Modulation of human neutrophils' oxidative burst by hydroxylated 2-styrylchromones: the relevance of the catechol group

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Introduction

2-Styrylchromones (2-SC, **Figure 1**) are a group of oxygen-containing heterocyclic compounds, which are characterized by the attachment of a styryl group to the C-2 position of their chromone core. 2-SC can be found in nature or can be chemically synthesized, in the laboratory. As their presence in nature is scarce, the synthetic origin is the most common. Over the years, several biological activities have been attributed to 2-SC, such as antioxidant, anti-inflammatory, antimicrobial, antiviral, and antitumoral. In the literature there are few studies that evaluate the effect of 2-SC against reactive pro-oxidant species, in *in vitro* non-cellular systems [1,2]. Neutrophils constitute the body's first line of defence against pathogens. During the inflammatory process, there is an increase in quantity of neutrophils. In order to destroy, remove and protect the organism against pathogens, these cells produce a series of reactive pro-oxidant species, namely reactive oxygen and nitrogen species (ROS and RNS, respectively), under a process designated as oxidative burst [3]. To the best of our knowledge, there are no reports in the literature about the modulatory effect of 2-SC on human neutrophils' oxidative burst.

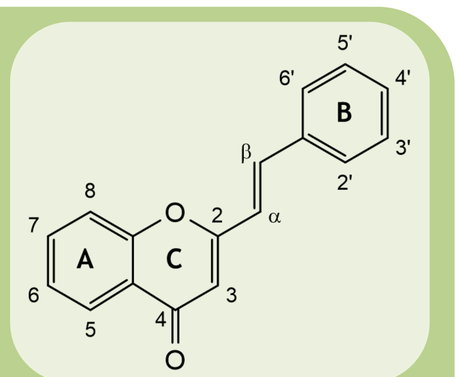


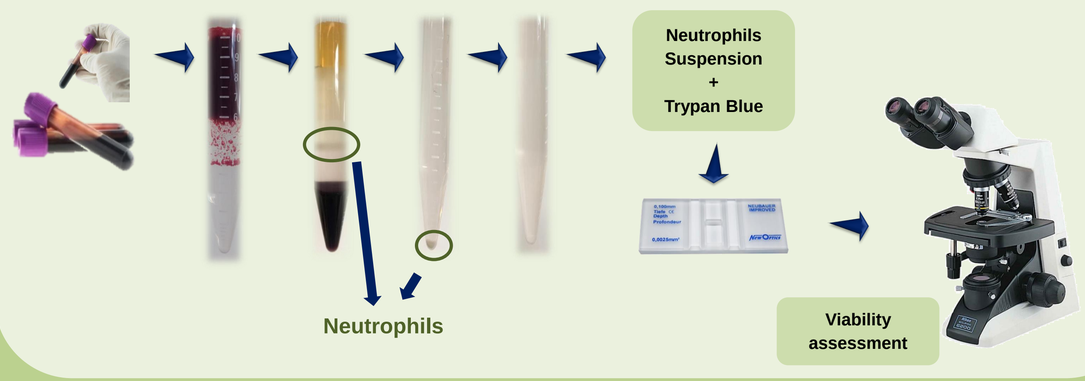
Figure 1 Chemical structure of 2-SC and the numbering system adopted.

Aim

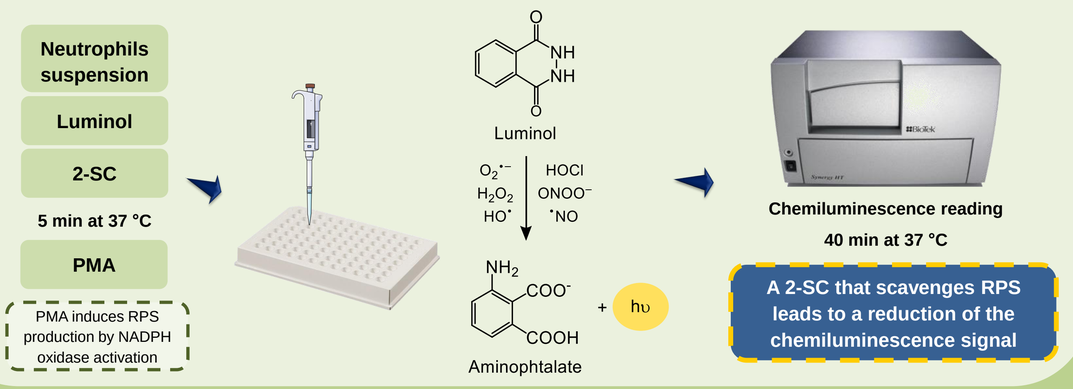
The present work aimed to evaluate the modulation of human neutrophils' oxidative burst by a panel of hydroxylated 2-SC (**Figure 2**), analysing the structure-activity relationships.

Methods

Isolation of human neutrophils by density gradient centrifugation method



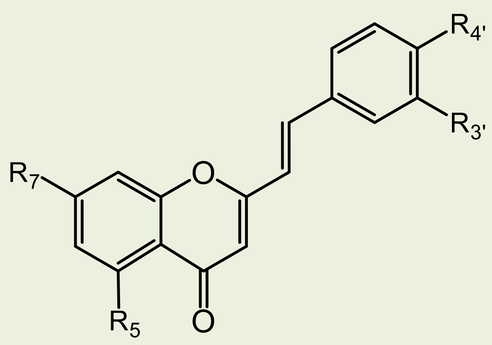
Modulation of human neutrophils' oxidative burst



PMA - phorbol-12-myristate-13-acetate
RPS - reactive pro-oxidant species

NADPH - nicotinamide adenine dinucleotide phosphate

Results



Group A

1 $R_5 = R_7 = R_{3'} = R_{4'} = OH$
2 $R_5 = H$; $R_7 = R_{3'} = R_{4'} = OH$
3 $R_5 = R_{3'} = R_{4'} = OH$; $R_7 = H$
4 $R_5 = R_7 = H$; $R_{3'} = R_{4'} = OH$

Group B

5 $R_5 = R_7 = R_{4'} = OH$; $R_{3'} = H$
6 $R_5 = R_{3'} = H$; $R_7 = R_{4'} = OH$
7 $R_5 = R_{4'} = OH$; $R_7 = R_{3'} = H$
8 $R_5 = R_7 = R_{3'} = H$; $R_{4'} = OH$

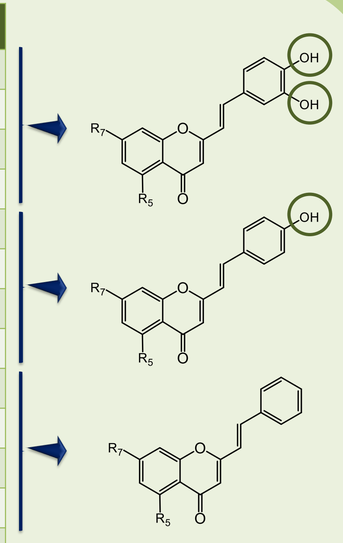
Group C

9 $R_5 = R_7 = OH$; $R_{3'} = R_{4'} = H$
10 $R_5 = R_{3'} = R_{4'} = H$; $R_7 = OH$
11 $R_5 = OH$; $R_7 = R_{3'} = R_{4'} = H$
12 $R_5 = R_7 = R_{3'} = R_{4'} = H$

Figure 2 Chemical structures of the tested 2-SC..

Table 1 Inhibition of human neutrophils' oxidative burst by the tested 2-SC and the positive control, quercetin, expressed as percentage of inhibition of luminol oxidation (% ± SEM) or IC_{50} values (μM, mean ± SEM).

Group	2-SC	Inhibitory activity
A	**1**	**0.8 ± 0.1**
	2	**0.7 ± 0.1**
	3	42 ± 10 % [0.75 μM]
	4	**0.8 ± 0.1**
B	**5**	3.1 ± 0.3
	6	4.1 ± 0.3
	7	6.2 ± 0.3
	8	7.5 ± 0.4
C	**9**	< 30% [50 μM]
	10	53 ± 4 % [75 μM]
	11	< 30% [25 μM]
	12	< 30% [75 μM]
Positive Control	**Quercetin**	0.8 ± 0.1



Note: The percentage of inhibition was expressed for the highest concentration (in superscript) that could be tested under the assay conditions to avoid interferences with the methodology (n≥4). SEM - standard error of the mean. The most active 2-SC tested are highlighted in **orange**.

Conclusions

- ✓ The 2-SC from group A, **1**, **2** and **4**, with a catechol group (C-3' and C-4') on B-ring, were the most active compounds, with $IC_{50} < 1$ μM.
- ✓ The 2-SC from group B, with an OH at C-4' on B-ring, showed a variable effect depending on the number and position of the OH substituents on A-ring.
- ✓ The 2-SC from group C, without any substitution on B-ring, showed very low or no inhibitory activity of reactive pro-oxidant species production.

The catechol on B-ring seems to play an important role in the modulation of human neutrophils' oxidative burst by 2-SC

Acknowledgements

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References

1. Gomes, A.; Freitas, M.; Fernandes, E.; Lima, J. L., Biological activities of 2-styrylchromones. Mini Rev Med Chem 2010, 10 (1), 1-7.
2. Santos, C. M. M.; Silva, A. M. S., An Overview of 2-Styrylchromones: Natural Occurrence, Synthesis, Reactivity and Biological Properties. Eur. J. Org. Chem. 2017, 2017 (22), 3115-3133.
3. Freitas, M.; Lima, J. L. F. C.; Fernandes, E., Optical probes for detection and quantification of neutrophils' oxidative burst. A review. Anal. Chim. Acta 2009, 649 (1), 8-23.