Prenyloxycoumarins with antioxidant and lipoxygenase inhibitory activity: Synthesis, bioactivity evaluation and encapsulation in biodegradable poly (lactic acid) nanoparticles

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ABSTRACT

Oxyprenylated natural products (isopentenyloxy-, geranyloxy- and the less spread farnesyloxy- compounds and their biosynthetic derivatives) represent a family of secondary metabolites that have been considered for years just as biosynthetic intermediates of C-prenylated derivatives. Prenyloxycoumarins are secondary metabolites commonly present in plants belonging to the families of Rutaceae and Umbelliferae. Several of these coumarins were shown to possess valuable pharmacological properties. Among them, auraptene (7-geranyloxycoumarin) which is isolated from the peel of citrus fruit has been reported to have chemopreventive effects on chemically induced carcinogenesis and possess antioxidant activity.

In this work, the synthesis of the natural product auraptene as well as its analogue, 4-methyl-7-geranyloxycoumarin will be presented. The compounds were evaluated in vitro for their antioxidant activity as well as for their ability to inhibit soybean lipoxygenase, as an indication of their potential anti-inflammatory activity. In addition, the compounds were encapsulated in biodegradable poly (lactic acid) (PLA) nanoparticles and this was achieved via the emulsification-solvent evaporation technique. Size, polydispersity index and ζ-potential of the nanoparticles were measured by Dynamic Light Scattering method whereas the Encapsulation Efficiency (EE) was determined indirectly, using UV-Vis spectroscopy.

INTRODUCTION

Coumarins are a class of compounds widely encountered in nature which possess numerous therapeutic prospects. Among the biological activities which have been attributed to simple coumarins and their analogues is the antimicrobial activity, antiviral activity, antitumor, enzyme inhibitory activity, anti-inflammatory, antioxidant and antithrombotic activity. \[1]\)

Specifically, the alkylation of aromatic secondary metabolites plays an important role in the biosynthesis of many molecules with significant biological activities. The first example of a prenylated secondary metabolite is auraptene or 7-geranyloxy-coumarin which was isolated in 1930 from the plant Citrus aurantium L. (Rutaceae) and its structure was assigned by Kariyone and Matsuo (Figure 1). \[2]\)

Figure 1. The structure of auraptene

The pharmacological actions of auraptene have been mentioned in literature since 1991 \[3]\) and up to date 300 oxy-prenylated derivatives, which have been synthesized or isolated, exhibit interesting biological properties.
Auraptene is a chemoprotective agent against skin, tongue, oesophagus and colon carcinogenesis in rodents \(^4\) and also, prevents the mutation of tumor cells, accelerating the action of metabolism and some enzymes, such as glutathione S-transferase. \(^5\) In addition to these properties, the anti-inflammatory activity of auraptene has been proven in vivo and in vitro. \(^6\)

Nanoencapsulation is of particular interest in the field of drug delivery, through particulate systems which operate as “carriers” of some bioactive compounds. These nanoparticles can provide protection against unwanted degradation, photo- and air-oxidation as well as modifying the aqueous solubility of compounds. \(^7\) Poly (lactic acid) (PLA) is a polymer with many important advantages, such as the renewability, biocompatibility, biodegradation and it is ideal for the encapsulation of hydrophobic compounds. \(^8\)

As a continuation of our studies toward the synthesis of bioactive coumarin analogues, \(^9\), \(^10\) we report here the synthesis and evaluation of antioxidant and anti-inflammatory activity of the natural product auraptene (3) and its 4-methylated analogue (4).

**EXPERIMENTAL**

(a) **Synthesis of compounds 3 and 4**

The synthesis of two geranyloxy-coumarins 3 and 4 was carried out by Williamson’s ether reaction, using the commercially available 7-hydroxy-coumarin (umbelliferone) (1) or the synthesized 4-methyl-7-hydroxy-coumarin (2) as starting materials. The appropriate coumarin reacts with geranyl bromide (Scheme 1) in a basic environment, to provide coumarins 3 and 4 in good yields and high purity.

The structures of the synthesized compounds were identified using NMR spectroscopy.

**Scheme 1.** The synthetic route for 7-geranyloxy-coumarins 3 and 4

(b) **Encapsulation of compounds 1-4 in PLA nanoparticles**

The produced geranyloxy-coumarins 3 and 4 were encapsulated in PLA nanoparticles via the emulsification-solvent evaporation technique, which forms simple emulsions and it is appropriate for hydrophobic compounds. According to this method, the polymer was dissolved in a volatile organic solvent, such as acetone, and was mixed with the solution of the compound which will be encapsulated. The new solution was emulsified in an aqueous phase containing an emulsifier (in this case poly(vinyl alcohol), PVA) which does not dissolve the polymer. The organic solvent was then evaporated under mild conditions (shaker or rotary evaporator at room
temperature) and the particles are collected by filtration or centrifugation and redispersed in distilled water.

(c) Evaluation of biological activity

The antioxidant activity of coumarins 1-4 was evaluated by the DPPH assay and their ability to inhibit lipid peroxidation of linoleic acid induced by the thermal free radical producer AAPH. Moreover, the ability of compounds 1-4 to inhibit the activity of soybean lipoxygenase was evaluated as an indication of their anti-inflammatory activity.

RESULTS AND DISCUSSION

In the present work, the natural product 7-geranyloxy-coumarin (auraptene) (3) and its 4-methyl analogue (4) were synthesized and evaluated for their antioxidant and soybean lipoxygenase inhibitory activity. The results of in vitro biological testing of compounds 1-4 are shown in Table 1.

Table 1. In vitro antioxidant and lipoxygenase inhibitory activities of compounds 1-4

<table>
<thead>
<tr>
<th>Compound</th>
<th>DPPH radical scavenging ability (%)</th>
<th>Inhibition of lipid peroxidation induced by AAPH (%)</th>
<th>Inhibition of soybean lipoxygenase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20/60 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Umbelliferone (1)</td>
<td>1/2</td>
<td>92</td>
<td>100</td>
</tr>
<tr>
<td>4-methyl-umbelliferone (2)</td>
<td>No/no</td>
<td>93</td>
<td>No</td>
</tr>
<tr>
<td>Auraptene (3)</td>
<td>26/6</td>
<td>52</td>
<td>44</td>
</tr>
<tr>
<td>4-methyl-auraptene (4)</td>
<td>21/No</td>
<td>20</td>
<td>78</td>
</tr>
<tr>
<td>Nordihydroguaiaretic acid (NDGA)</td>
<td>93</td>
<td>-</td>
<td>86</td>
</tr>
<tr>
<td>Trolox</td>
<td>-</td>
<td>63</td>
<td>-</td>
</tr>
</tbody>
</table>

The results indicate that the tested coumarin analogues do not possess satisfactory DPPH radical scavenging ability possibly for steric reasons. However, umbelliferone (1) and 4-methyl-umbelliferone (2) are potent lipid
peroxidation inhibitors presenting 92% and 93% inhibitory activity, respectively. Auraptene (3) and 4-methyl-auraptene (4) exhibit lower inhibition of lipid peroxidation (52% and 20%, respectively) than the corresponding 7-hydroxy analogues, indicating that the presence of the geranyl-moiety as well as the etherification of the 7-OH group, does not favor this activity. Umbelliferone (1) highly inhibits soybean lipoxygenase (100% at 100μM), which is the highest inhibition among the tested derivatives, followed by 4-methyl-auraptene (4) with 78% inhibition. It is noteworthy that the addition of a methyl group on position 4 of the umbelliferone structure (compound 2) leads to complete loss of activity whereas in the case of the 7-geranyloxy-analogues the 4-methyl-analogue (4) is more active than auraptene (3).

Compounds 1-4 were successfully encapsulated in biodegradable PLA nanoparticles. The size, polydispersity index and ζ-potential of the nanoparticles were determined by Dynamic Light Scattering method (DLS). The results are shown in the following table (Table 2).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Size(nm)</th>
<th>PDI</th>
<th>Z-potential(mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank NPs</td>
<td>225,3</td>
<td>0,253</td>
<td>-17,4</td>
</tr>
<tr>
<td>umbelliferone-NPs (1-NPs)</td>
<td>261,9</td>
<td>0,212</td>
<td>-9,05</td>
</tr>
<tr>
<td>4-methyl-umbelliferone-NPs (2-NPs)</td>
<td>209,5</td>
<td>0,235</td>
<td>-11,83</td>
</tr>
<tr>
<td>7-geranyloxy-coumarin-NPs (3-NPs)</td>
<td>210,8</td>
<td>0,251</td>
<td>-12,49</td>
</tr>
<tr>
<td>4-methyl-7-geranyloxy-coumarin-NPs (4-NPs)</td>
<td>227,4</td>
<td>0,223</td>
<td>-9,85</td>
</tr>
</tbody>
</table>

The Encapsulation Efficiency (EE) was determined indirectly, using UV-Vis spectroscopy, measuring at the maximum absorption wavelength for each compound. A standard calibration curve was first plotted using standard concentrations of each compound. Then, the amount of the non-encapsulated compound was quantified in the supernatant solutions, which were collected during the centrifugation. The mass of the non-encapsulated compound was calculated from the average absorption value for each supernatant. Eq. (1) gives the EE (%).[1]

\[
EE\% = \frac{[\text{total amount of compound} - \text{(non-encapsulated compound)}]}{\text{total amount of compound}} \times 100
\]

**Equation 1.** Encapsulation Efficiency

The results are shown in the following table (Table 3).
Table 3. Encapsulation Efficiency of compounds 1-4

<table>
<thead>
<tr>
<th>Compound</th>
<th>Encapsulation Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>57.72</td>
</tr>
<tr>
<td>2</td>
<td>47.17</td>
</tr>
<tr>
<td>3</td>
<td>92.6</td>
</tr>
<tr>
<td>4</td>
<td>78.02</td>
</tr>
</tbody>
</table>

The encapsulation of compounds 1-4 in nanoparticles of biodegradable poly(lactic acid) PLA was successful and the nanoparticles have the following characteristics:

- **Size**: (209 - 260) nm
- **PDI**: 0.212 - 0.253
- **Z-potential**: (-9) – (-17.4) mV

The synthesized nanoparticles are nanocapsules in size, are stable in suspension and exhibit uniform size dispersion. It is also obvious that 7-geranyloxy-coumarins 3 and 4, which are more hydrophobic compounds than the corresponding 7-hydroxy coumarins 1 and 2, exhibit higher encapsulation efficiency into nanoparticles of poly(lactic acid) PLA.

**CONCLUSIONS**

In this work, we present the synthesis and biological activity evaluation of a naturally occurring coumarin (auraptene) and its 4-methyl-analogue. The results indicate that 7-hydroxy coumarins 1 and 2 are potent inhibitors of lipid peroxidation. As far as the ability of the coumarins tested to inhibit soybean lipoxygenase, the results show that umbelliferone (1) is the most potent compound followed by 4-methyl-auraptene (4), therefore these compounds merit further investigation as potential agents possessing dual antioxidant and anti-inflammatory activity.

Moreover, coumarins 1-4 were successfully encapsulated in biodegradable PLA nanoparticles which possessed satisfactory physicochemical characteristics and encapsulation efficiency. The study of the release kinetics of the encapsulated compounds from the polymer matrix as well as the bioactivity of the loaded nanoparticles are currently underway.
REFERENCES


