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New heterocyclic polyphenols with skin anti-aging potential

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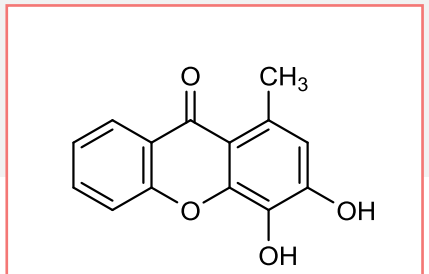
New heterocyclic polyphenols with skin anti-aging potential



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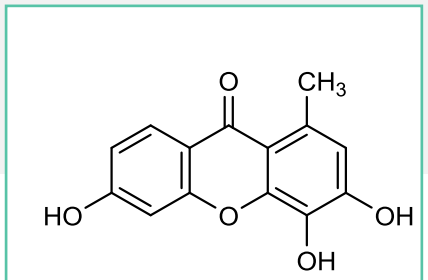

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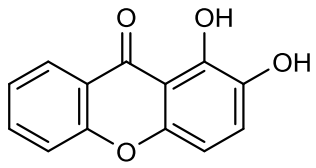

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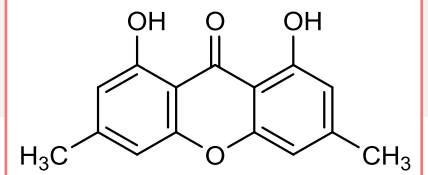

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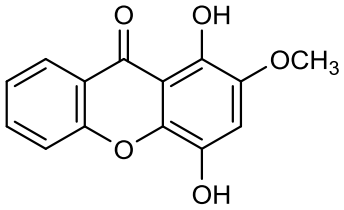

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Synthesis

- Benzophenone method
- Ullmann ether synthesis
- GSS method

Antioxidant Activity

- DPPH Scavenging Effect
- Metal chelating effect

Anti-aging Activity

- Anti-Tyrosinase
- Anti-Elastase
- Anti-Colagenase
- Anti-Hialuronidase

Stability

- pH Stability

Solubility

- Water
- Glycerol

Phototoxicity

- Evaluation in a human keratinocyte cell line (HaCaT)

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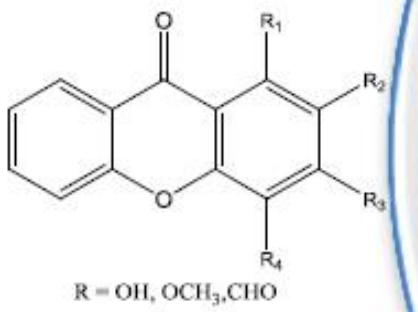
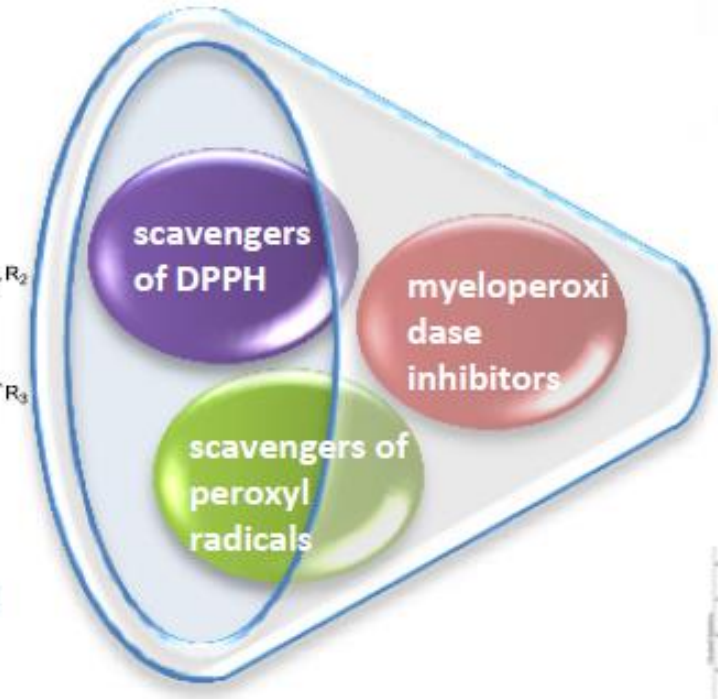
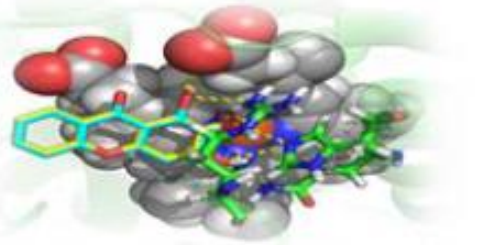
Abstract:
Xanthones or dibenzo-gamma-pyrones are heterocyclic polyphenolic compounds that can be found in microorganisms, fungi, lichens, and some higher plants. Structure-activity relationship studies emerged from a library of natural and synthetic polyoxygenated have suggested that xanthones with vicinal 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-9*H*-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

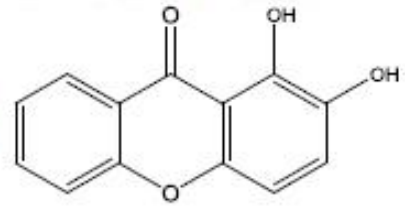
Previously…



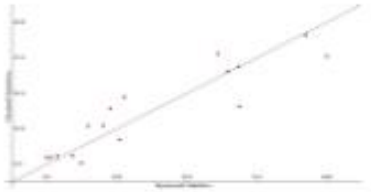
20 oxygenated xanthones



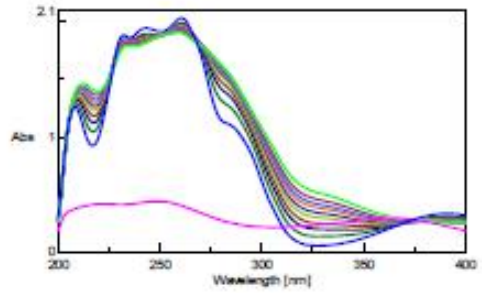




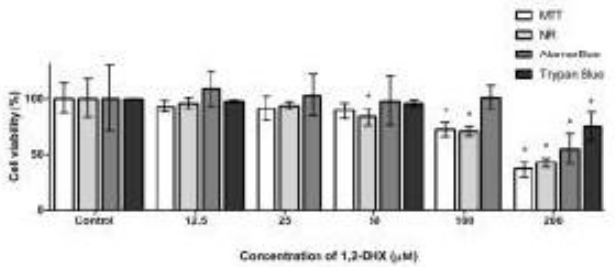
***Hit* compound**



maximal atomic partial charge



metal chelating activity

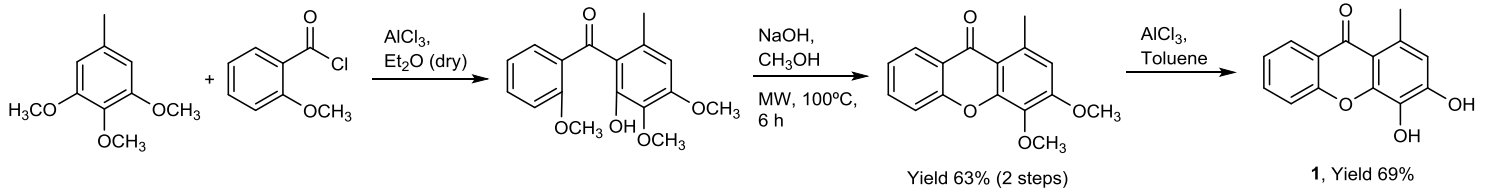


Cell viability of HaCaT cell line
potential application in skin

Cidade H, et al. Arab. J. Chem. ***2017****, https://doi.org/10.1016/j.arabjc.2017.01.006*

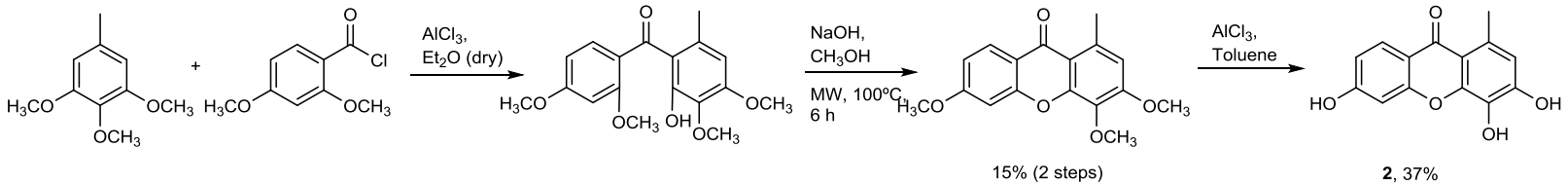
Synthesis of polyhydroxyxanthones

1 **Benzophenone method**



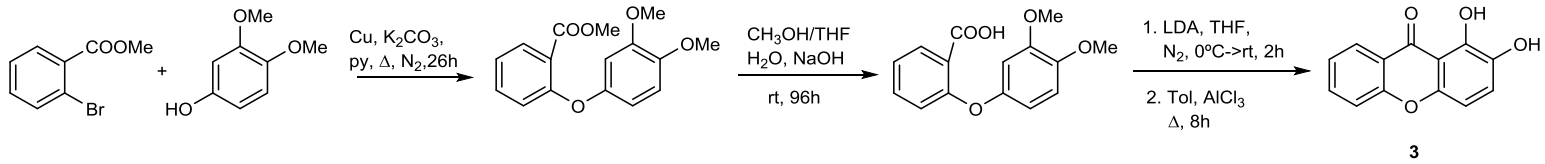
Resende, D. et al. Molecules ***2018****, 23 (10)*, 2617.

2 **Benzophenone method**



Resende, D. et al. Molecules ***2018****, 23 (10)*, 2617.

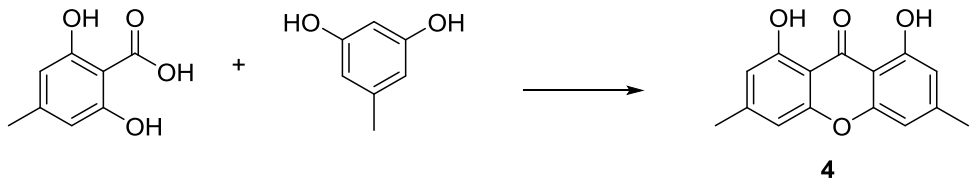
3 **Ullmann ether synthesis**



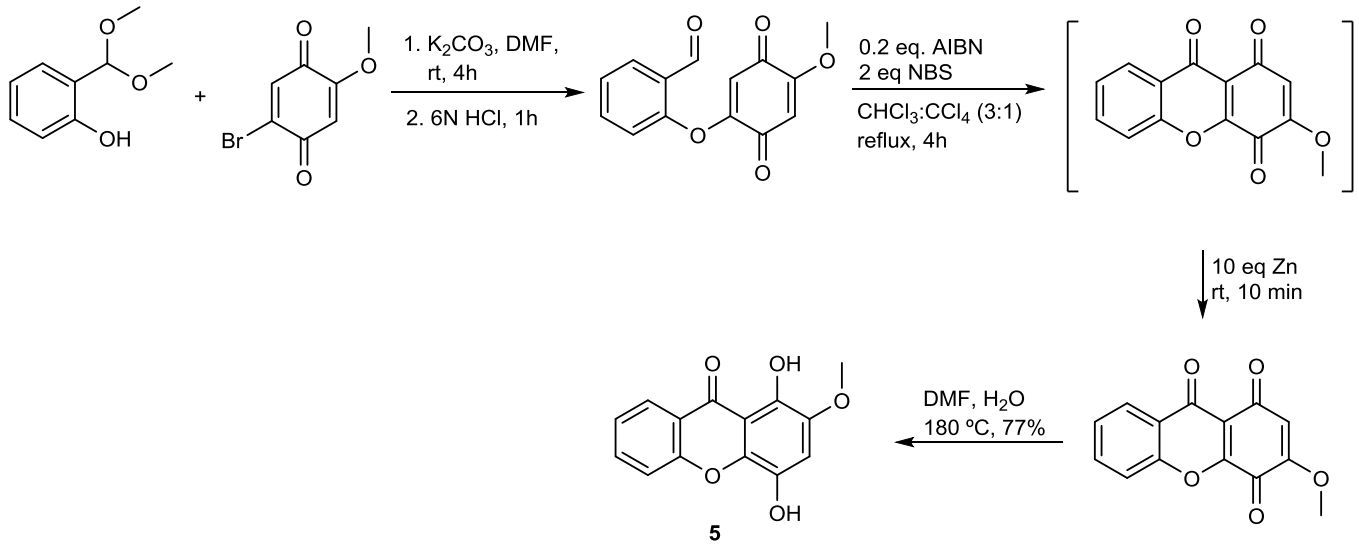
Sousa, E. P. et al. Helv. Chim. Acta ***2002****, 85 (9),* 2862-2876.

Synthesis of new polyhydroxyxanthones

4 GSS method



5 Benzophenone method



Adapted from Kraus, G. A.; Liu, F., *Tetrahedron Lett.* **2012**, *53* (2), 111-114.

Antioxidant Activity

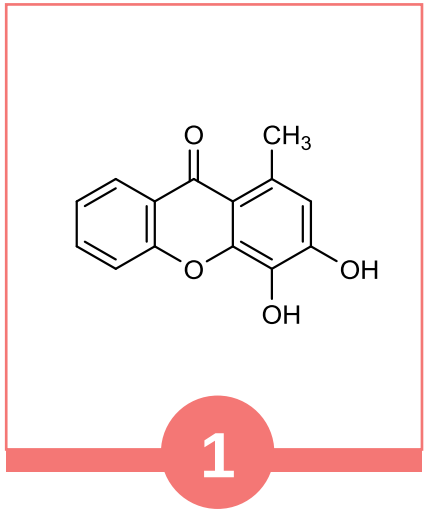
Results

DPPH SCAVENGING CAPACITY

$$\%\ scavenging\ of\ DPPH = 100 - \frac{Abs\ sample\ w\ /\ DPPH - Abs\ sample\ blank}{Abs\ DPPH - Abs\ EtOH} x100$$

Compound	IC_{50} μM (at 60min)	DPPH Scavenging effect (%) at 25 μM
Ascorbic Acid	40.0 ± 0.8	28.9 ± 0.3
Compound 1	**31.2 ± 4.8***	36.8 ± 4.9
Compound 2	47.3 ± 0.4	24.9 ± 1.3
Compound 3	**28.4 ± 0.2**	43.3± 1.5
Compound 4	Not determined	9.2 ± 2.4
Compound 5	Not determined	34.6 ± 3.2

*standard deviation derived from three independent experiments



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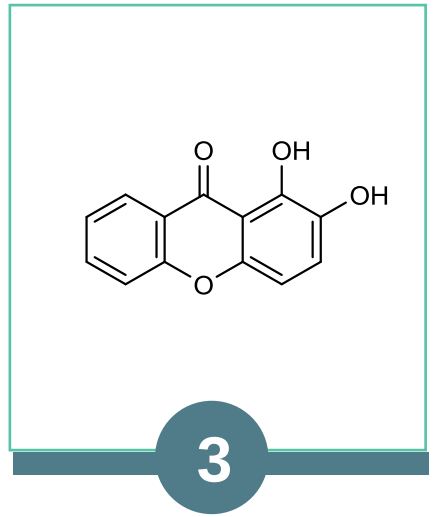


3

Antioxidant Activity

Results

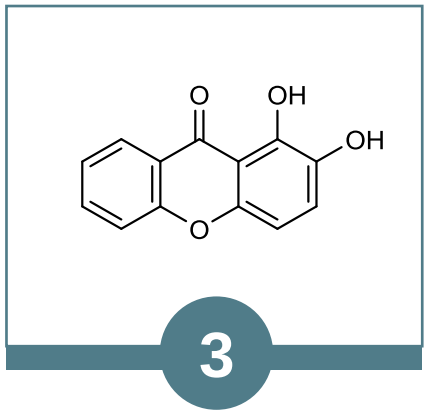
METALS CHELATING EFFECT

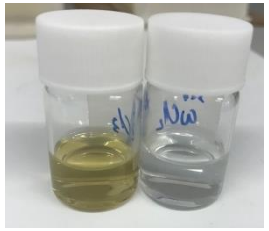
Summary of the observed shift in UV/Vis spectra

	$FeCl_3$	$CuCl_2$
Compound 1	B	N
Compound 2	B	N
Compound 3	B	B
Compound 4	B	N
Compound 5	B	B

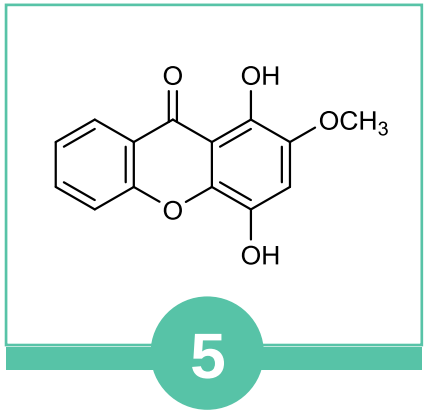
B* bathochromic effect, **N*** no relevant changing

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





Solutions of xanthone **5** after ten additions of $FeCl_3$ on the left and $CuCl_2$ on the right



Antiaging Activity

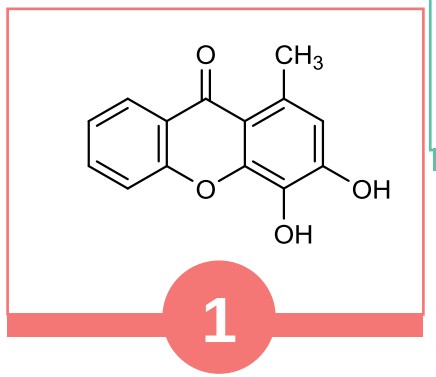
Results

DERMAL ENZYME INHIBITION ACTIVITIES

Anti-aging 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



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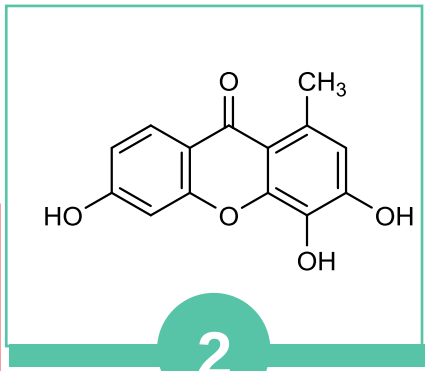

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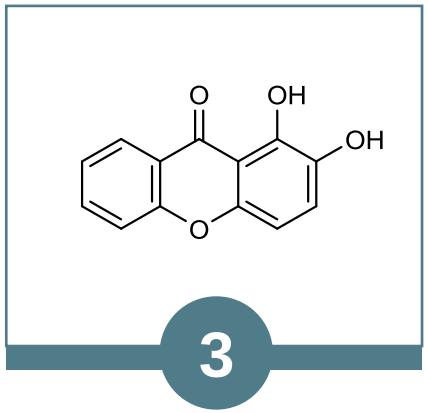


3

	Tyrosinase		Elastase		Colagenase		Hialuronidase
Compounds	% Inhibition (150 µM)	IC_{50} (µM)	% Inhibition (150 µM)	IC_{50} (µM)	% Inhibition (150 µM)	IC_{50} (µM)	% inhibition (150 µM)
1	84.05	8.93	10.85		26.83		n.a.
2	91.42	3.28	18.21		24.91		n.a.
3	96.17	7.8	35.2		35.77		n.a.
4	47.32	-	24.12		0.38		n.a.
Kojic acid		12.81					
MAAPVCK				0.26			
EDTA						102.95	

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

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

Stability

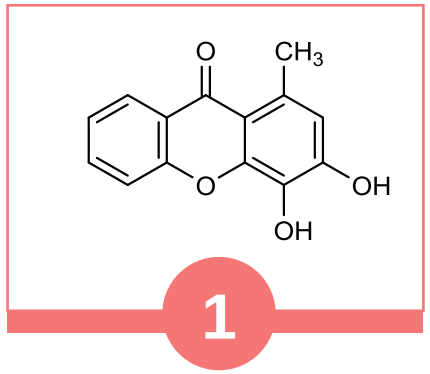
Results

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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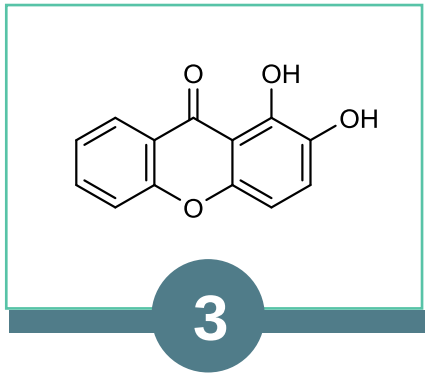

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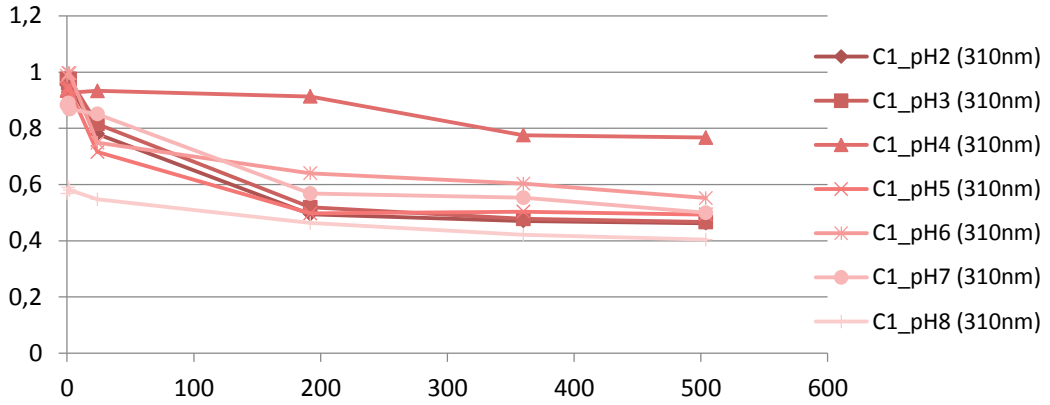
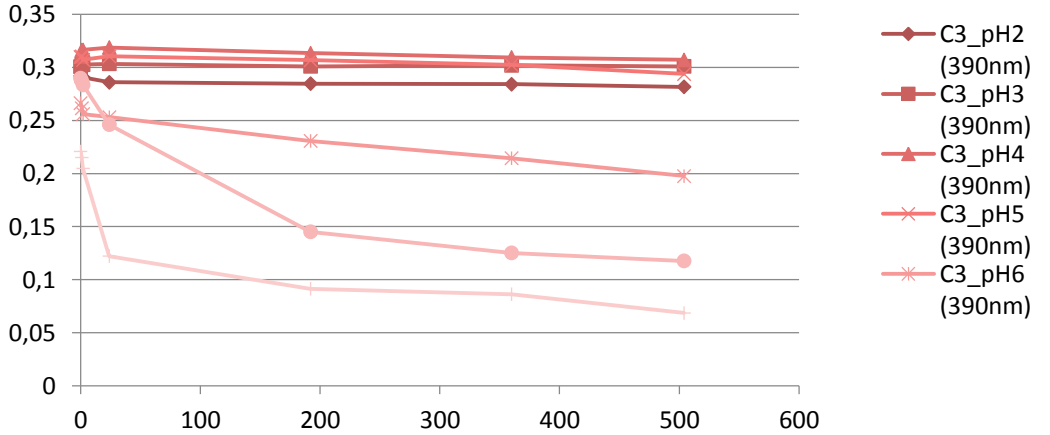


Xanthone **1** stability given by variation of absorbance in pH buffers over the time of analysis (0, 1, 2, 24, 192, 360 and 504 hours).



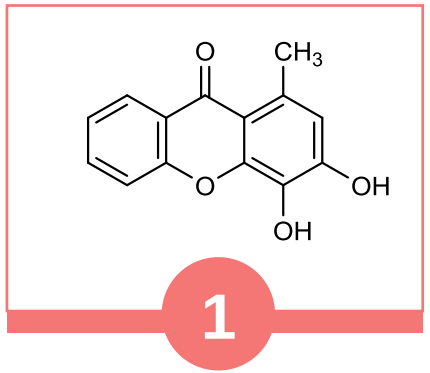
Xanthone **3** stability given by variation of absorbance in pH buffers over the time of analysis (0, 1, 2, 24, 192, 360 and 504 hours).

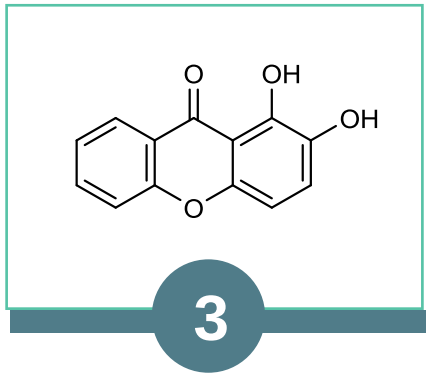
Results

SOLUBILITY

Solubility criteria European Pharmacopeia

Descriptive terms	Solubility (mg/mL)
Very soluble	>1000
Freely soluble	100-1000
Soluble	33-100
Sparingly soluble	10-33
Slightly soluble	1-10
Very slight soluble	0.1-1
Practically insoluble	<0.1

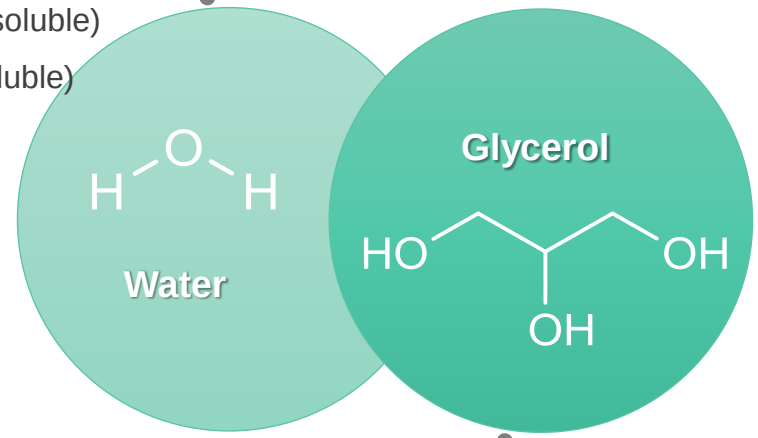




Solubility in Water (mg/mL)

Xanthone **1**: 0.001 (Practically insoluble)

Xanthone **3**: n.d. (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

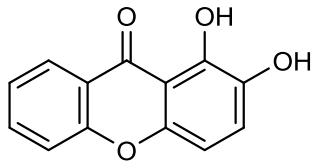
Solubility in Glycerol (mg/mL)

Xanthone **1**: n.d. (Practically insoluble)

Xanthone **3**: 0.019 (Practically insoluble)

Phototoxicity

Results



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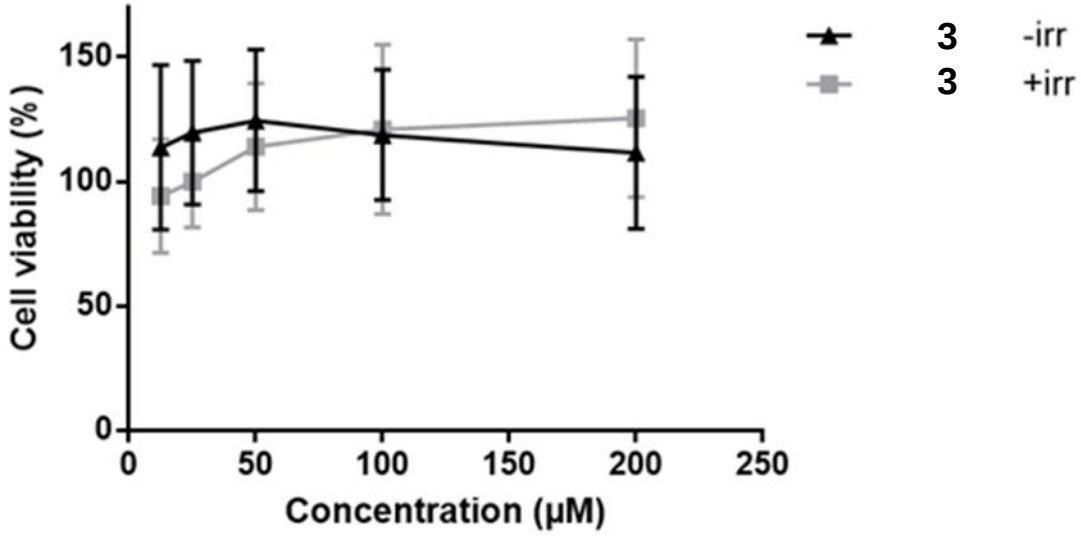
Adapted from OECD 432 guideline

	Photo Irritation Factor (PIF)
No Phototoxicity	<2
Probable Phototoxicity	2 – 5
Phototoxicity	>5

Compound	IC_{50} (-irr)	IC_{50} (+irr)	PIF
Xanthone 3	> 200 µM	> 200 µM	Not applicable



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-9*H*-xanthen-9-one (**4**)

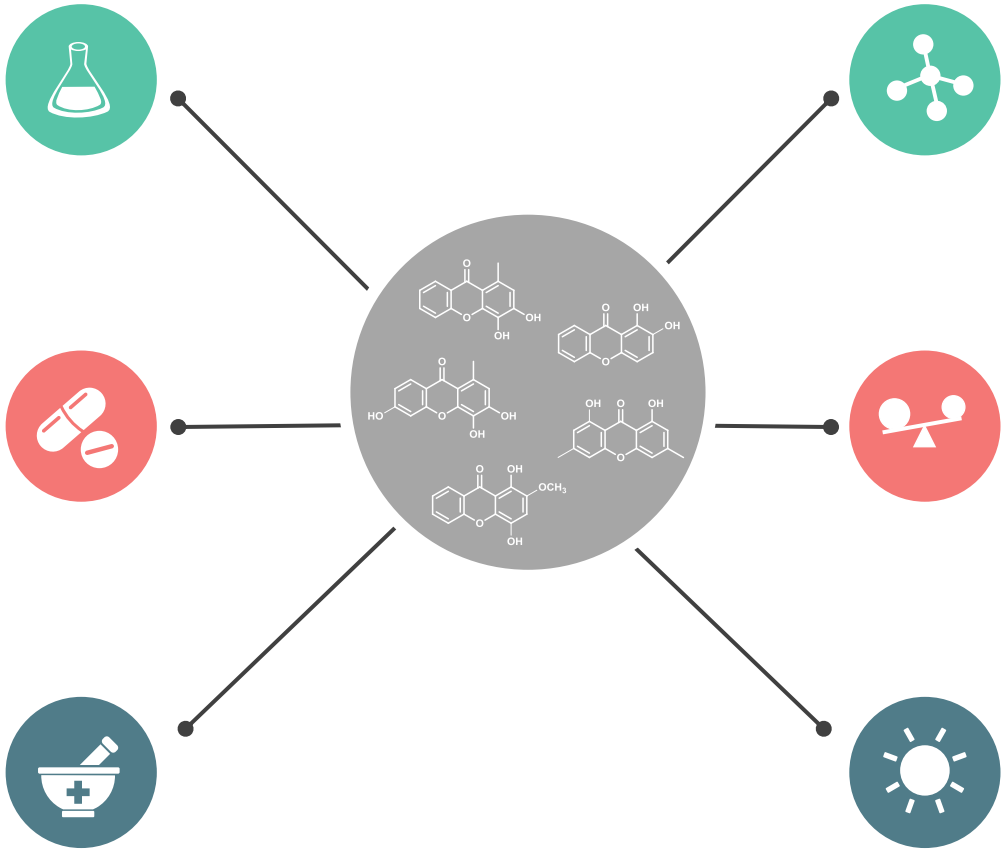
Antioxidant Activity

DPPH: Xanthones **1** and **3** showed IC_{50} values lower than the ones obtained for ascorbic acid

Metals: Xanthones **3** and **5** exhibited metal chelating ability

Anti-aging Activity

Almost all xanthones were excellent tyrosinase inhibitors, more active than control inhibitor kojic acid



Solubility

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

Stability

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

Phototoxicity

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

Acknowledgements

This work was developed under the Strategic Funding UID/Multi/04423/2019 and Project No. POCI-01-0145-FEDER-028736, co-financed by COMPETE 2020, Portugal 2020 and the European Union through the ERDF, and by FCT through national funds, QREN,FEDER, COMPETE, by funding the cE3c centre (Ref. UID/BIA/00329/2019) and Azores DRCT for funding ABG. This work was also supported by the Applied Molecular Biosciences Unit-UCIBIO which is financed by national funds from FCT/MCTES (UID/Multi/04378/2019.