The impact of B-ring substituents on the styrylchromones' ability to modulate the levels of reactive prooxidant species

Mariana Lucas[a*], Marisa Freitas[a], Joana L.C. Sousa[b], Artur M.S. Silva[b], Eduarda Fernandes[a], Daniela Ribeiro[a,c]

[a] LAQV, REQUIMTE, Laboratory of Applied Chemistry, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal.
[b] LAQV-REQUIMTE & Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal.
[c] Faculty of Agrarian Sciences and Environment, University of the Azores, 9700-042 Angra do Heroísmo, Açores, Portugal.
** email: mflucas@ff.up.pt*

INTRODUCTION

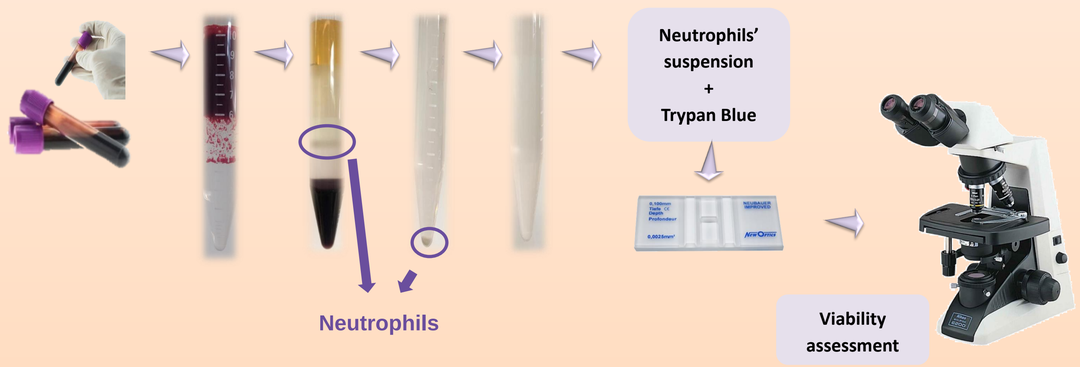
2-Styrylchromones (2-SC) are a small group of heterocyclic compounds that have a chromone core with a styryl group attached to it at C-2. Most of the know 2-SC are of synthetic origin and have demonstrated several bioactive properties, such as antioxidant, anti-inflammatory and antitumoral. Among the effects reported in the literature, 2-SC showed potential as prooxidant reactive species (PRS) scavengers in *in vitro* non-cellular systems [1,2]. Neutrophils are one of the main cells involved in the inflammatory response and have microbicidal functions, for which they use reactive oxygen and nitrogen species (ROS and RNS) produced during the oxidative burst. However, the overproduction of ROS and RNS may result in harmful effects to the body, as it can compromise endogenous antioxidant defenses, leading to an imbalance between the amount produced and its removal [3]. To the best of our knowledge, there are no reports in the literature about the effect of 2-SC on human neutrophils' viability and oxidative burst.

AIM

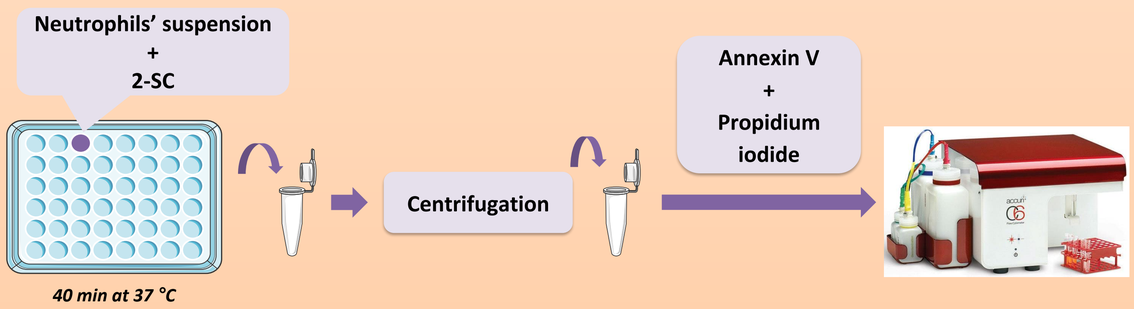
The present work aimed to evaluate the influence of B-ring substituents of 2-SC (Figure 1) on human neutrophils' viability and modulation of oxidative burst.

METHODS

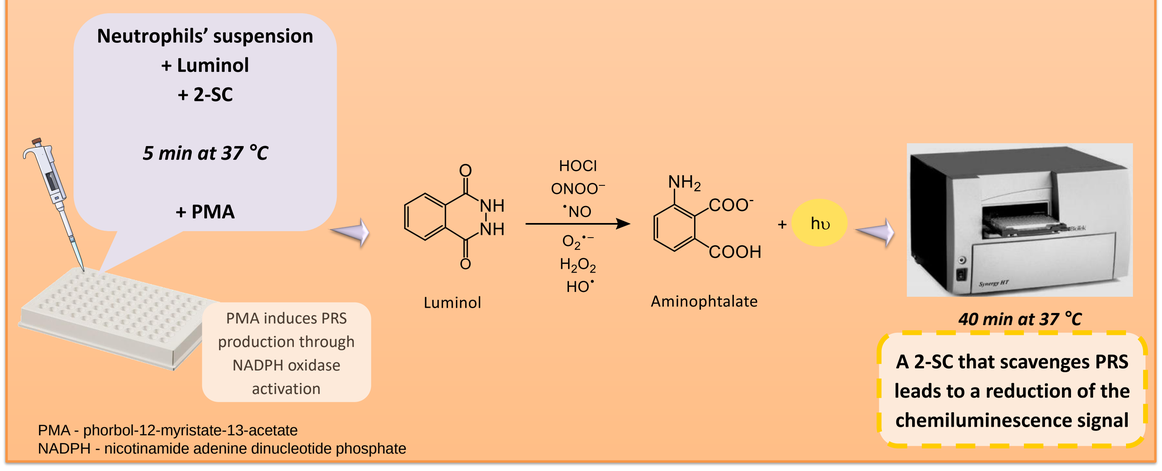
Isolation of human neutrophils by the density gradient centrifugation method [4]



Evaluation of neutrophils' viability by annexin V / propidium iodide assay [4]

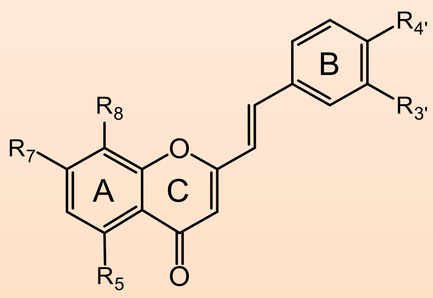


Modulation of human neutrophils' oxidative burst [4]



PMA - phorbol-12-myristate-13-acetate
NADPH - nicotinamide adenine dinucleotide phosphate

RESULTS



2-SC	R_5	R_7	R_8	$R_{3'}$	$R_{4'}$
1	-	-	-	OH	OH
2	OCH_3	OCH_3	-	OH	OH
3	-	OCH_3	OCH_3	OH	OH
4	-	-	-	OCH_3	OCH_3
5	OCH_3	OCH_3	-	OCH_3	OCH_3
6	-	OCH_3	OCH_3	OCH_3	OCH_3
7	-	-	-	OBn	OBn
8	OCH_3	OCH_3	-	OBn	OBn
9	-	OCH_3	OCH_3	OBn	OBn

Figure 1 Chemical structures of the tested 2-SC.

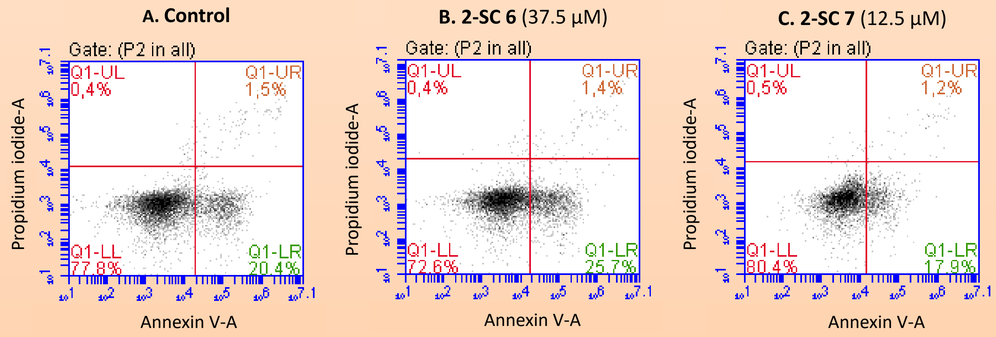
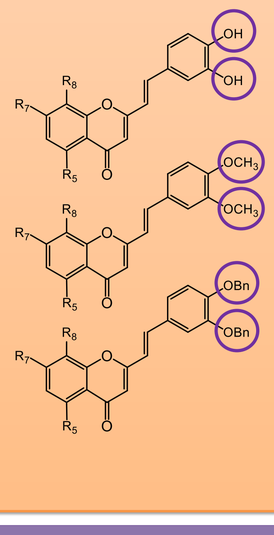


Figure 2 Examples of flow cytometry plots of annexin V (xx axis) / propidium iodide (yy axis) assay for 2-SC concentrations that did not affect neutrophils' viability: (**A**) control (without 2-SC), (**B**) 2-SC **6** (37.5 µM), and (**C**) 2-SC **7** (12.5 µM).

Table 1 2-SC concentrations that did not affect human neutrophils' viability and inhibition of oxidative burst by the tested 2-SC and the positive control.

Group	2-SC	Concentration (µM)	Inhibitory activity (% ± SEM) or IC_{50} (µM, mean ± SEM)
A	**1**	12.5	**1.0 ± 0.1**
	2	12.5	**0.8 ± 0.1**
	3	12.5	**1.0 ± 0.2**
B	**4**	25	< 30% [25 µM]
	5	75	54 ± 2 % [50 µM]
	6	37.5	20 ± 2
C	**7**	12.5	< 30% [12.5 µM]
	8	12.5	< 30% [12.5 µM]
	9	12.5	< 30% [12.5 µM]
Positive Control	Quercetin	5.0	0.8 ± 0.1



Note: The percentage of inhibition is expressed for the highest tested 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 **red**.

CONCLUSIONS

- None of the 2-SC affected neutrophils' viability, up to the maximum tested concentration.
- The 2-SC from group A were the most active compounds (IC_{50} ≈ 1 µM).
- The type of substituents present on B-ring, namely the catechol group, influence the modulation of neutrophils' oxidative burst and PRS production.

Acknowledgements

The work was supported by PT national funds (FCT/MCTES, Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) through grant UIDB/50006/2020 (LAQV-REQUIMTE Associate Laboratory) and from the European Union (FEDER funds through COMPETE POCI-01-0145-FEDER-029253). Mariana Lucas thanks FCT and ESF (European Social Fund) through Programa Operacional Regional do Norte (NORTE 2020) for her PhD grant (2021.06746.BD). Marisa Freitas further acknowledges her contract under the Scientific Employment Stimulus - Individual Call (CEEC Individual) 2020.04126.CEECIND/CP1596/CT0006.

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.
4. Freitas, M.; Costa, V.M.; Ribeiro, D.; Couto, D.; Porto, G.; Carvalho, F.; Fernandes, E., Acetaminophen prevents oxidative burst and delays apoptosis in human neutrophils. Toxicol. Lett. 2013, 219, 170-177

The 7th International Electronic Conference on Medicinal Chemistry
01–30 NOVEMBER 2021 | ONLINE