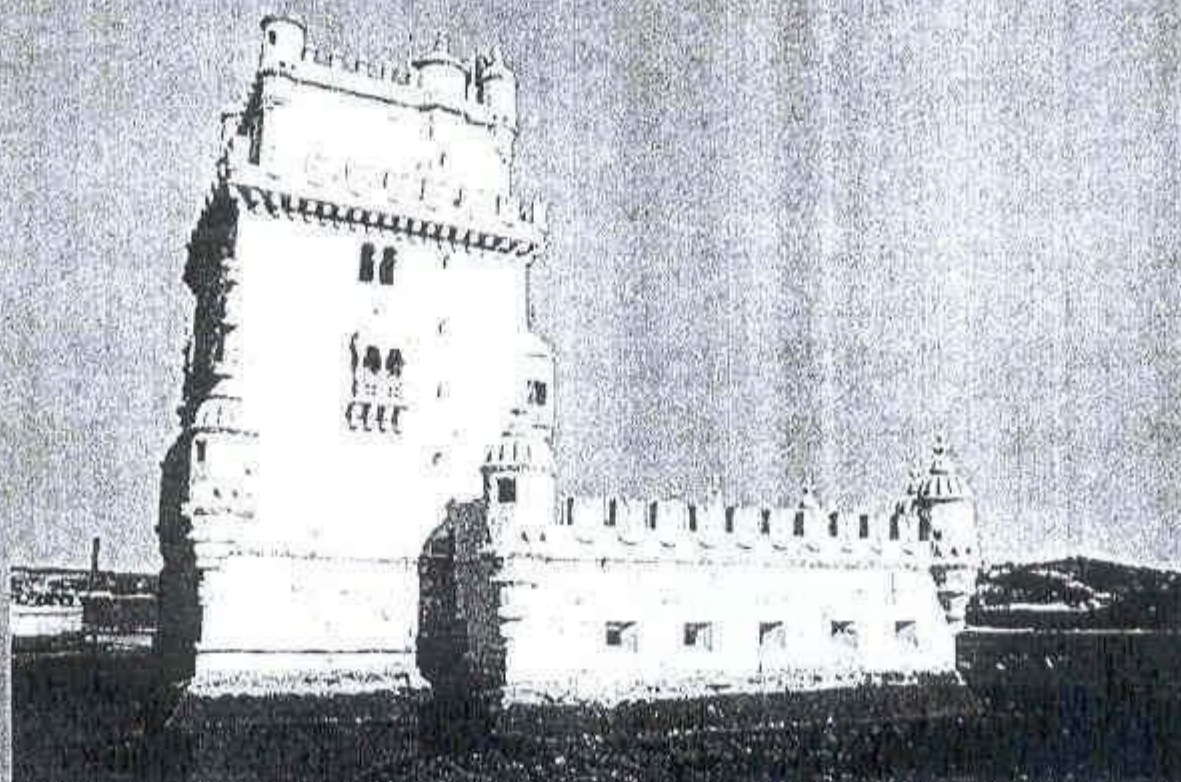


AOAC EUROPE SECTION INTERNATIONAL WORKSHOP

Enforcement of European Legislation on Food and
Water: Analytical and Toxicological Aspects

And

II ENCONTRO NACIONAL DE BROMATOLOGIA, HIDROLOGIA E TOXICOLOGIA



ABSTRACTS BOOK

Lisbon, 17th and 18th of April 2008

9TM

Toxicological Methods

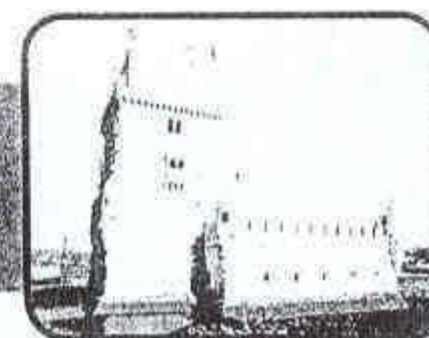
The ROS and RNS scavenging activity of 2-styrylchromones are effectively reproduced by cyclic voltammetry analysis

Ana Gomes^a, Eduarda Fernandes^a, M. Beatriz Q. Garcia^a, Artur M. S. Silva^b, Clementina M. M. Santos^b,
Diana C. G. A. Pinto^b, José A. S. Cavaleiro^b, and José L.F.C. Lima^a



ORDEM DOS FARMACÊUTICOS





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The ROS and RNS scavenging activity of 2-styrylchromones are effectively reproduced by cyclic voltammetry analysis

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^a REQUIMTE, Departamento de Química-Física, Faculdade de Farmácia, Universidade do Porto, Rua Aníbal Cunha, 164, 4099-030 Porto, Portugal.

^b Departamento de Química, Universidade de Aveiro, 3810-193 Aveiro, Portugal.

2-Styrylchromones (2-SC) are a chemical family of heterocyclic compounds, vinylogues of flavones (2-phenylchromones), whose occurrence in nature has been reported. Recently, several 2-SC derivatives were demonstrated to entail antioxidant properties, namely, xanthine oxidase inhibition, hepatoprotection against pro-oxidant agents in cellular and non cellular systems, and scavenging activity against reactive oxygen and reactive nitrogen species (ROS and RNS).

The capacity of phenolic compounds, especially the hydroxylated derivatives, to scavenge free radicals is intimately related to their effectiveness to donate hydrogen atoms (H.). Electrochemical oxidation can be used as a model for the scavenging reaction since both redox models involve the breaking of the same O-H bond and the donation of e⁻ and H⁺.

Thus, the aim of the present study was to compare the antioxidant effectiveness of 2-SC by different methodologies: the scavenging of ROS and RNS, studied by molecular spectroscopy (fluorescence, chemiluminescence, and UV-Vis absorption), and the electrochemical oxidation, studied by cyclic voltammetry.

The results from the scavenging assays were in agreement with those from the cyclic voltammetry, i.e., higher scavenging effects corresponded to lower values of oxidation potentials. Thus, in this family of compounds, oxidation potentials obtained by cyclic voltammetry seem to be applicable as indicators of radical scavenging ability.

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INTRODUCTION

2-Styrylchromones (2-SC) are a chemical family of heterocyclic compounds, vinylogues of flavones (2-phenylchromones), whose occurrence in nature has been reported. Recently, several 2-SC derivatives were demonstrated to entail antioxidant properties, namely, xanthine oxidase inhibition¹, hepatoprotection against pro-oxidant agents in cellular² and non cellular systems³, and scavenging activity against reactive oxygen and reactive nitrogen species (ROS and RNS)⁴.

The capacity of phenolic compounds, especially the hydroxylated derivatives, to scavenge free radicals is intimately related to their effectiveness to donate hydrogen atoms (H). Electrochemical oxidation can be used as a model for the scavenging reaction since both redox models involve the breaking of the same O-H bond and the donation of e⁻ and H⁺⁵.

Thus, the aim of the present study was to compare the antioxidant effectiveness of 2-SC by different methodologies: the scavenging of ROS and RNS⁴, studied by molecular spectroscopy (fluorescence, chemiluminescence, and UV-Vis absorption), and the electrochemical oxidation, studied by cyclic voltammetry.

EXPERIMENTAL PROCEDURES

Scavenging assays

The assays were performed in a microplate reader (Synergy HT, BIO-TEK) by different fluorescence, chemiluminescence and UV/Vis methodologies⁴.

Cyclic Voltammetry

Electrochemical measurements were carried out in an Autolab electrochemical system (Eco Chemie model PGSTAT 10) and data acquisition was accomplished through GPES software (Version 4.6). Voltammetric signals were recorded at room temperature. The working electrode was a glassy carbon electrode (3.0 mm diameter), a Ag/AgCl (KCl 3M) electrode was used as reference and a carbon electrode was used as auxiliar. The glassy carbon working electrode was polished with alumina 0.075 mm aqueous slurry before every experiment. Cyclic voltammograms were obtained by a single cycle performed at a scan rate of 100 mV s⁻¹.

STATISTICAL ANALYSIS

Pearson correlation test was performed using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com⁷.

RESULTS

Significant correlations were found for H₂O₂, hypochlorous acid (HOCl), singlet oxygen (¹O₂), and peroxynitrite, indicating that the scavenging mechanism against these reactive species is based on redox reactions. No significant correlation was found for superoxide radical (O₂⁻) (Table 2).

Table 1 – Chemical structures and oxidation potentials (E_{p_ox}) of the tested 2-SC


Compound	Chemical structure	R ¹	R ²	R ³	R ⁴	E _{p_ox} (V) vs Ag/AgCl	E _{p_ox} (V) vs Ag/AgCl
						1 st peak	2 nd peak
1A		OH	OH	OH	OH	-0.169	+0.162
1B		OH	OH	H	OH	-0.167	+0.060
2C		OH	OH	OH	H	-0.100	+0.019
1D		OH	OH	H	H	+0.171	-
2A		OH	H	OH	OH	+0.444	+0.772
2B		OH	H	H	OH	+0.485	-
2C		OH	H	OH	H	+0.452	-
2D		OH	H	H	H	+0.495	-
3A		H	H	OH	OH	+0.704	-
3B		H	H	H	OH	+0.787	-
3C		H	H	OH	H	+0.817	-
3D		H	H	H	H	-	-

Table 2 – Correlations (Pearson correlation coefficients) between the E_{p_ox} and the scavenging activity against ROS and RNS⁴ of the tested 2-SC

	O ₂ ⁻ a	H ₂ O ₂ ^b	HOCl ^c	¹ O ₂ ^d	ONOO ⁻ e (OH ⁻ / NaHCO ₃) ^f	ONOO ⁻ g (OH ⁻ / NaHCO ₃) ^f
E _{p_ox}	-0.027	-0.032**	-0.788*	-0.810**	-0.730*	-0.704**

^a) IC₂₇; ^b) % effect at 125 μM; ^c) IC₅₀; ^d) IC₃₀; *significant at p < 0.05. **significant at p < 0.01

CONCLUSION

The scavenging effects of the studied 2-SC are related to their electrochemical behaviour. Oxidation potentials can be used as a general indicator of radical scavenging ability.

REFERENCES

- Fernandes, E.; Carvalho, F.; Silva, A. M. S.; Santos, C. M. M.; Pinto, D. C. G. A.; Cavaleiro, J. A. S.; Bastos, M. L. *J. Enzyme Inhib. Med. Chem.* **2002**, *17*, 45.
- Fernandes, E.; Carvalho, M.; Carvalho, F.; Silva, A. M. S.; Santos, C. M. M.; Pinto, D. C. G. A.; Cavaleiro, J. A. S.; Bastos, M. L. *Arch. Toxicol.* **2003**, *77*, 500.
- Filipe, P.; Silva, A. M.; Morliere, P.; Brito, C. M.; Patterson, L. K.; Hug, G. L.; Silva, J. N.; Cavaleiro, J. A.; Maziere, J. C.; Freitas, J. P.; Santos, R. *Biochem. Pharmacol.* **2004**, *67*, 2207.
- Gomes, A.; Fernandes, E.; Silva, A. M.; Santos, C. M.; Pinto, D. C.; Cavaleiro, J. A.; Lima, J. L. *Bioorg. Med. Chem.* **2007**, *15*, 6027.
- van Acker, S. A.; van den Berg, D. J.; Tromp, M. N.; Griffioen, D. H.; van Bennekum, W. P.; van der Vijgh, W. J.; Bast, A. *Free Radic. Biol. Med.* **1996**, *20*, 331.