3º Encontro Nacional de Química Terapêutica

3rd Portuguese Meeting on Medicinal Chemistry
1st Portuguese-Spanish-Brazilian Meeting on Medicinal Chemistry.

Sociedade Portuguesa de Química

universidade de aveiro

Electrochemical behavior of hydroxyxanthones *versus* ROS and RNS scavenging activities

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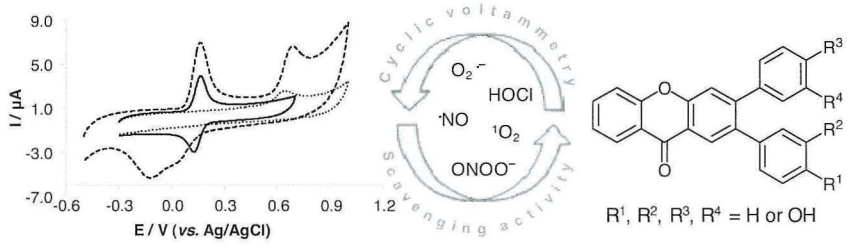
Xanthones are a class of naturally-occurring oxygenated heterocyclic compounds with a broad spectrum of biological activities of great interest for potential therapeutic applications.[1] Thus, over the last decades a great number of publications have emerged focusing on the isolation and synthesis of these natural compounds or even in the search for novel bioactive derivatives.[2]

Electrochemical methodologies can be applied to establish correlations between structure and oxidation potential of electroactive species and therefore to predict some of its biological activities. In fact, cyclic voltammetry has become an important and widely used electroanalytical technique in many relevant studies of redox processes for clarifying the electrochemical behavior of chemical and biochemical systems.[3]

Herein, we will report the electrochemical behavior of several phenolic and catecholic-substituted 2,3-diarylxanthones by cyclic voltammetry using a glassy carbon electrode, at pH 7.4, and the results obtained compared with their scavenging activities for reactive oxygen species (ROS) and reactive nitrogen species (RNS).[4] Useful considerations about oxidation mechanism will be highlighted and the electrochemical profile of xanthones will corroborate their biological properties.



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References

[1] (a) El-Seedi, H.R.; El-Ghorab, D.M.H.; El-Barbary, M.A.; Zayed, M.F.; Göransson, U.; Larsson, S.; Verpoorte, R. *Curr. Med. Chem.* **2009**, *16*, 2581. (b) Pinto, M. M. M.; Sousa, M. E.; Nascimento, M. S. J. *Curr. Med. Chem.* **2005**, *12*, 2517.

[2] (a) Pinto, M. M. M.; Castanheiro, R. A. P. *Curr. Org. Chem.* **2009**, *13*, 1215. (b) Pinto, M. M. M.; Sousa, M. E.; *Curr. Med. Chem.* **2005**, *12*, 2447.

[3] Teixeira. J. G.; Dias, C. B.; Teixeira, D. M. *Electroanalysis* **2009**, *21*, 2345. (b) Bozal, B.; Uslu, B.; Özkan, S. A. *Int. J. Electrochemistry* **2011**, 1. (c) Heinze, J. *Angew. Chem. Int. Ed. Engl.* **1984**, *23*, 831.

[4] Santos, C. M. M.; Freitas, M.; Ribeiro, D.; Gomes, A.; Silva, A. M. S.; Cavaleiro, J. A. S.; Fernandes, E. *Bioorg. Med. Chem.* **2010**, *18*, 6776.

ELECTROCHEMICAL BEHAVIOR OF HYDROXYXANTHONES *VERSUS* ROS AND RNS SCAVENGING ACTIVITIES

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INTRODUCTION

✹ Xanthones are a class of naturally-occurring oxygenated heterocyclic compounds presenting a broad spectrum of biological activities of great interest for potential therapeutic applications [1]. Thus, over the last decades a great number of publications have emerged focusing on the isolation and synthesis of these natural compounds or even in the search for novel bioactive derivatives [2].

✹ Electrochemical methodologies can be applied to establish correlations between structure and oxidation potential of electroactive species and therefore to predict some of its biological activities. In fact, cyclic voltammetry has become an important and widely used electroanalytical technique in many relevant studies of redox processes for clarifying the electrochemical behavior of chemical and biochemical systems [3].

✹ Herein, we report the electrochemical behavior of several phenolic and catecholic-substituted 2,3-diarylxanthones, by cyclic voltammetry, using a glassy carbon electrode, at pH 7.4. The obtained results are then compared with their scavenging activities for reactive oxygen species (ROS) and reactive nitrogen species (RNS) [4]. Useful considerations about oxidation mechanisms are highlighted and the electrochemical profile of xanthones discussed in light of their antioxidant properties.

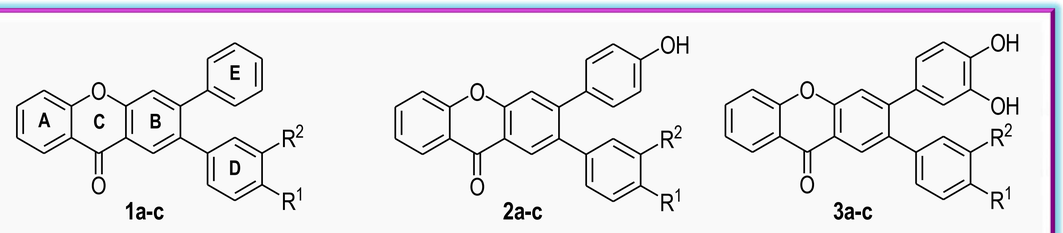
CYCLIC VOLTAMMETRY

✹ Voltammetric experiments were carried out using an Autolab electrochemical system (Eco Chemie model PGSAT 10). Data were acquired using GPES (General Purpose Electrochemical System) software, version 4.9.

✹ The working electrode was a glassy carbon electrode (GCE) (3.0 mm) whereas an Ag/AgCl (KCl 3 M) electrode and a carbon electrode were used as reference and auxiliary electrodes, respectively. Before use in electrochemical experiment and in order to obtain a clean renewed electrode surface, the glassy carbon working electrode was hand-polished with a 0.075 μm alumina aqueous slurry using a polishing cloth and washed with purified water.

✹ Xanthones **1-3** were synthesized according to previously reported procedures [5] and the chemical structures are presented in scheme 1. Stock solutions of **1a-c**, **2a-c** and **3a-c** were prepared in DMSO and diluted in sodium phosphate buffer solution pH 7.4 at the final concentration 0.1 mM, unless otherwise mentioned.

✹ The cell volume was 10 mL and cyclic voltammograms were obtained in a single cycle performed at a scan rate of 100 mV s^{-1}, at room temperature. For the scan rate studies, the scanning speed varied from 10 to 200 mV s^{-1}. Voltammetric scans were recorded in the voltage range between -0.5 and +1.0 V *versus* Ag/AgCl.



Scheme 1. Chemical structures of the studied 2,3-diarylxanthones

CONCLUSIONS

✓ The oxidation peak at higher potential values can be attributed to the phenolic groups. The absence of the corresponding reduction peaks also pointed to the irreversibility of the redox reaction of the oxidized compounds generated in the forward scan.

✓ Cyclic voltammograms of compounds **1c**, **2c**, **3a-c** showed the permanent presence of a low oxidation peak attributed to the catechol group oxidation, in an electrochemical reversible process.

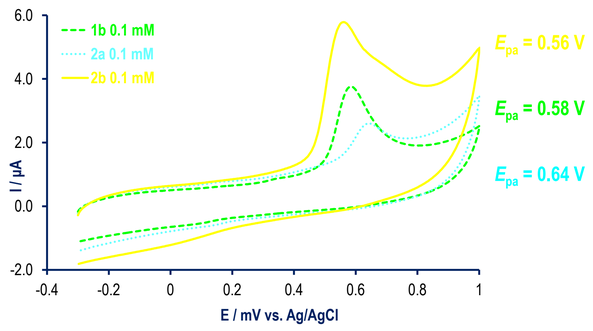
✓ In the Pearson test, excellent correlations are observed for $O_2^{\bullet -}$, $^{\bullet}NO$ and $ONOO^-$, as expected for scavenging reactions involving electron transfer mechanisms. Significant correlations are also found for HOCl and $^1O_2$, highly reactive oxygen species which are known for scavenging mechanisms involving structural features.

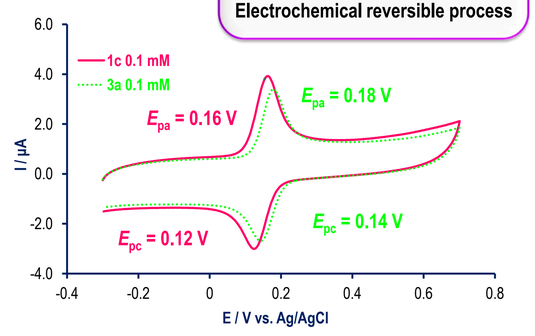
✓ Xanthone **3c** with two catechol units presented the lowest anodic potential voltage (E_{pa} = 0.15 V) and proved to be the most effective ROS and RNS scavenger.

ACKNOWLEDGEMENTS

Sincere thanks are expressed to Faculdade de Farmácia da Universidade do Porto, and to Universidade de Aveiro, Fundação para a Ciência e a Tecnologia (Portugal) and FEDER for funding the Organic Chemistry Research Unit (project PEst-C/QUI/UI0062/2011).

RESULTS

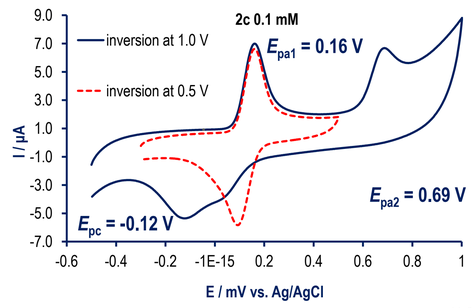


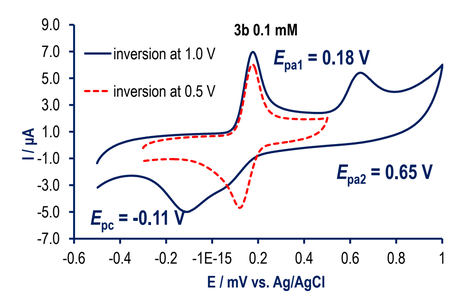


1c and **3a** $I_{pa} / I_{pc} = 1$

⇩

Electrochemical reversible process



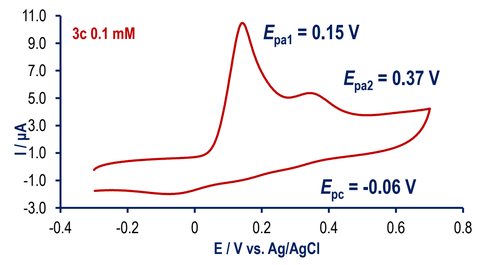


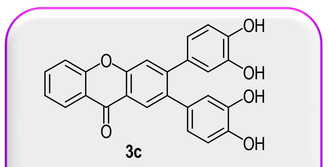
Inversion of potential scan before the second oxidation peak (at 0.5 V), **2c** and **3b** $I_{pa} / I_{pc} = 1$

⇩

Electrochemical reversible process

Compound 3c presents the lowest IC_{50} values for all ROS and RNS tested!





Lowest E_{pa} value!

Table 1

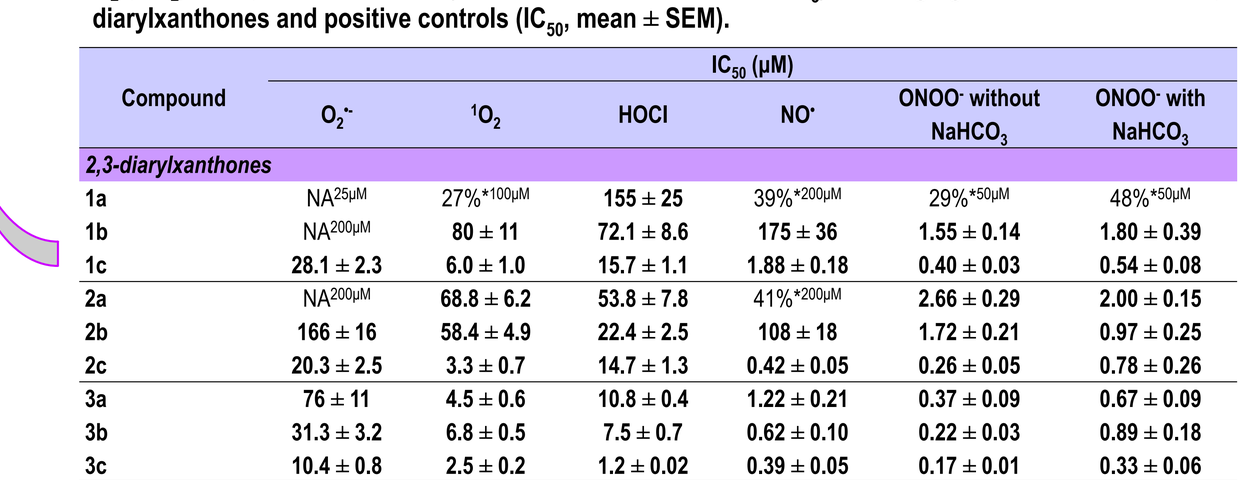
$O_2^{\bullet -}$, $^1O_2$, HOCl, $^{\bullet}NO$ and $ONOO^-$ (with and without 25 mM $NaHCO_3$) scavenging effects of the tested 2,3-diarylxanthones and positive controls (IC_{50}, mean ± SEM).

Compound	IC_{50} (μM)					
	$O_2^{\bullet -}$	$^1O_2$	HOCl	$^{\bullet}NO$	$ONOO^-$ without $NaHCO_3$	$ONOO^-$ with $NaHCO_3$
2,3-diarylxanthones						
1a	$NA^{25\mu M}$	$27\%^{*100\mu M}$	**155 ± 25**	$39\%^{*200\mu M}$	$29\%^{*50\mu M}$	$48\%^{*50\mu M}$
1b	$NA^{200\mu M}$	**80 ± 11**	**72.1 ± 8.6**	**175 ± 36**	**1.55 ± 0.14**	**1.80 ± 0.39**
1c	**28.1 ± 2.3**	**6.0 ± 1.0**	**15.7 ± 1.1**	**1.88 ± 0.18**	**0.40 ± 0.03**	**0.54 ± 0.08**
2a	$NA^{200\mu M}$	**68.8 ± 6.2**	**53.8 ± 7.8**	$41\%^{*200\mu M}$	**2.66 ± 0.29**	**2.00 ± 0.15**
2b	**166 ± 16**	**58.4 ± 4.9**	**22.4 ± 2.5**	**108 ± 18**	**1.72 ± 0.21**	**0.97 ± 0.25**
2c	**20.3 ± 2.5**	**3.3 ± 0.7**	**14.7 ± 1.3**	**0.42 ± 0.05**	**0.26 ± 0.05**	**0.78 ± 0.26**
3a	**76 ± 11**	**4.5 ± 0.6**	**10.8 ± 0.4**	**1.22 ± 0.21**	**0.37 ± 0.09**	**0.67 ± 0.09**
3b	**31.3 ± 3.2**	**6.8 ± 0.5**	**7.5 ± 0.7**	**0.62 ± 0.10**	**0.22 ± 0.03**	**0.89 ± 0.18**
3c	**10.4 ± 0.8**	**2.5 ± 0.2**	**1.2 ± 0.02**	**0.39 ± 0.05**	**0.17 ± 0.01**	**0.33 ± 0.06**
Positive controls						
Tiron	**273 ± 32**	–	–	–	–	–
Quercetin	–	**1.8 ± 0.1**	–	–	–	–
Dihydrolipoic acid	–	–	**2.3 ± 0.3**	–	–	–
Rutin	–	–	–	**2.53 ± 0.37**	–	–
Ebselen	–	–	–	–	**0.50 ± 0.03**	**2.01 ± 0.22**

^{NA}No activity was found up to the highest tested concentration (in superscript)

*Scavenging effect (mean %) at the highest tested concentration (in superscript)

Scheme 2. Correlations between the E_{pa} at pH 7.4 and the scavenging activity against ROS and RNS of hydroxyxanthones 1-3a-c.



REFERENCES

[1]. (a) El-Seedi, H.R.; El-Ghorab, D.M.H.; El-Barbary, M.A.; Zayed, M.F.; Göransson, U.; Larsson, S.; Verpoorte, R. *Curr. Med. Chem.* 2009, *16*, 2581. (b) Pinto, M. M. M.; Sousa, M. E.; Nascimento, M. S. J. *Curr. Med. Chem.* 2005, *12*, 2517.
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[4]. Santos, C. M. M.; Freitas, M.; Ribeiro, D.; Gomes, A.; Silva, A. M. S.; Cavaleiro, J. A. S.; Fernandes, E. *Bioorg. Med. Chem.* 2010, *18*, 6776.
[5]. Santos, C. M. M.; Silva, A. M. S.; Cavaleiro, J. A. S. *Eur. J. Org. Chem.* 2009, 2642.

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Clementina Maria Moreira dos Santos

Has participated at the 3rd Portuguese Meeting on Medicinal Chemistry and 1st Portuguese-Spanish-Brazilian Meeting on Medicinal Chemistry and has presented a poster communication entitled "Electrochemical behavior of hydroxyxanthones versus ROS and RNS scavenging activities"

Portugal, Aveiro, 28th to 30th November 2012

Artur Silva

Prof. Artur M. S. Silva
Chairman

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