Synthesis of 7-O-(2-Amino)ethyl Flavones and Their Antioxidant Activities

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It is becoming apparent that inadvertent overproduction of reactive oxygen species may result in oxidative tissue injury. Reactive oxygen species have been implicated in a variety of diseases including cardiovascular disease, ischemia, Alzheimer’s disease, and chronic gut inflammation. Fortunately, plants contain a wide variety of free radical scavenging molecules such as flavonoids, anthocyanins, carotenoids, dietary glutathione and vitamins. The diverse biological properties of flavonoids are suggested mainly due to their antioxidant activity by scavenging oxygen radicals and inhibiting lipid peroxidation. However, the antioxidant activity of the flavonoids varies considerably depending on their backbone structures as well as their substituents.

Luteolin (1) is one of the naturally occurring, common flavones which possesses potent antioxidant activities (Figure 1). However, the in vivo biological activities of luteolin depend on many parameters, including bioavailability. Low solubility of luteolin in oil results in its poor permeation across cell membranes. Thus, a luteolin and its phospholipid complex was prepared with the aim to improve the lipophilic properties while retaining its DPPH radical scavenging activity. Based on studies examining structure-activity relationships, the lower solubility of flavonoids in aqueous solution can also limit their bioavailability in vivo. To enhance water solubility over the parent compounds and thereby improving hydrophilic character, complexation of luteolin with cyclodextrins, and synthesis of sodium salt and ammonium salts of flavones have been reported along with investigation of their antioxidant properties. We also recently reported the synthesis of glucose-containing flavones (e.g. 2) in an attempt to improve the physicochemical properties including water solubility. The attachment of a glucose group to luteolin increased superoxide anion scavenging activities, but diminished lipid peroxidation inhibitory activities. We assumed that the reduced inhibition of lipid peroxidation was caused by the increased hydrophilic character. In this work, we describe the synthesis of 7-O-aminoethyl flavones 3a-3h and their antioxidant activities. We introduced amino groups to the structure of luteolin to increase water solubility with formation of hydrochloride salts. It was also thought that the alkyl groups in aminoalkyl flavones can also improve the lipophilic character by increasing alkyl chains and thereby enhance lipid peroxidation inhibitory activities. Aminoethyl was attached at the C-7 position of 1 via an ether bond because we previously found that modification at this position did not substantially influence antioxidant activity. The B-ring of luteolin was also varied by the introduction of two or three hydroxyl groups to examine the influence on antioxidant activity.

The synthesis of 7-O-aminoethyl flavones 3a-3h were accomplished by the procedures shown in Scheme 1. 1-(2-Hydroxy-4,6-bis(methoxymethoxy)phenyl)ethanone (4) was condensed with O-benzyl-protected aldehydes (5a, 5b) in the presence of NaH in DMF to provide the chalcones 6a and 6b. Oxidative cyclization of the chalcones to form flavone ring was accomplished by treatment with PIDA/KOH in methanol to afford 7a and 7b. The MOM-protecting group in 7a and 7b was removed using 10% HCl in methanol to afford 8a and 8b. The selective alkylation of 7-hydroxyl in 8a and 8b was performed using commercially available aminoethyl chlorides in the presence of K2CO3 in aceton to give 9a-9h. Finally, the benzyl-protecting groups in 9a-9h were removed by hydrogenolysis with Pd(OH)2 and cyclohexene in EtOH and then transformed to 3a-3h as hydrochloride salts by treating with HCl gas in ethanol at 0°C.

It has been reported that the low solubility of flavones both in oil and aqueous solution can limit their bioavailabilities in vivo. In the present study, the aminoethyl moiety
was introduced to the 7-position of luteolin via an ether bond to improve its physicochemical properties and enhance antioxidant activity. An additional hydroxyl group was also introduced in the 5'-position of the B-ring in luteolin to examine its influence on antioxidant activities. The antioxidant activities of the synthesized flavones 3a-3h were

**Table 1.** Yields and antioxidant activities of aminoethyl flavones 3a-3h

<table>
<thead>
<tr>
<th>Entry</th>
<th>R&lt;sup&gt;1&lt;/sup&gt;</th>
<th>R&lt;sup&gt;2&lt;/sup&gt;R&lt;sup&gt;3&lt;/sup&gt;N-</th>
<th>Overall yields (%) from 8a or 8b</th>
<th>DPPH radicals&lt;sup&gt;a&lt;/sup&gt;</th>
<th>O&lt;sub&gt;2&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt; radicals&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Lipid peroxidation&lt;sup&gt;c&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>3a</td>
<td>H</td>
<td>N</td>
<td>46</td>
<td>25.90 ± 0.48</td>
<td>3.10 ± 0.81</td>
<td>38.76 ± 2.42</td>
</tr>
<tr>
<td>3b</td>
<td>H</td>
<td>N</td>
<td>35</td>
<td>27.41 ± 3.24</td>
<td>3.45 ± 0.19</td>
<td>18.79 ± 4.07</td>
</tr>
<tr>
<td>3c</td>
<td>H</td>
<td>N</td>
<td>41</td>
<td>52.74 ± 5.34</td>
<td>3.07 ± 0.30</td>
<td>18.01 ± 2.50</td>
</tr>
<tr>
<td>3d</td>
<td>H</td>
<td>N</td>
<td>30</td>
<td>28.82 ± 2.31</td>
<td>3.22 ± 0.26</td>
<td>18.34 ± 1.99</td>
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<tr>
<td>3e</td>
<td>OH</td>
<td>N</td>
<td>38</td>
<td>19.51 ± 1.12</td>
<td>26.03 ± 12.89</td>
<td>15.11 ± 2.31</td>
</tr>
<tr>
<td>3f</td>
<td>OH</td>
<td>N</td>
<td>43</td>
<td>26.34 ± 0.52</td>
<td>30.12 ± 7.38</td>
<td>19.27 ± 1.61</td>
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<tr>
<td>3g</td>
<td>OH</td>
<td>N</td>
<td>48</td>
<td>41.89 ± 5.55</td>
<td>19.65 ± 3.58</td>
<td>19.52 ± 1.10</td>
</tr>
<tr>
<td>3h</td>
<td>OH</td>
<td>N</td>
<td>45</td>
<td>36.31 ± 5.55</td>
<td>17.56 ± 9.18</td>
<td>20.80 ± 1.34</td>
</tr>
<tr>
<td>1</td>
<td>luteolin</td>
<td></td>
<td>16.18 ± 1.85</td>
<td>8.66 ± 0.10</td>
<td>24.66 ± 4.75</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td>17.28 ± 0.27</td>
<td>3.28 ± 0.20</td>
<td>40.41 ± 5.75</td>
<td></td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td></td>
<td></td>
<td>40.99 ± 6.19</td>
<td>&gt; 50</td>
<td></td>
<td>71.44 ± 5.54</td>
</tr>
</tbody>
</table>

<sup>a</sup>DPPH radical scavenging activity. <sup>b</sup>Scavenging activity of superoxide anion radicals generated in the xanthine/xanthine oxidase system. <sup>c</sup>Iron-dependent lipid peroxidation inhibition activity using rat liver homogenate. <sup>d</sup>IC<sub>50</sub> values (defined as concentrations that inhibited activity by 50%) were calculated using GraphPad Prism using data obtained from at least three independent experiments and expressed as the means ± SD.

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**Scheme 1.** Reagents and conditions: (a) NaH, DMF, 0 °C ~ rt; (b) PIDA, KOH, MeOH, 0 °C ~ rt; (c) 10% HCl, MeOH, 60 °C; (d) R'R'NCH<sub>2</sub>Cl, K<sub>2</sub>CO<sub>3</sub>, acetone, reflux; (e) (i) Pd(OH)<sub>2</sub>/C, EtOH/cyclohexene (1/1), reflux; (ii) HCl (gas), EtOH.
evaluated by examining their effects on DPPH and superoxide anion radical scavenging and inhibition of lipid peroxidation (Table 1).\(^5\) Ascorbic acid and trolox were used as positive controls in our assay systems. The antioxidant activity data of the parent compound, luteolin (1) and 7-O-glucosyl-luteolin (2) were also included as positive controls to assess whether antioxidant activities were maintained or increased by these aminoethyl flavones. As shown in Table 1, the synthesized compounds showed varied DPPH radical scavenging activities (IC\(_{50}\) = 19.51-52.74 μM) that were similar to or 2-3-fold less potent than those of the parent compounds 1 and 2 (IC\(_{50}\) = 16.18 and 17.28 μM, respectively). The superoxide anion radical scavenging activities of 3a-3h were examined using a xanthine/xanthine oxidase system.\(^2\) Compounds 3a-3d exhibited potent superoxide anion scavenging activities (IC\(_{50}\) = 3.07-3.45 μM) and their potencies were higher than that of 1 (IC\(_{50}\) = 8.66 μM) and similar to that of 2 (IC\(_{50}\) = 3.28 μM). Interestingly, these activities were 2-3 times lower in compounds 3e-3h, indicating that an additional hydroxyl group in the 5’-position of the B-ring can alter superoxide anion scavenging activities significantly. The lipid peroxidation inhibitory effects of synthesized compounds were evaluated using rat liver homogenates.\(^3\) In our previous work, it has been found that glycosylation of luteolin reduced lipid peroxidation inhibitory activity slightly.\(^4\) However, every aminoethyl-flavones 3a-3h exhibited enhanced activities when compared to 1 and 2. The different amino groups at the 7-position and the degree of hydroxylation on the B-ring have no significant effects on lipid peroxidation inhibitory activity as suggested by similar activity of all compounds.

In conclusion, we synthesized aminoethyl flavones 3a-3h to increase the hydrophilic and lipophilic characteristics of luteolin while retaining antioxidant activity. The synthesized compounds showed DPPH radical scavenging activities, but their potencies were slightly lower than those of the parent compounds. However, compounds 3a-3d, which possess two hydroxyl groups in the 3’,4’-positions of the B-ring had significantly increased superoxide anion radical scavenging activities. Interestingly, the introduction of an additional hydroxyl group in the 5’-position of the B-ring of the flavones decreased these activities significantly. Irrespective of the types of amino groups examined and the degree of hydroxylation on the B-ring, almost all aminoethyl flavones exhibited potent lipid peroxidation inhibitory activities. Of the synthesized compounds, 3b and 3d exhibited the most potent and well-balanced antioxidant activities in the three antioxidant assay systems. Taken together, these findings suggest that the introduction of the aminoethyl moiety to luteolin may improve pharmacokinetic properties as well as antioxidant activities although their activities in cell-based assay systems remain to be investigated.

Experimental

\(^1\)H and \(^13\)C NMR spectra were recorded on a Gemini Varian-300 (300 and 75 MHz, respectively). Analytical thin layer chromatographies (TLC) were carried out by precoated silica gel (E. Merck Kieselgel 60F\(_{254}\), layer thickness 0.25 mm). Flash column chromatographies were performed with Merck Kieselgel 60 Art 9385 (230-400 mesh). All solvents used were purified according to standard procedures.

3-(3,4-Bis-benzoyloxy-phenyl)-1-(2-hydroxy-4,6-bis-methoxymethoxy-phenyl)-propene (6a): To a stirred solution of 4 (1.6 g, 6.24 mmol) in DMF (47 mL) was added NaH (60% dispersed in mineral oil, 0.98 g, 25.0 mmol). To this solution was added a solution of 5a (2.36 g, 7.4 mmol) in DMF (20 mL) dropwise at 0 °C. After stirring for 3 h at rt, the reaction was carefully quenched with water. DMF was evaporated under vacuum and the residue was dissolved in EtOAc before washing with water and brine. The organic layer was dried over anhydrous MgSO\(_4\), filtered, concentrated, and crystallized from EtOH to give 6a (3.33 g, 96% yield).\(^3\) \(^1\)H NMR (CDCl\(_3\)) \(\delta\) 7.80 (1H, \(J = 15.3\) Hz), 7.31 (1H, \(J = 15.3\) Hz), 7.35-7.50 (10H, m), 7.19-7.24 (2H, m), 6.99 (1H, d, \(J = 8.4\) Hz), 6.35 (1H, d, \(J = 2.1\) Hz), 6.27 (1H, d, \(J = 2.1\) Hz), 5.26 (2H, s), 5.25 (2H, s), 5.24 (2H, s), 5.22 (2H, s), 3.53 (3H, s), 3.52 (3H, s).

1-(2-Hydroxy-4,6-bis-methoxymethoxy-phenyl)-3-(3,4,5-tris-benzyloxy-phenyl)-propene (6b): Compound 6b was prepared from 4 and 5b using the procedure described for 6a. Yield 90%; \(^1\)H NMR (CDCl\(_3\)) \(\delta\) 7.83 (1H, d, \(J = 15.9\) Hz), 7.80 (1H, d, \(J = 15.9\) Hz), 7.29-7.47 (15H, m), 6.95 (1H, s), 6.94 (1H, s), 6.35 (1H, m), 6.24 (1H, m), 5.16-5.25 (8H, m), 3.51-3.52 (6H, m).

3’,4’-Dibenzyloxy-5,7-dimethoxymethyl-flavone (7a): To a stirred solution of the 6a (774 mg, 1.39 mmol) in MeOH (30 mL) was added a solution of KOH (583 mg, 10.39 mmol) in MeOH (10 mL) slowly at 0 °C. After stirring for 10 min, phenylidene diacetate (PIDA, 1.16 g, 3.6 mmol) was added in three portions and the resulting mixture was stirred at rt for 24 h. The solution was concentrated and water was added to the residue. The mixture was extracted with CH\(_2\)Cl\(_2\) and the combined organic layer was dried over anhydrous MgSO\(_4\), filtered, evaporated, and purified by flash column chromatography (EtOAc/n-hexane = 1:2) to afford 7a (263 mg, 37% yield).\(^3\) \(^1\)H NMR (CDCl\(_3\)) \(\delta\) 7.29-7.54 (12H, m), 7.03 (1H, d, \(J = 9.0\) Hz), 6.85 (1H, d, \(J = 2.4\) Hz), 6.77 (1H, d, \(J = 2.4\) Hz), 6.54 (1H, s), 5.37 (2H, s), 5.30 (2H, s), 5.27 (4H, s), 3.59 (3H, s), 3.56 (3H, s).

3’,4’,5’-Tribenzyloxy-5,7-dimethoxymethyl-flavone (7b): Compound 7b was prepared from 6b using the procedure described for 7a. Yield 61%; \(^1\)H NMR (CDCl\(_3\)) \(\delta\) 7.29-7.51 (15H, m), 7.18 (2H, s), 6.85 (1H, d, \(J = 2.4\) Hz), 6.79 (1H, d, \(J = 2.4\) Hz), 5.38 (2H, s), 5.31 (2H, s), 5.20 (4H, s), 5.17 (2H, s), 3.60 (3H, s), 3.58 (3H, s).

2-(3,4-Bis(benzyloxy)phenyl)-5,7-dihydroxy-4H-chromen-4-one (8a): A stirred solution of 7a (1.2 g, 2.16 mmol) in MeOH (150 mL) was treated with 10% hydrochloric acid (38 mL) and heated at reflux for 5 h. The reaction mixture was diluted with water and the resulting precipitate was separated and washed with MeOH to afford 8a (0.96 g, 94%). \(^1\)H NMR (DMSO-d\(_6\)) \(\delta\) 7.71 (1H, d, \(J = 2.0\) Hz), 7.65 (1H, dd, \(J = 2.0\) Hz), 7.62-7.52 (10H, m), 7.21 (1H, d, \(J = 2.0\) Hz), 7.29 (1H, d, \(J = 2.0\) Hz), 7.23 (1H, d, \(J = 2.0\) Hz), 7.20 (1H, d, \(J = 2.0\) Hz), 7.18 (1H, d, \(J = 2.0\) Hz), 7.15 (1H, d, \(J = 2.0\) Hz), 7.10 (1H, d, \(J = 2.0\) Hz), 7.05 (1H, d, \(J = 2.0\) Hz), 7.02 (1H, d, \(J = 2.0\) Hz), 6.98 (1H, d, \(J = 2.0\) Hz), 6.93 (1H, d, \(J = 2.0\) Hz), 6.89 (1H, d, \(J = 2.0\) Hz), 6.84 (1H, d, \(J = 2.0\) Hz), 6.77 (1H, d, \(J = 2.0\) Hz), 6.63 (1H, d, \(J = 2.0\) Hz), 6.50 (1H, d, \(J = 2.0\) Hz), 6.27 (1H, d, \(J = 2.0\) Hz), 6.18 (1H, d, \(J = 2.0\) Hz), 5.42 (2H, s), 5.27 (4H, s), 3.59 (3H, s), 3.56 (3H, s).

the procedure described for (DMSO-chromatography (CH concentrated. The residue was purified by flash column at 0 dissolved in absolute EtOH and then saturated with HCl gas filtered through Celite and concentrated. The residue was refluxed for the period of time (TLC monitoring), precipitate was filtered to afford phenyl)-4

1.74 (1H, m), 1.58-2.04 (4H, bs). 1.58-1.66 (2H, m).

7-(2-(Piperidin-1-yl)ethoxy)-5-hydroxy-2-(3,4,5-trihydroxy-phenyl)-4-chromen-4-one Hydrochloride (3b): Yield 35%; 1H NMR (CDOD) δ 7.46 (1H, d, J = 2.1 Hz), 7.43 (1H, dd, J = 8.4, 2.1 Hz), 6.94 (1H, d, J = 8.4 Hz), 6.78 (1H, d, J = 2.1 Hz), 6.64 (1H, s), 6.49 (1H, d, J = 2.1 Hz), 4.48 (2H, t, J = 5.1 Hz), 3.72 (2H, t, J = 5.1 Hz), 3.50 (4H, bs), 2.17-2.19 (4H, m).

7-(2-(Pyrrolidin-1-yl)ethoxy)-5-hydroxy-2-(3,4,5-trihydroxy-phenyl)-4-chromen-4-one Hydrochloride (3c): Yield 41%; 1H NMR (CDOD) δ 7.45 (1H, m), 7.42 (1H, m), 6.94 (1H, d, J = 8.4 Hz), 6.77 (1H, d, J = 2.4 Hz), 6.64 (1H, s), 6.49 (1H, d, J = 2.1 Hz), 4.54 (2H, bs), 3.99 (4H, bs), 3.68 (2H, bs), 3.44 (4H, bs).

7-(2-(Diethylamino)ethoxy)-5-hydroxy-2-(3,4,5-trihydroxy-phenyl)-4-chromen-4-one Hydrochloride (3e): Yield 30%; 1H NMR (CDOD) δ 7.43 (1H, m), 7.42 (1H, m), 6.94 (1H, d, J = 8.1 Hz), 6.76 (1H, d, J = 1.5 Hz), 6.63 (1H, s), 6.47 (1H, d, J = 1.5 Hz), 4.51 (2H, t, J = 4.8 Hz), 3.69 (2H, t, J = 4.8 Hz), 3.39-3.42 (4H, m), 1.41 (6H, t, J = 7.3 Hz).

7-(2-(Piperidin-1-yl)ethoxy)-5-hydroxy-2-(3,4,5-trihydroxy-phenyl)-4-chromen-4-one Hydrochloride (3f): Yield 38%; 1H NMR (CDOD) δ 7.02 (2H, s), 6.71 (1H, d, J = 2.1 Hz), 6.56 (1H, s), 4.49 (2H, bs), 3.60 (2H, bs), 1.94 (4H, bs), 1.74 (2H, bs).

7-(2-(Pyrrolidin-1-yl)ethoxy)-5-hydroxy-2-(3,4,5-trihydroxy-phenyl)-4-chromen-4-one Hydrochloride (3b): Yield 48%; 1H NMR (CDOD) δ 7.04 (2H, s), 6.75 (1H, d, J = 1.5 Hz), 6.60 (1H, s), 5.00 (1H, d, J = 1.8 Hz), 4.56 (2H, bs), 4.02 (4H, bs), 3.71 (2H, bs), 3.71 (4H, bs).

7-(2-(Morpholinoethoxy)-5-hydroxy-2-(3,4,5-tri hydroxy-phenyl)-4-chromen-4-one Hydrochloride (3g): Yield 45%; 1H NMR (D2O + DMSO-d6) δ 7.10 (2H, s), 6.83 (1H, d, J = 2.4 Hz), 6.69 (1H, s), 6.54 (1H, d, J = 2.4 Hz), 4.55 (2H, t, J = 4.8 Hz), 3.69 (2H, t, J = 4.8 Hz), 3.68-3.44 (4H, m), 1.44 (6H, t, J = 7.2 Hz).

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References