New heterocyclic polyphenols with skin anti-aging potential


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New heterocyclic polyphenols with skin anti-aging potential

1. Synthesis
   - Benzophenone method
   - Ullmann ether synthesis
   - GSS method

2. Antioxidant Activity
   - DPPH Scavenging Effect
   - Metal chelating effect

3. Stability
   - pH Stability

4. Solubility
   - Water
   - Glycerol

5. Phototoxicity
   - Evaluation in a human keratinocyte cell line (HaCaT)

Anti-aging Activity
- Anti-Tyrosinase
- Anti-Elastase
- Anti-Colagenase
- Anti-Hyaluronidase
**Abstract:**
Xanthones or dibenzo-gamma-pyrones are heterocyclic polyphenolic compounds that can be found in microorganisms, fungi, lichens, and some higher plants. Structure-activity relationship studies emerged from a library of natural and synthetic polyoxygenated have suggested that xanthones with vicinal diol groups have promising antioxidant activity. Antioxidants have long been used in the cosmetic industry to prevent or minimize skin aging which is mediated by oxidative stress, making the search for new antioxidant agents highly desirable in this field.

Considering the structure-activity relationship studies, it was hypothesized that trioxygenated xanthones could be promising antioxidants with potential as skin anti-aging ingredients. Hence, the synthesis of trioxygenated xanthones was attempted by the Smiles rearrangement pathway and also via acyl radical cyclization. The Smiles rearrangement pathway failed to yield the ester intermediate that was essential in this approach and was therefore abandoned. In the acyl radical cyclization method it was possible to obtain the 1,4-dihydroxy-3-methoxy-9H-xanthen-9-one.

The antioxidant activity of this new xanthone as well as of four other polyoxygenated xanthones was evaluated by the DPPH assay, and two new derivatives showed IC$_{50}$ values in the same range as the ascorbic acid. Almost all of the compounds were excellent tyrosinase inhibitors, were weak to moderate collagenase inhibitors, and showed no activity against elastase. The stability in presence of metal ions and dependence of the pH was also studied, as well as their solubility in water and glycerol. Finally, the phototoxicity of the most promising xanthone was evaluated in a human keratinocyte cell line and no phototoxicity was observed in the concentration range tested, which is an important requirement for topical ingredients.

**Keywords:** Xanthones; antioxidants; synthesis; skin-degrading enzymes; stability, phototoxicity
Previously...

20 oxygenated xanthones

- Scavengers of DPPH
- Myeloperoxidase inhibitors
- Scavengers of peroxyl radicals

Hit compound

Metal chelating activity

Maximal atomic partial charge

Cell viability of HaCaT cell line
Potential application in skin

Synthesis of polyhydroxyxanthones

1. Benzophenone method

\[
\text{H}_2\text{CO} + \text{H}_2\text{CO} \xrightarrow{\text{AlCl}_3, \text{Et}_2\text{O} (dry)} \text{H}_2\text{CO} \xrightarrow{\text{H}_2\text{CO}, \text{CH}_2\text{OH}} \text{H}_2\text{CO} \xrightarrow{\text{NaOH, CH}_2\text{OH}, \text{MW, 100°C, 6 h}} \text{H}_2\text{CO} \xrightarrow{\text{AlCl}_3, \text{Toluene}} \text{H}_2\text{CO}
\]

Yield 63% (2 steps)

1, Yield 59%


2. Benzophenone method

\[
\text{H}_2\text{CO} + \text{H}_2\text{CO} \xrightarrow{\text{AlCl}_3, \text{Et}_2\text{O} (dry)} \text{H}_2\text{CO} \xrightarrow{\text{H}_2\text{CO}, \text{CH}_2\text{OH}} \text{H}_2\text{CO} \xrightarrow{\text{NaOH, CH}_2\text{OH}, \text{MW, 100°C, 6 h}} \text{H}_2\text{CO} \xrightarrow{\text{AlCl}_3, \text{Toluene}} \text{H}_2\text{CO}
\]

15% (2 steps)

2, 37%


3. Ullmann ether synthesis

\[
\text{Br} + \text{H}_2\text{CO} \xrightarrow{\text{Cu, KOH, py, N}_2, 26h} \text{H}_2\text{CO} \xrightarrow{\text{CH}_2\text{OH/THF, H}_2\text{O, NaOH, rt, 95h}} \text{H}_2\text{CO} \xrightarrow{1. \text{LDA, THF, N}_2, \text{60°C, rt, 2h}} \text{H}_2\text{CO} \xrightarrow{2. \text{Tol, AlCl}_3, \Delta, 8h} \text{H}_2\text{CO}
\]

Synthesis of new polyhydroxyxanthones

GSS method

![Reaction](image)

Benzophenone method

![Reaction](image)

## Results

% scavenging of DPPH = \(100 - \frac{Abs \ sample \ w/ \ DPPH - Abs \ sample \ blank}{Abs \ DPPH - Abs \ EtOH} \times 100\)

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC\textsubscript{50} (\mu)M (at 60min)</th>
<th>DPPH Scavenging effect (% at 25 (\mu)M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic Acid</td>
<td>40.0 ± 0.8</td>
<td>28.9 ± 0.3</td>
</tr>
<tr>
<td>Compound 1</td>
<td>31.2 ± 4.8*</td>
<td>36.8 ± 4.9</td>
</tr>
<tr>
<td>Compound 2</td>
<td>47.3 ± 0.4</td>
<td>24.9 ± 1.3</td>
</tr>
<tr>
<td>Compound 3</td>
<td>28.4 ± 0.2</td>
<td>43.3 ± 1.5</td>
</tr>
<tr>
<td>Compound 4</td>
<td>Not determined</td>
<td>9.2 ± 2.4</td>
</tr>
<tr>
<td>Compound 5</td>
<td>Not determined</td>
<td>34.6 ± 3.2</td>
</tr>
</tbody>
</table>

*standard deviation derived from three independent experiments
### Results

**METALS CHELATING EFFECT**

Summary of the observed shift in UV/Vis spectra

<table>
<thead>
<tr>
<th>Compound</th>
<th>FeCl$_3$</th>
<th>CuCl$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound 1</td>
<td>B</td>
<td>N</td>
</tr>
<tr>
<td>Compound 2</td>
<td>B</td>
<td>N</td>
</tr>
<tr>
<td>Compound 3</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>Compound 4</td>
<td>B</td>
<td>N</td>
</tr>
<tr>
<td>Compound 5</td>
<td>B</td>
<td>B</td>
</tr>
</tbody>
</table>

B* bathochromic effect, N* no relevant changing

Bathochromic shift on the UV/Vis spectra indicates the formation of a complex between the hydroxyl groups and the metals.

Antioxidant Activity

**Figure 7:** Solutions of xanthones (starting from the left) 1-4 after ten additions of FeCl$_3$ on the left and CuCl$_2$ on the right.

Solutions of xanthone 5 after ten additions of FeCl$_3$ on the left and CuCl$_2$ on the right.
### Results

**Antiaging Activity**

- Anti-tyrosinase
- Anti-elastase
- Anti-collagenase
- Anti-hyaluronidase

After exposure to sunlight, these enzymes are induced, leading to wrinkle formation, skin pigmentation, and skin sagging.

#### DERMAL ENZYME INHIBITION ACTIVITIES

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Tyrosinase</th>
<th>Elastase</th>
<th>Collagenase</th>
<th>Hialuronidase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Inhibition (150 µM)</td>
<td>IC₅₀ (µM)</td>
<td>% Inhibition (150 µM)</td>
<td>IC₅₀ (µM)</td>
</tr>
<tr>
<td>1</td>
<td>84.05</td>
<td>8.93</td>
<td>10.85</td>
<td>IC₅₀ (µM)</td>
</tr>
<tr>
<td>2</td>
<td>91.42</td>
<td>3.28</td>
<td>18.21</td>
<td>IC₅₀ (µM)</td>
</tr>
<tr>
<td>3</td>
<td>96.17</td>
<td>7.8</td>
<td>35.2</td>
<td>IC₅₀ (µM)</td>
</tr>
<tr>
<td>4</td>
<td>47.32</td>
<td>-</td>
<td>24.12</td>
<td>IC₅₀ (µM)</td>
</tr>
<tr>
<td>Kojic acid</td>
<td>12.81</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAAPVCK</td>
<td></td>
<td></td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>EDTA</td>
<td></td>
<td></td>
<td>102.95</td>
<td></td>
</tr>
</tbody>
</table>

Results from three independent experiments; results of three independent experiments; *standard deviation not shown

n.a. - Not active (0% inhibition)
Stability

pH

Xanthones 1 and 3 were submitted under a range of pH buffers to know what is the pH where each one is more stable.

**pH** is a **significant parameter** regarding skin compatibility of the cosmetic formulations.

The pH of human skin normally ranges from **4.5** to **6.0**. A pH **closer** to this range is desirable. These results are also of utmost importance for the formulation of a suitable vehicle, that maximizes the chemical stability of the actives incorporated.

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![Diagram of Xanthones 1 and 3](image)

**Results**

Xanthone 1 stability given by variation of absorbance in pH buffers over the time of analysis (0, 1, 2, 24, 192, 360 and 504 hours).

Xanthone 3 stability given by variation of absorbance in pH buffers over the time of analysis (0, 1, 2, 24, 192, 360 and 504 hours).
Solubility criteria European Pharmacopeia

<table>
<thead>
<tr>
<th>Descriptive terms</th>
<th>Solubility (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very soluble</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>Freely soluble</td>
<td>100-1000</td>
</tr>
<tr>
<td>Soluble</td>
<td>33-100</td>
</tr>
<tr>
<td>Sparingly soluble</td>
<td>10-33</td>
</tr>
<tr>
<td>Slightly soluble</td>
<td>1-10</td>
</tr>
<tr>
<td>Very slight soluble</td>
<td>0.1-1</td>
</tr>
<tr>
<td>Practically insoluble</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>

**Solubility in Water (mg/mL)**

- Xanthone 1: 0.001 (Practically insoluble)
- Xanthone 3: n.d. (Practically insoluble)

**Solubility in Glycerol (mg/mL)**

- Xanthone 1: n.d. (Practically insoluble)
- Xanthone 3: 0.019 (Practically insoluble)

The absorbance of saturated samples (suspension was shaken until the equilibrium solubility was achieved) was evaluated by HPLC at 310 nm and 255 nm for xanthone 1 and at 390 nm and 285 nm for xanthone 3. n.d. not detected.
Results

Adapted from OECD 432 guideline

<table>
<thead>
<tr>
<th>Photo Irritation Factor (PIF)</th>
<th>Compound</th>
<th>IC(_{50}) (-irr)</th>
<th>IC(_{50}) (+irr)</th>
<th>PIF</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Phototoxicity</td>
<td>Xanthone 3</td>
<td>&gt; 200 μM</td>
<td>&gt; 200 μM</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Probable Phototoxicity</td>
<td>Xanthone 3</td>
<td>Not applicable</td>
<td>Not applicable</td>
<td></td>
</tr>
<tr>
<td>Phototoxicity</td>
<td>Xanthone 3</td>
<td>Not applicable</td>
<td>Not applicable</td>
<td></td>
</tr>
</tbody>
</table>

Xanthone 3 was not cytotoxic to HaCaT cells even after irradiation. The IC\(_{50}\) values and consequently the PIF could not be obtained. However, as the cell viability was not decreased after UV exposure, the compound is deemed non phototoxic up to 200 μM.
Conclusions

**Synthesis**
It was possible to synthesize one new compound, 1,8-dihydroxy-3,6-dimethyl-9H-xanthen-9-one (4).

**Solubility**
Xanthones 1 and 3 were practically insoluble in water and glycerol.

**Antioxidant Activity**
DPPH: Xanthones 1 and 3 showed IC<sub>50</sub> values lower than the ones obtained for ascorbic acid.
Metals: Xanthones 3 and 5 exhibited metal chelating ability.

**Stability**
pH: Xanthone 3 presented a stable profile in the range of pH from 3 to 5.

**Anti-aging Activity**
Almost all xanthones were excellent tyrosinase inhibitors, more active than control inhibitor kojic acid.

**Phototoxicity**
Xanthone 3 is non phototoxic up to 200 µM.
Acknowledgements

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