Xanthones constitute an important class of heterocyclic compounds that commonly occur in nature. The inclusion of aryl groups has only been reported for a few synthetic derivatives, the 2,3-diaryl moiety being recently introduced by our group. The pharmacological properties of both natural and synthetic xanthones have attracted great attention namely as antioxidant, anti-allergic, anti-inflammatory, antimalarial and antitumour protectors. The antioxidant activity deserves special mention, considering the potent scavenging activity for reactive oxygen species (ROS) and reactive nitrogen species (RNS), as well as metal chelating activity already reported for some compounds bearing the xanthone scaffold. Thus, the main goal of this work was to evaluate the putative scavenging effect on \( \cdot \text{NO}, \text{ONOO}^- \) and \( \cdot \text{O}_2^- \) by the new synthetic 2,3-diarylxanthones (1), using in vitro non-cellular systems. The obtained results revealed that the tested 2,3-diarylxanthones are endowed with outstanding ROS and RNS scavenging properties, considering the nanomolar to micromolar range of the IC\textsubscript{50} values found. The xanthones with two catechol rings were the most potent scavengers of all tested ROS and RNS. In conclusion, the new 2,3-diarylxanthones are promising molecules to be used for their potential antioxidant properties.

Acknowledgements. Sincere thanks are expressed to Faculdade de Farmácia da Universidade do Porto, and also to Universidade de Aveiro, Fundação para a Ciência e a Tecnologia (Portugal) and FEDER for funding the Organic Chemistry Research Unit. Marisa Freitas and Ana Gomes acknowledge Fundação para a Ciência e a Tecnologia (FCT) and Fundo Social Europeu (FSE) their PhD (SFRH/BD/28502/2006) and post-doctoral (SFRH/BPD/63179/2009) grant, respectively.

**INTRODUCTION**

- Xanthones constitute an important class of heterocyclic compounds that commonly occur in nature. The inclusion of aryl groups has only been reported for a few synthetic derivatives, being the 2,3-diaryl moiety recently introduced by our group.
- The pharmacological properties of both natural and synthetic xanthones have attracted great attention as they act as antioxidant, anti-allergic, anti-inflammatory, antimarial and antitumour protectors. The antioxidant activity deserves special mention, considering the potent scavenging activity for reactive oxygen species (ROS) and reactive nitrogen species (RNS), as well as metal chelating activity already reported for some compounds bearing the xanthone scaffold.
- Thus, the major goal of this work was to evaluate the putative scavenging effect on 'NO, ONOO' and O_2·^- of the new synthetic 2,3-diarylxanthones (Figure 1), using in vitro non-cellular systems.

**METHODS**

**Superoxide radical (O_2·^-)**

The O_2·^- was generated by the NADH/PMS system and the O_2·^- scavenging activity was determined by monitoring the O_2·^--induced reduction of nitroblue tetrazolium chloride (NBT).

The effect of the tested compounds dissolved in DMSO, was determined spectrophotometrically at 560 nm during 2 min. The antioxidant action was used as positive control. The results were expressed as the percentage inhibition of NBT reduction to dimethylbenzene (Table 1, Figure 2).

**Nitric oxide ('NO')**

The 'NO scavenging activity was measured by monitoring the 'NO-induced oxidation of non-fluorescent 4,5-diaminofluorescein (DAF-2) to the fluorescent thiazolidofluorescein (DAF-2T).

'NO was generated by NOC-6, the tested compounds dissolved in DMSO and the luminometric signal was detected after a 20 min incubation period, at 37°C. Rutin was used as positive control. The results were expressed as the percentage inhibition of 'NO-induced oxidation of DAF-2 (Table 2, Figure 3).

**Peroxynitrite (ONOO^-)**

The ONOO^- scavenging activity was measured by monitoring the ONOO^- -induced oxidation of non-fluorescent dityrosine amine 123 (DHR) to fluorescent rhodamine 123.

The assays were performed at 37°C, the tested compounds dissolved in DMSO and the fluorometric signal detected after a 2 min incubation period. Ebselen was used as positive control. In a parallel set of experiments, the assays were performed in the presence of 25 mM NaHCO_3 in order to simulate the physiological CO_2 concentrations in vivo. The results were expressed as the percentage inhibition of ONOO^- -induced oxidation of DHR (Table 3, Figure 4 and 5).

**RESULTS**

**Table 1**

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC_50 (µM)</th>
<th>O_2·^-</th>
<th>NO</th>
<th>ONOO^- without NaHCO_3</th>
<th>ONOO^- with NaHCO_3</th>
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<tbody>
<tr>
<td>2,3-diarylxanthones</td>
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**CONCLUSIONS**

- The obtained results revealed that the tested 2,3-diarylxanthones are endowed with outstanding ROS/RNS scavenging properties, considering the nanomolar to micromolar range of the IC_50 values found.
- The results seem to be largely affected by the number and position of hydroxyl groups in D and E rings of the xanthone core.
- The xanthones with two catechol rings were the most potent scavengers of all tested ROS and RNS.
- In conclusion, the new 2,3-diarylxanthones are promising molecules to be used for their potential antioxidant properties.

**ACKNOWLEDGEMENTS**

Sincere thanks are expressed to Faculdade de Farmácia da Universidade do Porto, and also to Universidade de Aveiro, Fundação para a Ciência e Tecnologia (Portugal) and FEDER funding for the Organic Chemistry Research Unit. Maria Freitas and Ana Gomes acknowledge Fundação para a Ciência e Tecnologia (FCT) and Fundo Social Europeu (FSE) their PhD (SRHBD28982/2006) and post-doctoral (SRH/BPD/31759/2009) grant, respectively.

**REFERENCES**