmaceutical evaluation of diversely functionalized pyridine[1] and quinolone-substituted derivatives [2–4], as multitarget small molecules (MTSM) [5,6] for the potential treatment of Alzheimer’s disease (AD) [7], using in some cases selected multicomponent reactions (MCR) [8–10], as suitable synthetic protocols.

Among the many neuropathological AD hallmarks (e.g. cholinergic deficit, intracellular neurofibrillary tangles and amyloid-β (Aβ) deposits), oxidative stress plays a fundamental role in the biological events leading to this neurodegenerative disease [11]. For instance, the histopathological data obtained from extracellular Aβ deposits have revealed that the release of reactive oxygen species (ROS) can heavily damage the mitochondria and other cellular contents of the neurons [12,13]. Taking into account these observations, the search for new and more efficient antioxidants for the therapy of AD has been very intense over the last years [14].

From a synthetic angle, MCRs have been now established as powerful synthetic tools for creating molecular complexity and diversity-oriented synthesis of various heterocycles [15–18]. Among the known MCRs, the isocyanide-based Ugi four-component reaction (U-4CR) [19] allows the condensation, in one-step, of isocyanides, aldehydes, primary amines and carboxylic acids (Scheme 1). Recently, D’Arrigo and colleagues have reported the multicomponent synthesis of polyphenol-based α-acylamino carbamboxamides mimicking natural polyphenols (e.g. quercetin, resveratrol, (+)-epigallocatechin gallate) that were able to inhibit Aβ oligomerization [20]. The polyphenolic nature of these adducts allows the disruption of protein-protein interactions, thus hampering the aggregation of Aβ fibrils. One can also foresee that this set of polyphenol-based Ugi adducts could also have antioxidant activity, even if no data regarding this matter have been reported.

In this context, we have recently embarked in a project targeted to the synthesis of (E)-N-benzyl-N-[2-(benzylamino)-2-oxoethyl]-3-(aryl) acrylamides of type I bearing phenyl, pyridine and quinoline motifs (Scheme 1), assembled by U-4CR [19], where we have incorporated...
typical antioxidant motifs from the corresponding naturally-occurring ferulic (FA), caffeic (CA) and lipoic (LA) acids, that have been shown to be effective antioxidant agents [21,22]. In addition, we have incorporated halogens in our design due to their capacity to interact with a number of biological targets as hydrophobic moieties, to form halogen bonds with suitable functional motifs, [23,24] or increase their antioxidant activity [25], hypothesizing that the new synthetic derivatives would enhance the in vitro antioxidant effect compared to the parent acids.

Thus, in this report we describe the synthesis of adducts 1–12 (Scheme 1), and their antioxidant properties using the DPPH and ORAC assays. As a result, we have identified adducts 8 and 9 as potent antioxidant agents showing in addition strong A\beta_{1−40} self-aggregation inhibition.

2. Results and discussion

2.1. Synthesis

As shown in scheme 1, product 1 was obtained from FA, formaldehyde, benzyl isocyanide, and benzylamine. The use of FA/benzyl isocyanide/benzylamine afforded molecules 2–7, when using quinoline-3-carbaldehyde, 2-chloroquinoline-3-carbaldehyde, quinoline-4-carbaldehyde, 2-bromonicotinaldehyde, benzaldehyde, and 2-chlorobenzaldehyde, respectively. Similarly, the U-4CR of CA/benzyl isocyanide/benzylamine afforded the molecules 8–10, when quinoline-3-carbaldehyde, 2-chloroquinoline-3-carbaldehyde, and quinoline-4-carbaldehyde, respectively, have been reacted. Finally, selected and related analogues to adducts 1 and 8, such as 11 and 12 have been prepared by U-4CR between \((R)-LA\), formaldehyde (or quinoline-3-carbaldehyde), benzyl isocyanide, and benzylamine.

As described below and following the general protocol for the U-4CR, by stirring the solution of reagents in methanol, at room temperature (rt) for 24 h, the Ugi adducts were easily isolated by recrystallization from suitable solvents or flash column chromatography. All new compounds gave analytical and spectroscopic data in good agreement with their structures (see Section 5).

2.2. Antioxidant analysis

With pure compounds 1–12 in hand, we next tested their antioxidant capacity using the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) [26] and the Oxygen Radical Capacity (ORAC) [27] methods. These assays are well-established techniques for determining the radical scavenging activity (RSA) of a compound. ROS, such as the hydroxyl or superoxide radicals, cause lipid peroxidation, protein oxidation and DNA damage which can lead to organelles malfunction and further cellular disorders.
The antioxidant power of compounds 1–12 was then evaluated using the DPPH test, using FA, CA and LA as positive controls. As shown in Fig. 1, at 200μM dose, the investigated compounds showed a RSA percentage ranging from 7.4 for 11 to 89.1 for 8. Compounds 1–7 were more active than LA (9.1%), but still less active than FA and CA. Molecules 8–10, bearing a CA motif, showed a RSA percentage higher than 77%, being 8 the most active compound, 2.3 and 1.5-fold more active than FA and CA, respectively. Regarding structure-activity relationship (SAR), the most potent antioxidant compounds were those bearing CA followed by adducts bearing a feruloyl motif and, finally, those bearing a lipoyl moiety. For FA compound’ss, it seems that those bearing phenyl (6), 2-chlorophenyl (7) or without substituent (1) are better antioxidant than their analogues 2–5 while in the caffeic group the quinoline attached by its C3 (8) give the best effect and was thus the most antioxidant.

The ORAC test afforded the results shown in Fig. 2, expressed as Trolox equivalents (TE), in relation to radical scavenging properties of Trolox. Compounds 1–7 bearing a feruloyl motiff showed good radical scavenging properties with ORAC values, ranging from 2.19 TE for compound 6 to 3.48 TE for product 7, but remaining less active than FA. However, for caffeoyl-containing molecules 8–10, the ORAC values were equal to 5.48 TE, 6.86 TE and 4.98 TE, respectively, being more active than FA (3.7 TE). Lipoyl-containing compounds 11 and 12 gave low ORAC values as similarly observed in the DPPH assay. Regarding the SAR, the same trend observed in the DPPH test was found in the ORAC analysis, as the CA compounds are better antioxidants than those with FA and LA. From the FA group, compound 7, bearing 2-chlorobenzene motif, favourably compared to the other six ones, which did not show meaningful difference activity, regardless of the substituent incorporated. For the CA substituted quinolines 8–10 the presence of the chlorine atom at C2 in compound 9 is key to have a higher ORAC antioxidant effect.

### 2.3. Inhibition of Aβ1–40 aggregation by compounds 7–10

The four best antioxidants 7–10 were then evaluated as inhibitors of self Aβ1–40 aggregation at 20μM, curcumine being used as positive control. As shown in Fig. 3, the feruloyl-containing compound 7 has a very low activity as inhibitor of Aβ1–40 self-aggregation, but the three molecules bearing caffeoyl motif 8–10 displayed a strong inhibition
2.4. Neuroprotective activity of compounds 8–10 on H2O2 (150 µM)-induced cell death in SH-SY5Y cells

The ability to prevent the human neuroblastoma cell line SH-SY5Y cell death induced by hydrogen peroxide (H2O2) for the generation of exogenous free radicals, was then assayed to determine the neuroprotective capacity of the three most balanced antioxidants and Aβ1–40 self aggregation inhibitors, compounds 8–10, at non-toxic concentration 10 µM, against SH-SY5Y. As shown in table 1, only compound 9 showed a significant neuroprotection around 25% against H2O2 insult at 10 µM.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Dose</th>
<th>H2O2 (%)</th>
</tr>
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<tbody>
<tr>
<td>8</td>
<td>10 µM</td>
<td>np</td>
</tr>
<tr>
<td>9</td>
<td>10 µM</td>
<td>21.87 ± 0.01 *</td>
</tr>
<tr>
<td>10</td>
<td>10 µM</td>
<td>np</td>
</tr>
</tbody>
</table>

Data are expressed as % neuroprotection ± SEM of quadruplicates from at least three different cultures.
np: not protective.

* p < 0.05, as compared to the control cultures (one-way ANOVA).

Table 1
Neuroprotective activity of compounds 8–10 on H2O2 (at 150 µM)-induced cell death in SH-SY5Y cells.

Fig. 3. Inhibition of Aβ1–40 self-aggregation (%) by compounds 7–10. The Thioflavin-T fluorescence method was used. The values are expressed as the mean ± SD of at least three independent measurements. All values were obtained at a compound concentration of 20 µM.
4.2.1. (E)-N-Benzyl-N-(2-(benzylamino)-2-oxo-1-(quinolin-3-yl)ethyl)-3-(4-hydroxy-3-methoxyphenyl)acrylamide (1)

Following the general procedure, a solution of benzylamine (110 µL, 1 mmol), paraformaldehyde (30 mg, 1 mmol), ferulic acid (194 mg, 1 mmol) and benzyl isocyanide (120 µL, 1 mmol) in MeOH (1 mL) gave, after flash column chromatography [cyclohexane/AcOEt (7:3, v/v)], adduct 1 (305 mg, 71%) as a white foam: mp 163-4°C; IR (ATR) ν 3258, 3061, 1582, 1509 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) δ 7.70 (d, J = 15.0 Hz, 1H, H-c), 7.36-7.09 (m, 10H), 7.03-7.00 (m, 2H), 6.92-6.86 (m, 2H), 6.69 and 6.54 (d, J = 15.0 Hz, 1H, H-b), 6.06 (bs, 1H, NH), 4.80 and 4.65 (s, 2H, H-d), 4.44 and 4.31 (s, 2H, H-g), 4.12 (s, 2H, H-e), 3.86 (s, 3H, OMe); ¹³C NMR (CDCl₃, 75 MHz) δ 169.3 (C-i), 168.5 (C-a), 148.0, 147.0, 145.0 (C-c), 136.4, 129.2, 128.8, 128.2, 127.7, 127.5, 126.9, 122.6, 115.0 (C-b), 113.8, 110.2, 52.8 (C-e), 51.5 (C-d), 43.6 (C-g). Anal. Calcd. for C₃₅H₃₁N₃O₄: C, 75.39; H, 5.60; N, 7.54. Found: C, 75.20; H, 5.47; N, 7.66.

4.2.2. (E)-N-Benzyl-N-(2-(benzylamino)-2-oxo-1-(quinolin-3-yl)ethyl)-3-(4-hydroxy-3-methoxyphenyl)acrylamide (2)

Following the general procedure, a solution of benzylamine (110 µL, 1 mmol), 3-quinoline carboxaldehyde (157 mg, 1 mmol), ferulic acid (194 mg, 1 mmol) and benzyl isocyanide (120 µL, 1 mmol) in MeOH (1 mL) gave, after recrystallization with Et₂O with few drops of MeOH, adduct 2 (317 mg, 68%) as a white solid: mp 183-4°C; IR (ATR) ν 3260, 3058, 1646 1576, 1512 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) δ 8.79 (d, J = 2.2 Hz, 1H, 8.29 (bs, 1H, OH), 8.0 (d, J = 8.0 Hz, 1H), 7.73-7.66 (m, 3H + H-e), 7.52 (t, J = 7.0 Hz, 1H), 7.28-7.26 (m, 5H), 7.12-7.03 (m, 5H + H-b), 6.94 (dd, J = 1.5 Hz, J = 8.0 Hz, 1H), 6.84 (d, J = 8.0 Hz, 1H), 6.77 (d, J = 1.5 Hz, 1H), 6.59 (d, J = 15.4 Hz, 1H, H-b), 6.34 (bs, 1H, NH), 5.99 (s, 1H, H-e), 4.92 (d, J = 17.4 Hz, 1H, H-d), 4.77 (d, J = 17.4 Hz, 1H, H-d), 4.57 (dd, J = 14.1 Hz, 1H, H-g), 4.47 (dd, J = 14.1 Hz, J = 5.4 Hz, 1H, H-g), 4.31 (s, 3H, OMe); ¹³C NMR (CDCl₃, 75 MHz) δ 169.2 (C-i), 169.0 (C-a), 151.6, 148.1, 147.8, 147.0, 145.1 (C-c), 137.8, 137.0, 130.2, 129.4, 129.0, 128.9, 128.3, 128.0, 127.8, 127.2, 126.7, 122.9, 115.3, 115.0 (C-b), 110.1, 61.0 (C-e), 56.2 (OMe), 50.5 (C-d), 44.1 (C-g). Anal. Calcd. for C₃₉H₃₅N₂O₂C, 75.39; H, 5.60; N, 7.54. Found: C, 75.20; H, 5.54; N, 7.41.

4.2.3. (E)-N-Benzyl-N-(2-(benzylamino)-2-oxo-1-(2-chloroquinolin-3-yl)ethyl)-3-(4-hydroxy-3-methoxyphenyl)acrylamide (3)

Following the general procedure, a solution of benzylamine (110 µL, 1 mmol), 2-bromonicotinaldehyde (186 mg, 1 mmol), ferulic acid (194 mg, 1 mmol) and benzyl isocyanide (120 µL, 1 mmol) in MeOH (1 mL) gave, a solid that after washing with methanol (10 mL) and n-hexane (2 × 20 mL), afforded adduct 5 (451 mg, 77%) as a white solid:
mp 169–171 °C; IR (ATR) ν 3220, 3045, 1667 1530, 1519 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) δ 8.13–8.02 (m, 2H), 7.68 (d, J = 15.0 Hz, 1H, H-c), 7.26–7.11 (m, 11H), 6.93 (m, 2H), 6.84–6.79 (m, 2H), 6.61 (d, J = 15.0 Hz, 1H, H-b), 6.40 (bs, 1H, OH), 6.20 (d, J = 15.0 Hz, 1H, H-a), 4.91 (d, J = 17.0 Hz, 1H, H-d), 4.72 (d, J = 17.0 Hz, 1H, H-d), 4.53–4.47 (m, 1H, H-g), 4.37–4.32 (m, 1H, H-g), 3.80 (s, 3H, OMe); ¹³C NMR (CDCl₃, 75 MHz) δ 168.8, 168.6, 166.8, 165.8, 165.7, 154.2 (C-c), 139.9, 137.8, 137.2, 132.4, 128.8, 128.7, 127.9, 127.6, 126.5, 122.8, 115.0, 114.8 (C-b), 110.1, 61.5 (C-e), 56.1 (OMe), 49.5, 44.0 (C-g). Anal. Calcd. for C₃₉H₃₉BrN₂O₄C: C, 71.04; H, 5.40; N, 5.18. Found: C, 71.18; H, 5.32; N, 5.23.

4.2.8. (E)-N-Benzyl-N-(2-(benzylamino)-2-oxo-1-(quinolin-3-yl)ethyl)-3-(3,4-di-hydroxy phenyl) acrylamide (8)

Following the General procedure, a solution of benzylamine (110 µL, 1 mmol), 3-quinolinecarboxaldehyde (157 mg, 1 mmol), caffeic acid (180 mg, 1 mmol) and benzyl isocyanide (120 µL, 1 mmol) in MeOH (1 mL) gave, after recrystallization from Et₂O/CH₂Cl₂ (8:2, v/v), adduct 6 (737 mg, 73%) as a white solid: mp 166–7 °C; IR (ATR) ν 3355, 3022, 1674, 1634, 1499, 1419 cm⁻¹; ¹H NMR (acetone-d₆, 300 MHz) δ 8.84 (s, 1H), 8.27–8.22 (m, 3H + 2OH), 8.03 (d, J = 6.0 Hz, 1H), 7.93 (d, J = 8.1 Hz, 1H), 7.83 (d, J = 8.1 Hz, 1H), 7.70 (dd, J = 7 Hz, J = 1.2 Hz, 1H), 7.63–7.52 (m, 2H, CH₃om + Cc), 7.31–7.19 (m, 5H), 7.08–6.99 (m, 6H), 6.89 (d, J = 8.1 Hz, 1H), 6.76 (m, 2H, CH₃om + Bc), 6.53 (s, 1H, H-e), 5.12 (d, J = 20.0 Hz, 1H, H-d), 4.86 (d, J = 20.0 Hz, 1H, H-d), 4.52 (dd, J = 20.0 Hz, J = 6.2 Hz, 1H, H-g), 4.42 (dd, J = 20.0 Hz, J = 6.2 Hz, 1H, H-g); ¹³C NMR (acetone-d₆, 75 MHz) δ 170.1 (C-f), 168.8 (C-a), 153.0, 148.5, 148.3, 146.3, 144.5 (C-c), 140.2, 139.9, 137.4, 137.4, 137.3, 137.3, 130.6, 130.5, 129.9, 129.3, 129.1, 128.7, 128.6, 128.5, 127.9, 127.6, 127.2, 116.5, 116.4, 115.2 (C-b), carbon signal of C-e missing, 50.4 (C-d), 44.1 (C-g). Anal. Calcd. for C₃₉H₃₉N₂O₄C: C, 75.12; H, 5.38; N, 7.73. Found: C, 75.29; H, 5.43; N, 7.79.

4.2.9. (E)-N-Benzyl-N-(2-(benzylamino)-1-(2-chloroquinolin-3-yl)-2-oxoethyl)-3-(3,4-di-hydroxyphenyl) acrylamide (9)

Following the general procedure, a solution of benzylamine (110 µL, 1 mmol), 2-chloro-3-quinolinecarboxaldehyde (191 mg, 1 mmol), caffeic acid (180 mg, 1 mmol) and benzyl isocyanide (120 µL, 1 mmol) in MeOH (1 mL) gave, after washing with methanol (2 mL) Et₂O (10 mL), and n-hexane (10 mL) adduct 9 (358 mg, 62%) as a white solid: mp 159–161 °C; IR (ATR) ν 3244, 3030, 1677, 1638, 1540, 1413 cm⁻¹; ¹H NMR (acetone-d₆, 300 MHz) δ 8.35 (s, 1H), 8.22–8.14 (m, 1H), 7.92 (m, 1H), 7.79–7.72 (m, 2H), 7.67–7.59 (m, 2H), 7.32–7.22 (m, 4H), 7.08–6.91 (m, 7H), 6.84–6.79 (m, 3H), 5.13 (d, J = 19.2 Hz, 1H), 4.89 (d, J = 19.2 Hz, 1H), 4.52–4.50 (dd, J = 15.2 Hz, J = 7.1 Hz, 1H, H-g), 4.43–4.47 (dd, J = 15.2 Hz, 7.1 Hz, 1H, H-g); ¹³C NMR (acetone-d₆, 75 MHz) δ 169.9 (C-f), 168.6 (C-a), 152.2, 148.3, 148.0, 146.3, 144.7 (C-c), 147.0, 147.0, 131.7, 129.7, 129.3, 129.0, 126.9, 128.7, 128.2, 127.9, 127.4, 127.2, 122.4, 116.5, 116.3, 115.2 (C-b), 60.2 (C-e), 50.4 (C-d), 44.1 (C-g). Anal. Calcd. for C₃₉H₃₉N₂O₄C: C, 70.64; H, 4.88; N, 7.27. Found: C, 70.69; H, 4.92; N, 7.33.
4.2.10. (E)-N-Benzyl-N-(2-(benzylamino)-2-oxo-1-(quinolin-4-yl)ethyl)-3-(3,4-dihydroxy phenyl)acrylamide (10)

Following the general procedure, a solution of benzylamine (110 µL, 1 mmol), 4-quinoilinecarboxaldehyde (157 mg, 1 mmol), ferulic acid (194 mg, 1 mmol) and benzyl isocyanide (120 µL, 1 mmol) in MeOH (1 mL), gave, after washing with methanol (2 mL), CH2Cl2 (10 mL), and n-hexane (10 mL), adduct 10 (280 mg, 69%) as a white solid: mp 187–8°C; IR (ATR) ν 3296, 3061, 2923, 1639, 1538, 1358 cm\(^{-1}\); \(^1\)H NMR (CDCl\(_3\), 300 MHz) δ 8.73 (d, \(J = 2.4\) Hz, 1H), 8.24 (s, 1H), 8.00 (d, \(J = 8.7\) Hz, 1H), 7.71–7.67 (m, 2H), 7.55–7.50 (t, \(J = 8.7\) Hz, 1H), 7.34–7.24 (m, 5H), 7.12–7.08 (m, 3H), 6.97–6.95 (m, 2H), 6.43 (t, \(J = 6.0\) Hz, 1H, NH), 6.14 (s, 1H, H-e), 4.77 (d, \(J = 17.0\) Hz, 1H, H-d), 4.63 (d, \(J = 17.0\) Hz, 1H, H-d), 4.56–4.50 (dd, \(J = 14.8\) Hz, 6.0 Hz, 1H, H-g), 3.48–3.46 (q, \(J = 6.7\) Hz, 2H, H-h), 3.20–3.17 (m, 2H), 2.99–2.95 (t, \(J = 5.2\) Hz, 2H, H-i); 13C NMR (CDCl\(_3\), 75 MHz) δ 174.4 (C-f), 169.1 (C-a), 141.89, 138.7, 137.9, 129.4, 129.0, 128.1, 127.3, 127.2, 126.9, 126.7, 126.6, 126.2, 125.9, 125.6, 125.3, 125.5, 123.6, 121.4, 121.0, 115.5, 114.3 (C-b), 56.0 (C-e), 48.5 (C-d), 42.4 (C-g). Anal. Calcd. for C\(_{33}\)H\(_{35}\)N\(_3\)O\(_4\): C, 69.49; H, 5.32; N, 7.80.

4.2.11. (R)-N-Benzyl-N-(2-(benzylamino)-2-oxoethyl)-5-(1,2-dithiolan-3-yl)pentanamide (11)

Following the general procedure, a solution of benzylamine (110 µL, 1 mmol), paraformaldehyde (30 mg, 1 mmol), lipic acid (206 mg, 1 mmol) and benzyl isocyanide (120 µL, 1 mmol) in MeOH (1 mL), was automatically shaken prior to each reading. All the reaction were made in triplicate and at least three different assays were performed for each sample. Antioxidant curves (fluorescence versus time) were first normalized to the curve of the blank (without antioxidant) and then, the area under the fluorescence decay curve (AUC) was calculated as: AUC = 1 + \(\sum (f_i/f_0)\), where \(f_0\) is the initial fluorescence reading at 0 min and \(f_i\) is the fluorescence value at time \(i\).

The net AUC corresponding to a sample was calculated as follows:

\[
\text{Net AUC} = \text{AUC}_{\text{antioxidant}} - \text{AUC}_{\text{blank}}
\]

Regression equations were calculated by plotting the net AUC against the antioxidant concentration. The ORAC value was obtained by dividing the slope of the latter curve between the slope of the Trolox curve obtained in the same assay. Final ORAC values were expressed as trolox equivalents (µmol of Trolox equivalent/µmol of adduct). Data are expressed as means ± SD.
4.5. Inhibition of \( \alpha_\text{B} \)-40 aggregation

For the inhibition of self-mediated \( \alpha_\text{B} \)-40 aggregation experiment, the \( \alpha_\text{B} \) stock solution was diluted with 50 mM phosphate buffer (pH 7.4) to 50 μM before use. A mixture of the peptide (10 μL, 25 μM, final concentration) with or without the tested compound (10 μL, 20 μM, final concentration) was incubated at 37°C for 48 h. Blanks using 50 mM phosphate buffer (pH 7.4) instead of \( \alpha_\text{B} \) with or without inhibitors were also carried out. The sample was diluted to a final volume of 200 μL with 50 mM glycine-NaOH buffer (pH 8.0) containing thioflavin T (5 μM). Then the fluorescence intensities were recorded five minutes later (excitation, 450 nm; emission, 485 nm). The percent inhibition of aggregation was calculated by the expression:

\[
\text{inhibition of aggregation} = \frac{1 - (IFi/IFc)} \times 100%,
\]

in which IFi and IFc are the fluorescence intensities obtained for Aβ in the presence and absence of inhibitors after subtracting the background, respectively.

4.6. Effect of compounds 8–10 on \( \text{H}_2\text{O}_2 \) (150 μM)-induced cell death in SH-SY5Y cells

SH-SY5Y cells were seeded in 96-well culture plates at a density of 8 x 10^4 cells per well in DMEM/F12 (1:1) medium supplemented with 10% fetal bovine serum, 1 x non-essential amino acids, 100 units/mL penicillin and 10 mg/mL streptomycin (Dutsch, France). After 48 h of incubation, the cultures were treated with 100 μL of the test compounds or DMSO (0.1%) in the same medium. Following 24 h, the cells were co-incubated with H2O2 (150 μM) with or without the tested compounds at noncytotoxic concentrations for an additional period of 24 h. The percent of cell viability was measured using CellTiter 96 AQueous Non-Radioactive Cell Proliferation (MTS) Assay (Promega, France).

Acknowledgments

LI thanks the Regional Council of Franche-Comté (2016YC-04540 and 04560) for financial support and for the award of a PhD grant to MB.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2018.12.029.

References


