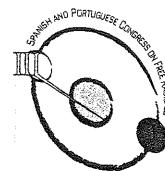


19-21 MAY 2010

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INTERNATIONAL SYMPOSIUM ON THE PATHOPHYSIOLOGY
OF REACTIVE OXYGEN AND NITROGEN SPECIES

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PS 5. RONS IN PLANTS

PS5-15

ROS AND RNS SCAVENGING ACTIVITY BY 2,3-DIARYLXANTHONES

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Xanthones constitute an important class of heterocyclic compounds that commonly occur in nature.¹ 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.² 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.³ Thus, the main goal of this work was to evaluate the putative scavenging effect on 'NO, ONOO⁻ and O₂⁻ by the new synthetic 2,3-diarylxanthones (1), using *in vitro* non-cellular systems.⁴ 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 IC₅₀ 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.

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2. C. M. M. Santos, A. M. S. Silva, J. A. S. Cavaleiro, *Eur. J. Org. Chem.*, 2009, 2642.
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ROS AND RNS SCAVENGING ACTIVITY BY 2,3-DIARYLXANTHONES

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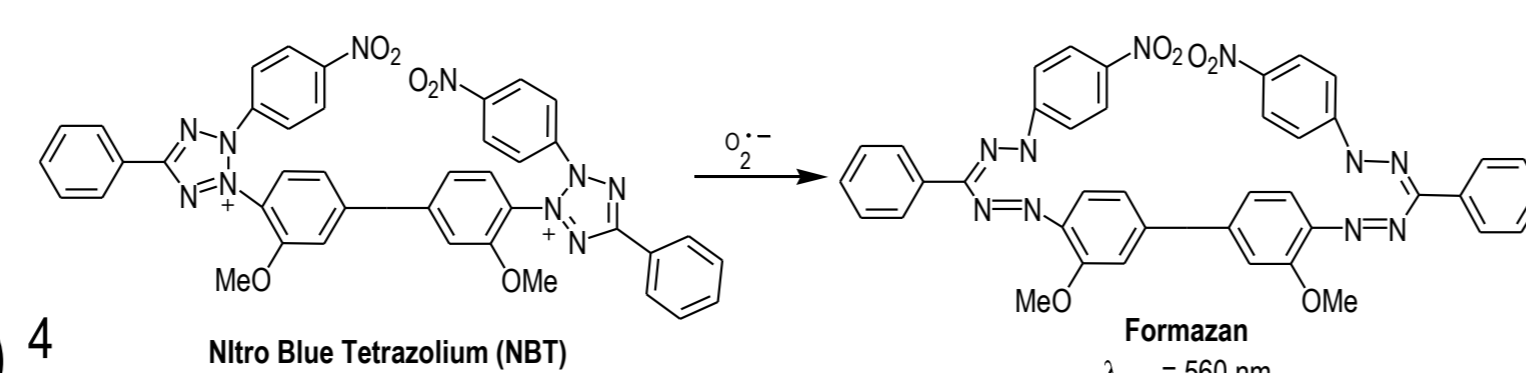
INTRODUCTION

- Xanthenes constitute an important class of heterocyclic compounds that commonly occur in nature.¹ 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.²
- The pharmacological properties of both natural and synthetic xanthenes 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.³
- Thus, the main goal of this work was to evaluate the putative scavenging effect on $\cdot\text{NO}$, $\text{ONOO}\cdot$ and $\text{O}_2^{\cdot-}$ of the new synthetic 2,3-diaryl xanthenes (Figure 1), using *in vitro* non-cellular systems.⁴

METHODS

Superoxide radical ($\text{O}_2^{\cdot-}$)

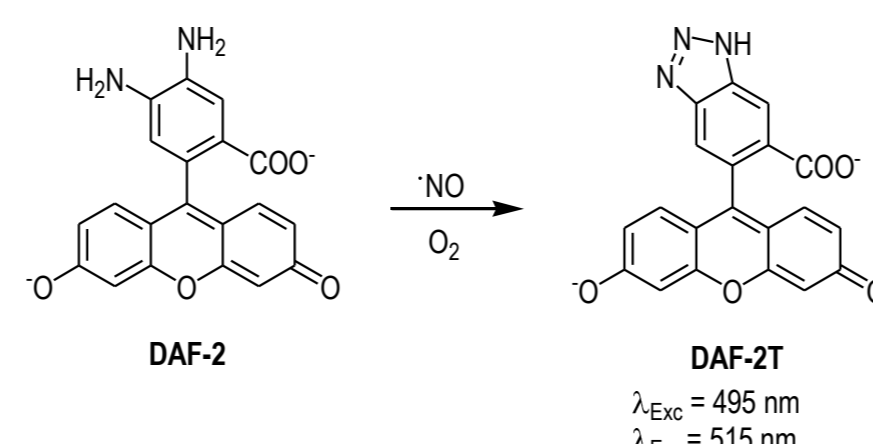
The $\text{O}_2^{\cdot-}$ was generated by the NADH/ PMS system and the $\text{O}_2^{\cdot-}$ scavenging activity was determined by monitoring the $\text{O}_2^{\cdot-}$ -induced reduction of nitroblue tetrazolium chloride (NBT).⁴



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

Nitric oxide ($\cdot\text{NO}$)

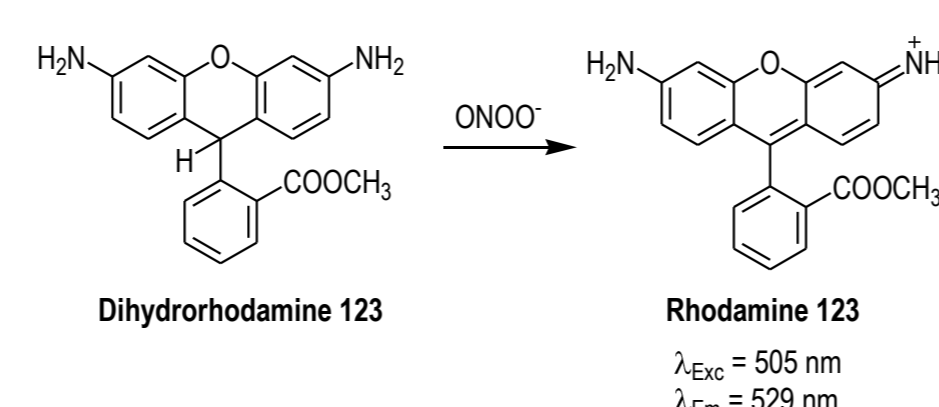
The $\cdot\text{NO}$ scavenging activity was measured by monitoring the $\cdot\text{NO}$ -induced oxidation of non-fluorescent 4,5-diaminofluorescein (DAF-2) to the fluorescent triazolofluorescein (DAF-2T).⁴



$\cdot\text{NO}$ was generated by NOC-5, the tested compounds dissolved in DMSO and the fluorimetric signal was detected after a 30 min incubation period, at 37°C. Rutin was used as positive control. The results were expressed as the percentage inhibition of $\cdot\text{NO}$ -induced oxidation of DAF-2 (Table 1, Figure 3).

Peroxynitrite ($\text{ONOO}\cdot$)

The $\text{ONOO}\cdot$ scavenging activity was measured by monitoring the $\text{ONOO}\cdot$ -induced oxidation of non-fluorescent dihydrorhodamine 123 (DHR) to fluorescent rhodamine 123.⁴



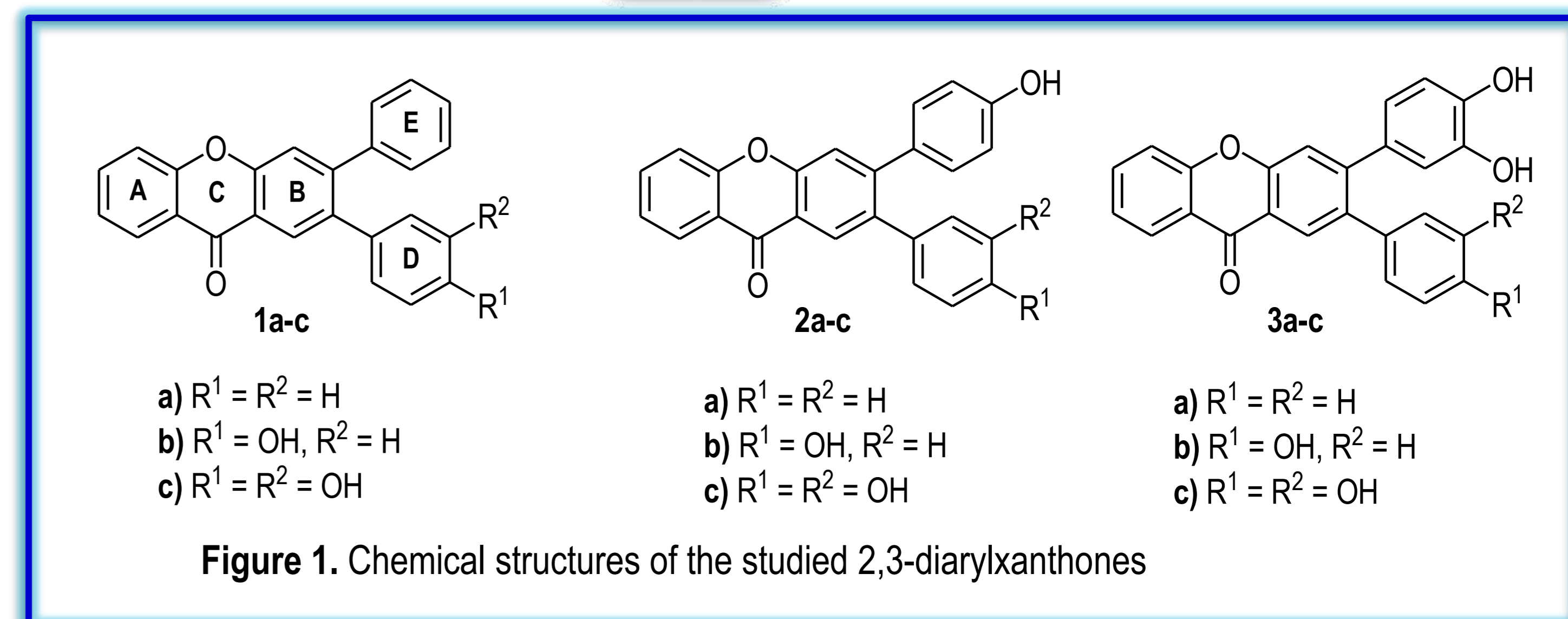
The assays were performed at 37°C, the tested compounds dissolved in DMSO and the fluorimetric signal detected after a 2 min incubation period. Ebselen was used as positive control. In a parallel set of experiments, the assays were performed in the presence of 25 mM NaHCO_3 in order to simulate the physiological CO_2 concentrations *in vivo*. The results were expressed as the percentage inhibition of $\text{ONOO}\cdot$ -induced oxidation of DHR. (Table 1, Figure 4 and 5).

CONCLUSIONS

- The obtained results revealed that the tested 2,3-diaryl xanthenes 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 xanthenes with two catechol rings were the most potent scavengers of all tested ROS and RNS.
- In conclusion, the new 2,3-diaryl xanthenes 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.



RESULTS

Table 1

$\text{O}_2^{\cdot-}$, $\cdot\text{NO}$ and $\text{ONOO}\cdot$ (with and without 25 mM NaHCO_3) scavenging effects (IC_{50} , mean SEM) of the tested 2,3-diaryl xanthenes and positive controls.

Compound	IC_{50} (μM)			
	$\text{O}_2^{\cdot-}$	$\cdot\text{NO}$	$\text{ONOO}\cdot$ without NaHCO_3	$\text{ONOO}\cdot$ with NaHCO_3
2,3-diaryl xanthenes				
1a	NA ²⁵ μM	39%* ²⁰⁰ μM	29%* ⁵⁰ μM	48%* ⁵⁰ μM
1b	NA ²⁰⁰ μM	175 36	1.55 0.14	1.80 0.39
1c	28.1 2.3	1.88 0.18	0.40 0.03	0.54 0.08
2a	NA ²⁰⁰ μM	41%* ²⁰⁰ μM	2.66 0.29	2.00 0.15
2b	166 16	108 18	1.72 0.21	0.97 0.25
2c	20.3 2.5	0.42 0.05	0.26 0.05	0.78 0.26
3a	76 11	1.22 0.21	0.37 0.09	0.67 0.09
3b	31.3 3.2	0.62 0.10	0.22 0.03	0.89 0.18
3c	10.4 0.8	0.39 0.05	0.17 0.01	0.33 0.06
Positive controls				
Tiron	273 32	—	—	—
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)

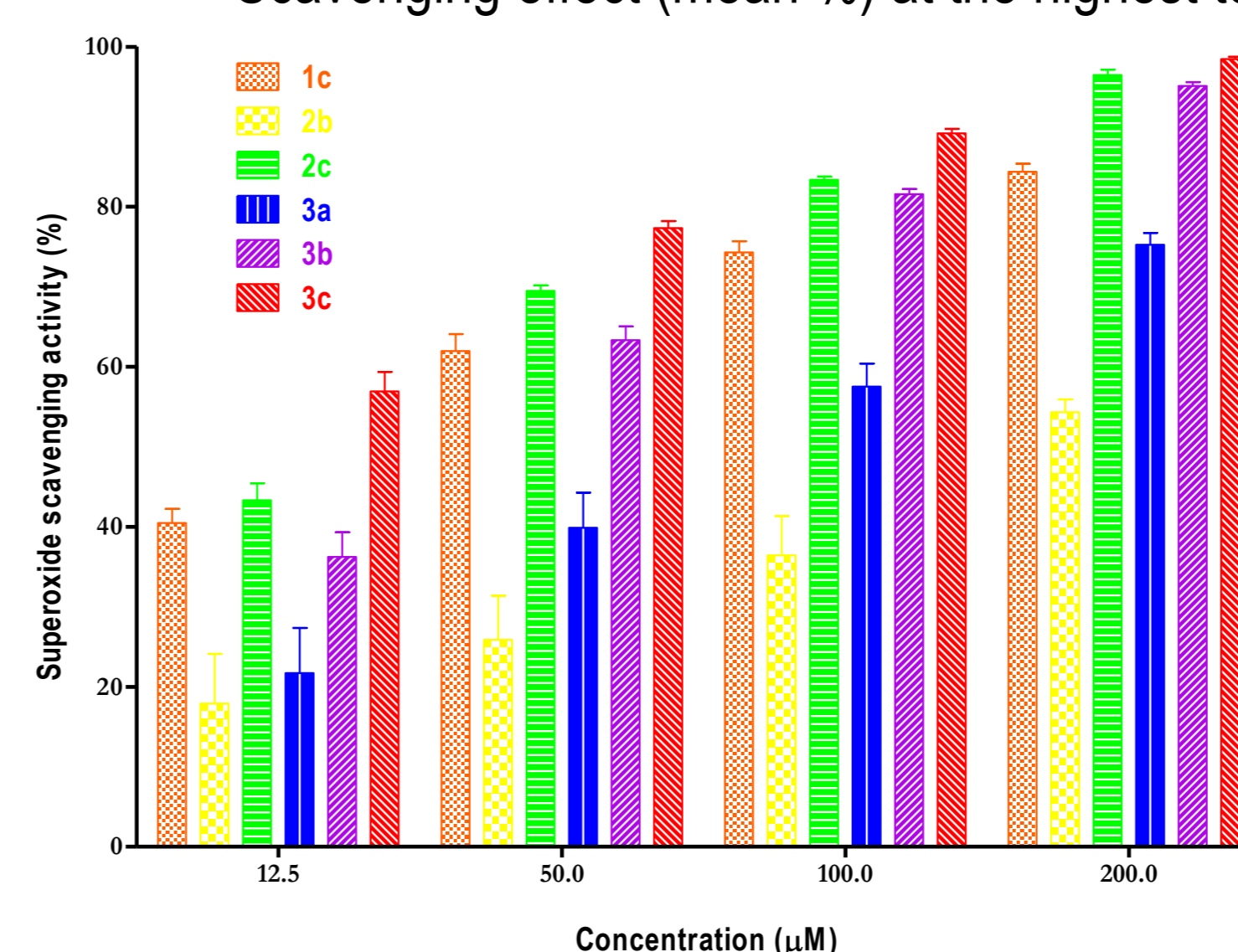


Figure 2. $\text{O}_2^{\cdot-}$ scavenging activity of 2,3-diaryl xanthenes 1c, 2c, 3c. Each column represents the values obtained from five experiments, performed in triplicate (mean SEM).

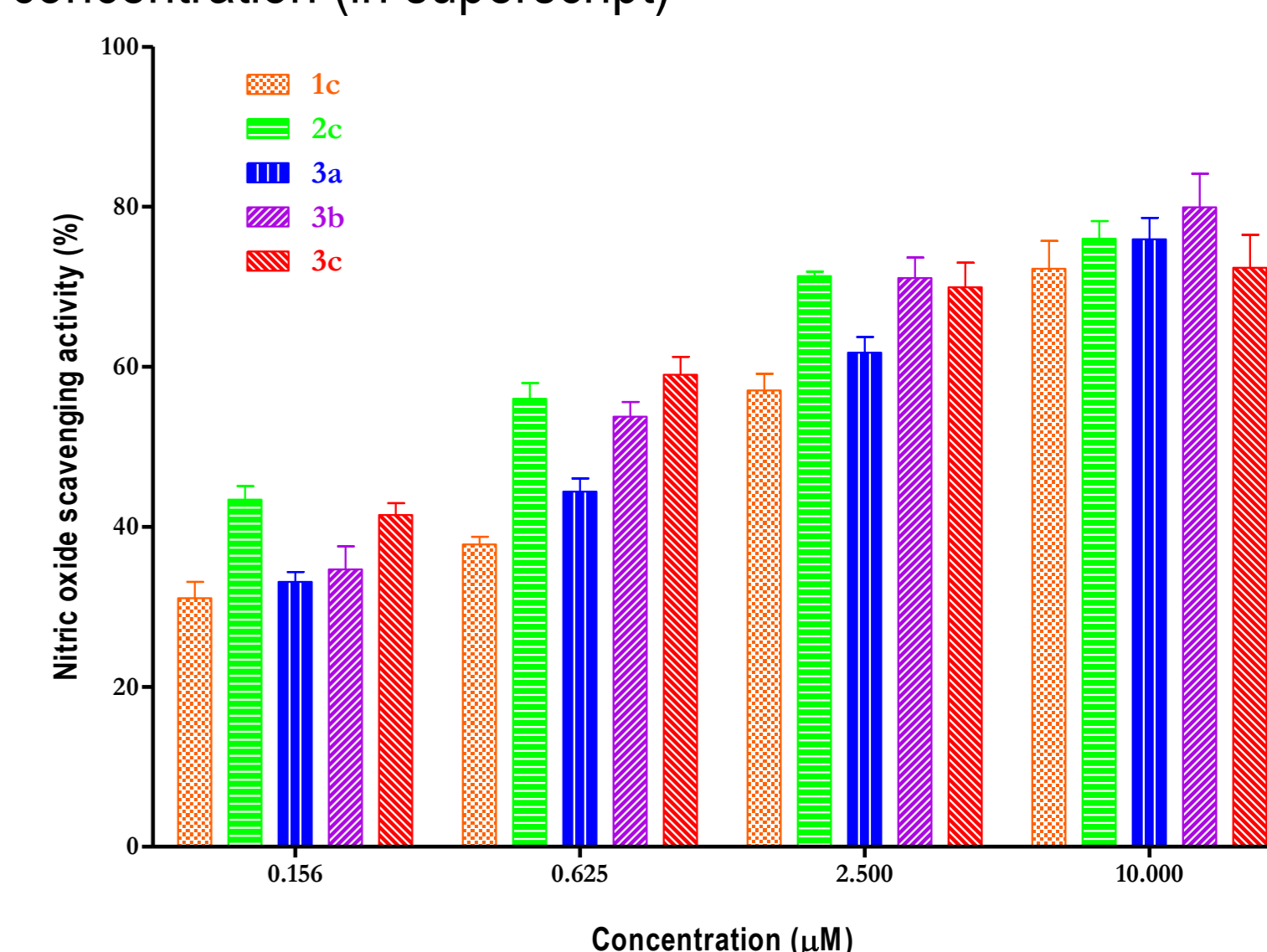


Figure 3. $\cdot\text{NO}$ scavenging activity of 2,3-diaryl xanthenes 1c, 2c, 3a-c. Each column represents the values obtained from five experiments, performed in triplicate (mean SEM).

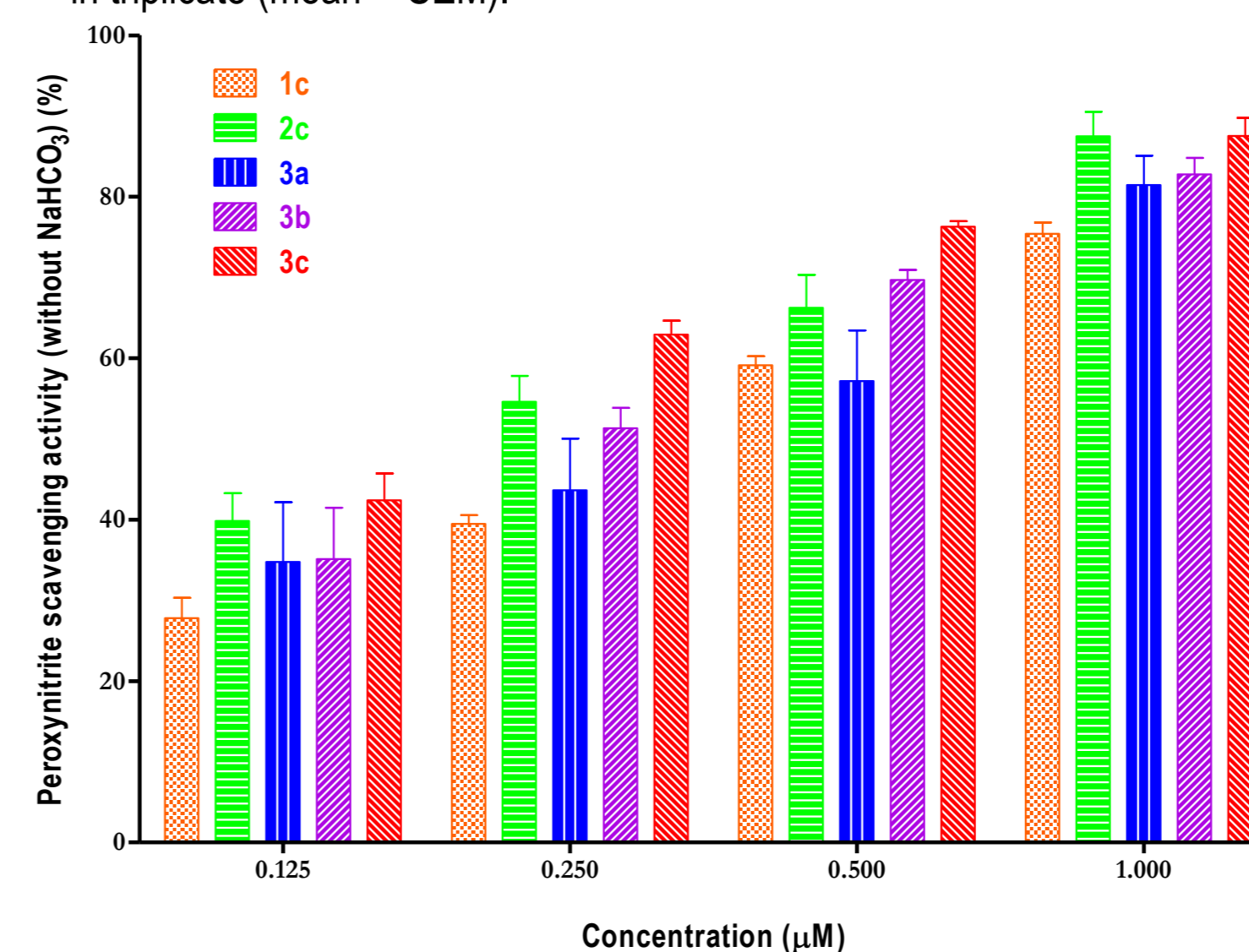


Figure 4. $\text{ONOO}\cdot$ scavenging activity of 2,3-diaryl xanthenes 1c, 2c, 3a-c. Each column represents the values obtained from five experiments, performed in triplicate (mean SEM).

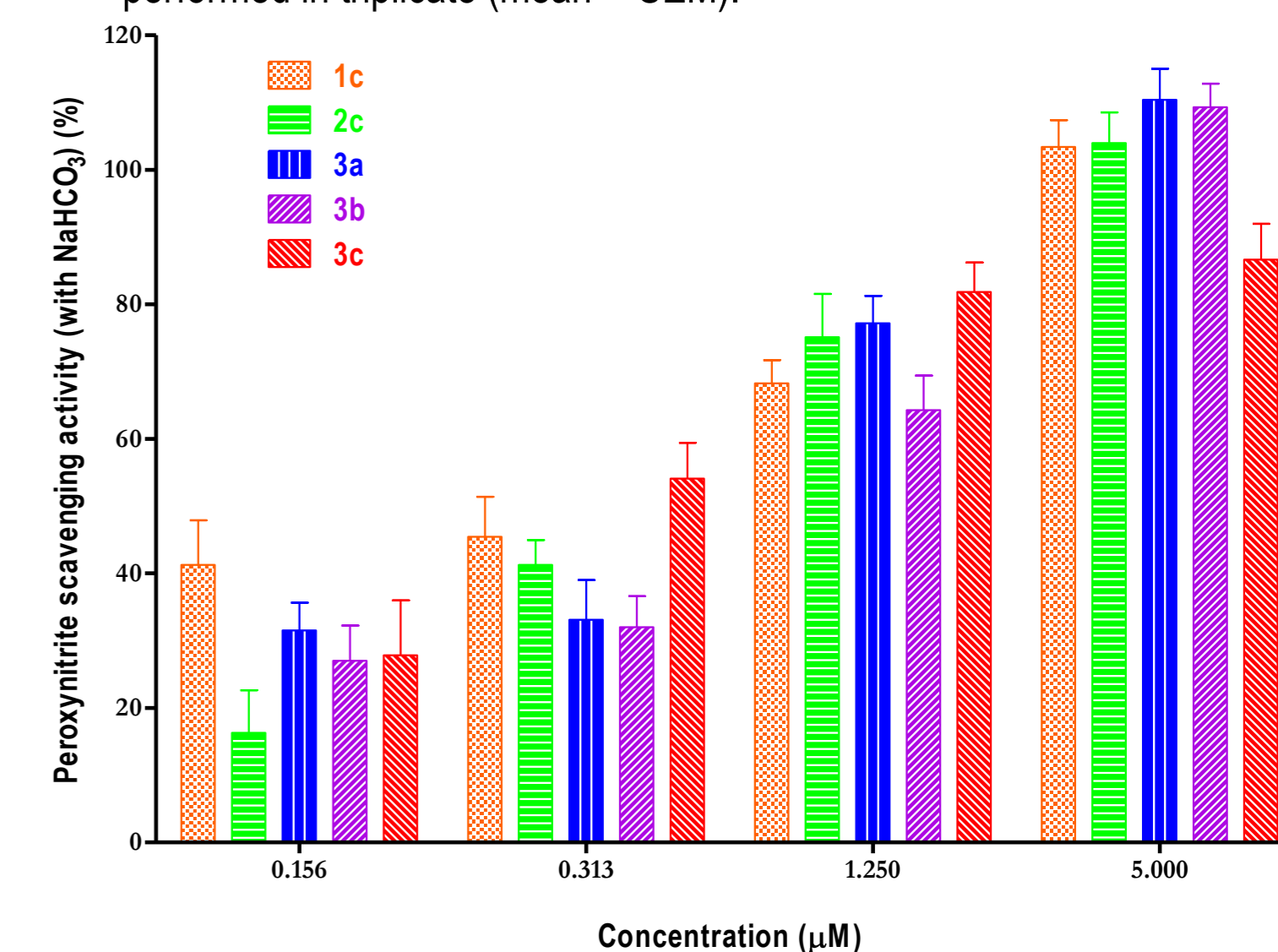


Figure 5. $\text{ONOO}\cdot$ scavenging activity of 2,3-diaryl xanthenes 1c, 2c, 3a-c, in the presence of NaHCO_3 , 25 mM. Each column represents the values obtained from five experiments, performed in triplicate (mean SEM).

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