

Anti-inflammatory potential of 2-styrylchromones regarding their interference with arachidonic acid metabolic pathways

Ana Gomes^a, Eduarda Fernandes^{a,*}, Artur M.S. Silva^b, Diana C.G.A. Pinto^b,
Clementina M.M. Santos^{b,c}, José A.S. Cavaleiro^b, José L.F.C. Lima^a

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

^bDepartamento de Química & QOPNA, Universidade de Aveiro, 3810-193 Aveiro, Portugal

^cDepartamento de Produção e Tecnologia Vegetal, Escola Superior Agrária de Bragança, Campus de Santa Apolónia, 5301-855 Bragança, Portugal

ABSTRACT

Cyclooxygenases (COXs) are the key enzymes in the biosynthesis of prostanoids. COX-1 is a constitutive enzyme while the expression of COX-2 is highly stimulated in the event of inflammatory processes, leading to the production of large amounts of prostaglandins (PGs), in particular PGE₂ and PGI₂, which are pro-inflammatory mediators.

Lipoxygenases (LOXs) are enzymes that produce hydroxy acids and leukotrienes (LTs). 5-LOX metabolizes arachidonic acid to yield, among other products, LTB₄, a potent chemoattractant mediator of inflammation.

The aim of the present work was to evaluate the anti-inflammatory potential of 2-styrylchromones (2-SC), a chemical family of oxygen heterocyclic compounds, vinylogues of flavones (2-phenylchromones), by studying their COX-1 and COX-2 inhibitory capacity as well as their effects on the LTB₄ production by stimulated human polymorphonuclear leukocytes (PMNL).

Some of the tested 2-SC were able to inhibit both COX-1 activity and LTB₄ production which makes them dual inhibitors of the COX and 5-LOX pathways. The most effective compounds in this study were those having structural moieties with proved antioxidant activity (3',4'-catechol and 4'-phenol substituted B-rings).

This type of compounds may exhibit anti-inflammatory activity with a wider spectrum than that of classical non-steroidal anti-inflammatory drugs (NSAIDs) by inhibiting 5-LOX product-mediated inflammatory reactions, towards which NSAIDs are ineffective.

1. Introduction

Arachidonic acid is the most abundant polyunsaturated fatty acid in the phospholipid bilayer of cell membranes. This second-messenger molecule is released by phospholipase A₂ in activated cells and further metabolized in different eicosanoids by the cyclooxygenase and lipoxygenase pathways (Fig. 1). Cyclooxygenases (COXs) are the key enzymes in the biosynthesis of prostanoids. In mammalian cells, COXs exist in at least two isoforms (COX-1 and COX-2). COX-1 is a constitutive enzyme, expressed in almost every cell type, responsible for the physiological production of prostaglandins (PGs) i.e., PGI₂, PGE₂, PGF_{2α}, and PGD₂, in diverse organs and thromboxane A₂ in platelets. COX-

2, on the other hand, is usually absent in most tissues (with the exception of kidney, parts of the brain, and gravid uterus where COX-2 is constitutive) but its expression can be readily induced by numerous stimuli such as growth factors, tumor promoters, or cytokines [1,2]. The earliest expression of COX-2 is detected in stromal cells, but it also can be found in multiple cells, like epithelial, endothelial, and stromal cells in several types of tumors [3]. In addition, COX-2 is highly expressed by cells that are involved in inflammation (e.g., neutrophils, macrophages, monocytes, mast cells, synoviocytes), emerging as the isoform primarily responsible for the synthesis of the prostanoids involved in pathological processes, such as acute and chronic inflammatory states. These inflammation-related cell types can be stimulated by lipopolysaccharide, phorbol esters, cytokines, or growth factors, producing large amounts of PGs, in particular PGE₂ and PGI₂, which are pro-inflammatory mediators that increase vascular permeability and promote edema at the sites of inflammation. Furthermore, these

* Corresponding author. Tel.: +351 222078968; fax: +351 222078961.
E-mail address: egracas@ff.up.pt (E. Fernandes).

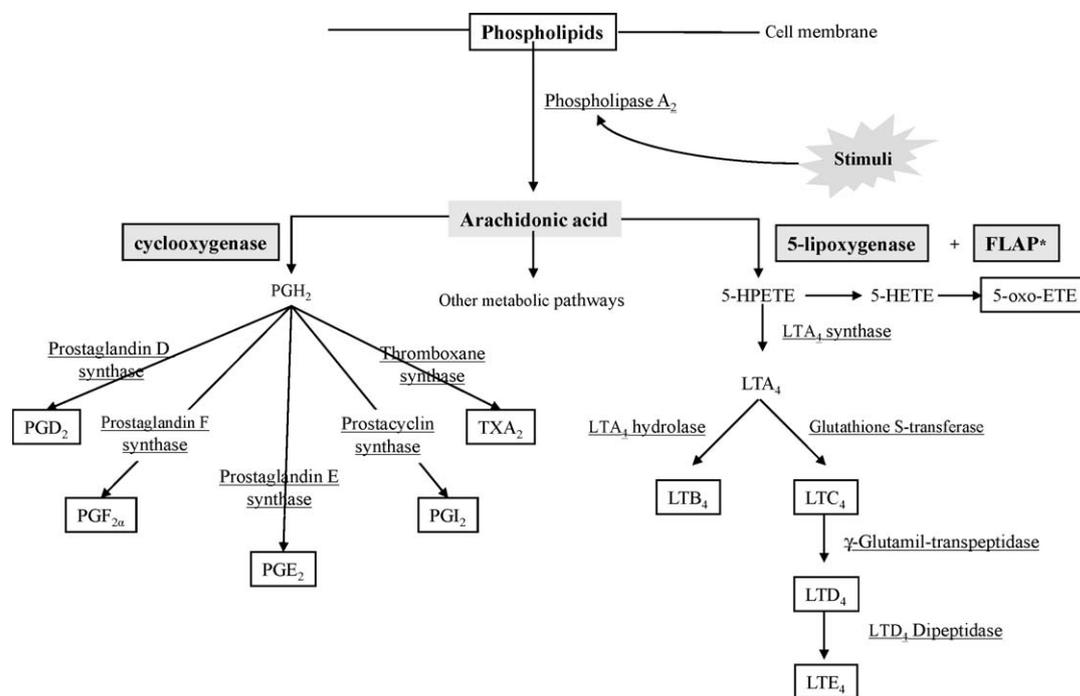


Fig. 1. Metabolism of arachidonic acid by the cyclooxygenase and 5-lipoxygenase pathways. (*) 5-Lipoxygenase-activating protein.

PGs are involved in the nociception of the inflammatory pain [4]. Thus, COX-2 isoform has been associated with acute and chronic inflammatory disorders [5] as well as in the carcinogenesis and tumor progression [3].

Lipoxygenases (LOXs) are enzymes that produce hydroxy acids and leukotrienes (LTs). From the LOXs existent in the mammalian tissues, 5-LOX, which is mainly found in cells of myeloid origin, i.e., polymorphonuclear leukocytes (PMNL), mast cells, macrophages, is the most implicated in inflammatory and allergic disorders [6]. 5-LOX metabolizes arachidonic acid to yield 5(S)-hydroxy-6,8,11,14-eicosatetraenoic acid (5-HETE), which is further metabolized to the bioactive 5-oxo-6,8,11,14-eicosatetraenoic acid (5-oxo-EETE) by PMNL 5-hydroxyeicosanoid dehydrogenase [7], and various LTs (LTA_4 - LTE_4) [8]. 5-oxo-EETE and LTB_4 , produced by PMNL, are potent chemoattractant mediators of inflammation. LTB_4 stimulates neutrophil chemotaxis, enhances neutrophil-endothelial interactions, and stimulates neutrophil activation, leading to degranulation and the release of mediators, enzymes, and superoxide radicals [9], while 5-oxo-EETE is much more active than LTB_4 as an eosinophil chemoattractant [10]. LTC_4 , LTD_4 and LTE_4 , also known as Cys-leukotrienes, have shown to be essential mediators in asthma pathophysiology [11].

Although the major pathophysiological implication of LTs was considered to be the bronchial asthma, these eicosanoids contribute to the pathogenesis of other human inflammatory diseases such as rheumatoid arthritis, inflammatory bowel disease, Crohn's disease, and psoriasis (see [11–13] for reviews). Recently, the 5-LOX pathway has also been associated with atherosclerosis, osteoporosis and certain types of cancer like prostate cancer [12].

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most commonly used remedy in inflammatory disorders. However, they cause several adverse effects, the most important being gastric injury up to gastric ulceration, renal failure and asthma [14]. On the other hand, the COX-2 selective drugs, generically known as COXIBs, have recently been a cause of controversy due to the enhanced cardiovascular risk they carry [13]. Thus, alternative therapeutic solutions, with similar anti-inflammatory potency but with fewer side effects, are needed, especially for the control of chronic inflammatory diseases, which implicate longer therapies.

2-Styrylchromones (2-SC) are a chemical family of oxygen heterocyclic compounds, vinylogues of flavones (2-phenylchromones), whose occurrence in nature has been reported [15,16]. Natural and synthetic 2-styrylchromones have been tested in different chemical and biological systems, showing activities with potential therapeutic applications [17–24]. The anti-inflammatory potential of 2-styrylchromones, concerning their interference with the arachidonic acid metabolic pathways, has not been explored so far. However, this may represent a promising field of research considering the potent inhibition of eicosanoids production by certain flavonoids containing structural similarities with 2-SC (see [25] for review). Therefore, the purpose of this work was to evaluate the inhibition of COX-1 and COX-2 activities, in a cell-free system, as well as the inhibition of LTB_4 production, in human PMNL, by a group of 2-SC (Fig. 2).

2. Material and methods

2.1. Reagents

All the chemicals and reagents were of analytical grade. Hanks' balanced salt solution (HBSS), Dulbecco's phosphate buffered saline, DMSO, nordihydroguaiaretic acid (NDGA), calcium ionophore (A23187), indomethacin, iron(II) chloride, iron(III) chloride, ascorbic acid, and arachidonic acid were obtained from Sigma-Aldrich (Steinheim, Germany). The "Leucotriene B₄ Enzyme Immunoassay (EIA) Kit" and "COX Inhibitor Screening Assay" were obtained from Cayman Chemical Co., Ann Arbor, MI, USA. Celecoxib was an offer from Pfizer. Potassium ferricyanide [$K_3Fe(CN)_6$] and trichloroacetic acid were obtained from Merck (Darmstadt, Germany).

2-Styrylchromones 1A–1D, 2A–2D and 3A–3D were synthesised by the three step Baker–Venkataraman method, starting from the O-protected 2'-hydroxyacetophenones and cinnamoyl chlorides [26]. The first step involves the O-acylation of appropriate 2'-hydroxyacetophenones with cinnamoyl chloride derivatives to give 2'-cinnamoyloxyacetophenones. These intermediates were converted into 5-aryl-3-hydroxy-1-(2-hydroxyaryl)-2,4-pentadien-1-ones by the base-catalysed Baker–Venkataraman rearran-

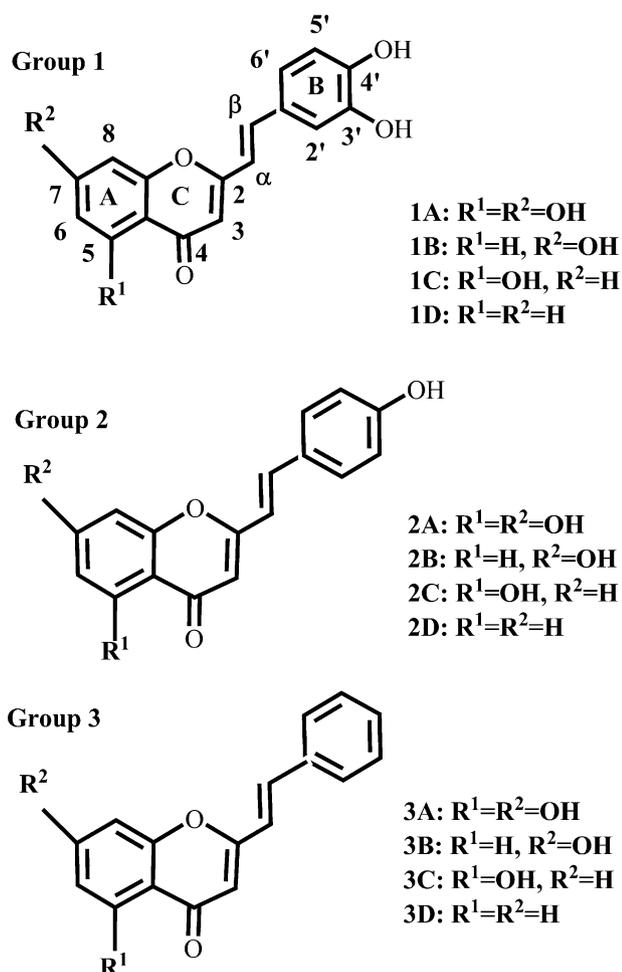


Fig. 2. Chemical structures of the tested 2-SC.

gement [27–29]. The cyclodehydration of these β -diketones with a mixture of DMSO and a catalytic amount of iodine or *p*-toluenesulfonic acid gives polybenzyloxy-2-SC, which were debenzylated by treatment with hydrogen bromide in acetic acid at reflux, to give the expected hydroxy-2-SC 1A–1D, 2A–2D and 3A–3D.

2.2. Equipment

A microplate reader (Synergy HT, BIO-TEK), was used to perform the spectrophotometric readings in all the assays.

2.3. Determination of LTB_4 production by human PMNL

Human PMNL were isolated from peripheral blood of healthy volunteers as previously described [30]. Neutrophil suspensions (5×10^6 cells/mL) in HBSS were pre-incubated at 37 °C for 10 min with the 2-SC (25 and 10 μ M) or with the lipoxygenase inhibitor, NDGA (1 μ M). The cells were subsequently incubated with A23187 (5 μ M) and arachidonic acid (10 μ g/mL) for 8 min. The reactions were stopped by the addition of cold methanol. Samples were subsequently centrifuged at $13,000 \times g$ for 1 min, and the supernatants were stored at -70 °C until analysis. The amount of LTB_4 in the samples was measured using the above mentioned commercial EIA kit, according to the manufacturer's instructions. The results were expressed as the percent inhibition of control LTB_4 production. At least four determinations were done for each experiment.

2.4. COX-1 and COX-2 inhibition assays

The inhibition of COX-1 (ovine) and COX-2 (human recombinant) by 2-SC was determined in a cell-free system by quantifying the levels of $PGF_{2\alpha}$, produced by catalysis of arachidonic acid, using the above mentioned specific EIA kit according to the manufacturer's instructions. The COX inhibitors indomethacin and celecoxib were used as positive controls. The results were expressed as the percent inhibition of control COX-1 or COX-2 activity. At least four determinations were done for each experiment.

2.5. Determination of the reducing power

The reductive potential of 2-SC was determined according to a previously described method [31]. Test compounds, in 1 mL of a ultrapure water/DMSO 3:1 solution, were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 mL, 1% w/v). The mixture was incubated at 50 °C for 20 min. A portion (2.5 mL) of trichloroacetic acid (10% w/v) was added to the mixture, which was then centrifuged for 10 min at 3000 g. The upper layer of the solution (2.5 mL) was mixed with ultrapure water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1% w/v), and the absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicated greater reducing power. Ascorbic acid was used as standard compound.

2.6. Determination of metal chelating activity

The metal chelating activity was assessed by monitoring the changes in UV–vis spectrum of 2-SC after addition of Fe(II) [32]. Titration experiments were performed by sequential additions of 2 μ L of $FeCl_2$ solution (300 μ M stock solution) to 300 μ L of a 10 μ M (final concentration) 2-SC solution. The titrations were performed in phosphate buffer 20 mM, pH 7.2.

2.7. Cytotoxicity

The effect of the 2-SC on the cell viability was assessed by the trypan blue exclusion method and by the release of LDH (as a measure of cell membrane integrity). The LDH activity was determined by following the rate of oxidation of NADH at 340 nm.

2.8. Statistical analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Dunnett's multicomparison test using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California, USA, <http://www.graphpad.com>. When comparing with control group, values of *P* less than 0.05 were considered significant.

Pearson correlation tests were performed using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California, USA, <http://www.graphpad.com>.

3. Results

3.1. Inhibition of LTB_4 production by human PMNL

All the tested 2-SC showed to have inhibitory effects on the LTB_4 production by human PMNL. 2-SC from group 1 were more effective inhibitors of LTB_4 production than the correspondent compounds from the other groups. Compounds 1A, 1C, and 1D, at the concentration of 25 μ M, were able to inhibit the LTB_4 production with a $\approx 90\%$ effect, while the compound 1B showed a $66.0 \pm 12.4\%$ effect at the same concentration (Fig. 3). These

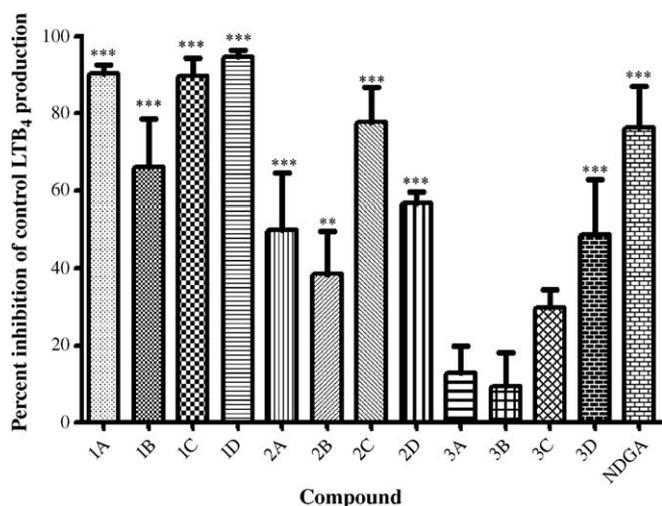


Fig. 3. Inhibition of human PMNL production of LTB₄ by 2-SC (25 μM) and NDGA (1 μM) determined by EIA. Each value represents mean ± SEM of at least four experiments performed in duplicate. (***) $P < 0.001$, (**) $P < 0.01$, (*) $P < 0.05$, significantly different from control.

compounds were still very effective at the lowest tested concentration (10 μM), 1C being the strongest inhibitor (80.9 ± 10.1% effect, $P < 0.001$) and 1B the weakest inhibitor (38.5 ± 11.8% effect, $P < 0.01$). 1A showed a 63.9 ± 9.2% effect ($P < 0.001$) and 1D showed a 62.3 ± 7.9% effect ($P < 0.001$). The compounds from group 2 were more effective inhibitors than the correspondent compounds from group 3. 2C was the most effective compound from group 2, reaching a 78.0 ± 9.0% effect at the concentration of 25 μM, while 3D was the most effective compound from group 3, showing a 48.9 ± 10.3% effect at the same concentration (Fig. 3). These compounds were still significantly effective at the concentration of 10 μM, where 2C showed an effect of 50.6 ± 11.9% ($P < 0.001$) and 3D showed an effect of 42.2 ± 9.6% ($P < 0.01$). The compounds 1B, 2B, and 3B were the weakest inhibitors in the respective groups. The 5-LOX inhibitor NDGA (1 μM) reached a 76.1 ± 11.0% inhibitory effect (Fig. 3).

3.2. Inhibition of COX-1 and COX-2 in a cell-free system

All 2-SC from group 1 were able to inhibit COX-1. 1A significantly inhibited the enzyme's activity at the concentrations of 100 μM (46.0 ± 5.6%) and 250 μM (74.4 ± 12.8%). 1B inhibition was only significant at 250 μM (54.2 ± 12.6%). Due to solubility issues, compounds 1C and 1D could only be tested at a maximum concentration of 100 μM, with 1D showing a significant inhibition (24.6 ± 6.4%). From group 2, only 2A and 2B where shown to inhibit COX-1, when tested at the concentration of 250 μM, 2A being a significant inhibitor (66.5 ± 11.4%) (Fig. 4). No other 2-SC inhibited COX-2 at the tested concentrations (100 μM and 250 μM). Indomethacin (1 μM) inhibited COX-1 (26.6 ± 6.6%) and COX-2 (92.6 ± 2.1%) and the selective COX-2 inhibitor celecoxib (10 μM) could only inhibit this isoenzyme (68.2 ± 3.0%).

3.3. Reducing power

From the tested 2-SC, compounds 1A–1D and 2B were able to reduce, significantly, ferric ion. Compounds from group 1 were shown to be more efficient reducers than ascorbic acid (Fig. 5).

3.4. Metal chelating activity

The UV–vis absorption spectrum of 2-SC 1A–1D showed a common absorption peak at 380 nm which presented bath-

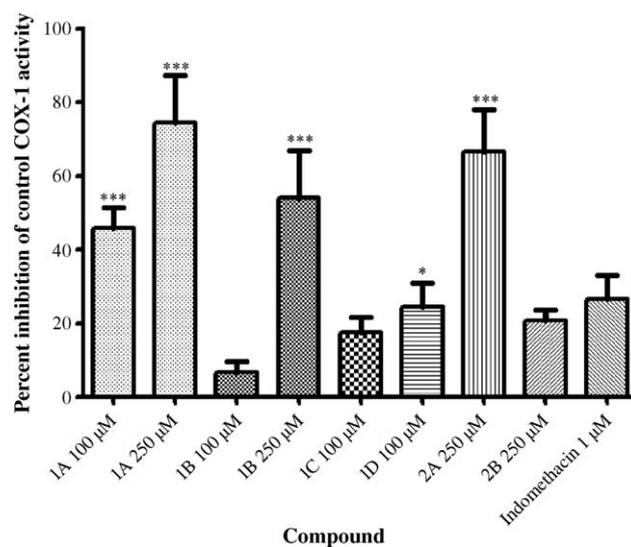


Fig. 4. Inhibition of COX-1 activity by 2-SC, determined by EIA. Each value represents mean ± SEM of at least 4 experiments performed in duplicate. (***) $P < 0.001$, (*) $P < 0.05$, significantly different from control.

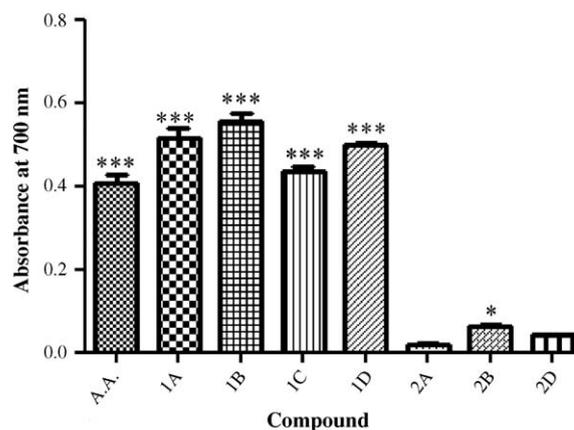


Fig. 5. Reducing activity of 2-SC and ascorbic acid (AA). All the compounds were tested at the final concentration of 25 μM. Each value represents mean ± SEM of triplicate measurements. (***) $P < 0.001$, (*) $P < 0.05$, significantly different from control.

ochromic shift upon addition of growing amounts of Fe(II), increasing gradually until the 400 nm (Fig. 6).

No relevant changes were observed in the UV–vis spectrum of 2-SC from groups 2 and 3 upon addition of Fe(II).

3.5. Cytotoxicity

None of the 2-SC caused an increased in the release of LDH compared to the control. The cell viability, assessed by the trypan blue exclusion method, was above 95% for all the compounds.

3.6. Correlations between the inhibition of LTB₄ production and scavenging activity

Significant correlations were found between the inhibition of LTB₄ production, described in Section 3.1, and the scavenging activity for singlet oxygen (¹O₂), and peroxynitrite anion (ONOO⁻) (these assays were performed in a previous work [24]).

4. Discussion

In this work, 2-SC were tested for their anti-inflammatory potential, through their capacity to interfere with the arachidonic

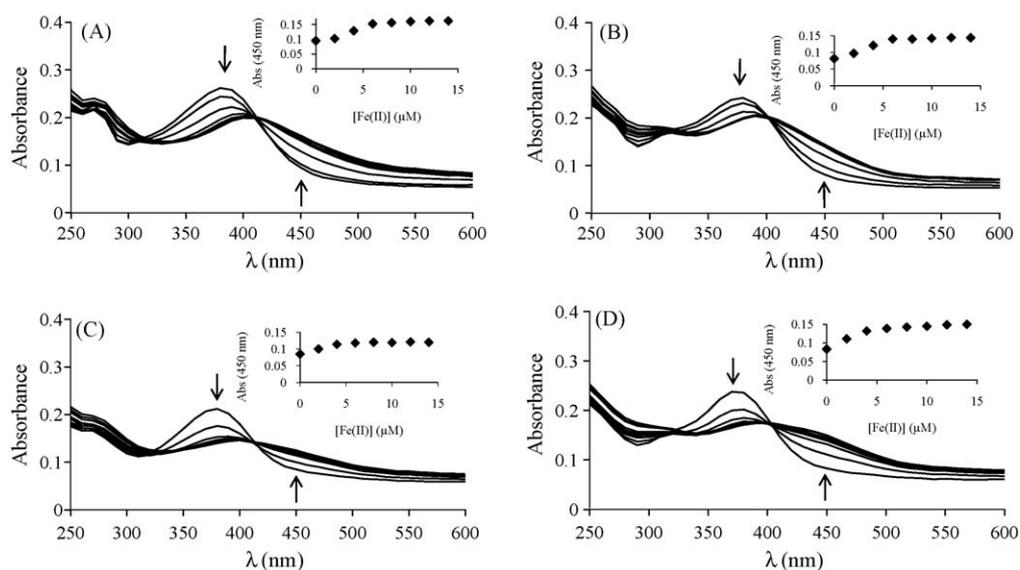


Fig. 6. UV-vis absorption spectrum of 10 μM of compounds (A) 1A, (B) 1B, (C) 1C, and (D) 1D in the presence of 0, 2, 4, 6, 8, 10, 12, 14, and 16 μM of Fe(II), in 20 mM phosphate buffer, pH 7.2. The direction of the arrows indicates crescent amounts of Fe(II). Insets: titration curves.

acid metabolic pathways, in particular, the COX-1 and COX-2 pathways, studied in a cell-free system, and 5-LOX pathway, studied in a cellular system using human PMNL. All the studied compounds were shown to inhibit the LTB_4 production by human leukocytes, especially those with a catechol substituted B-ring. This biological effect of 2-SC is shown for the first time, in the present study. As previously mentioned, 2-SC are vinylogues of flavones. Accordingly, Yoshimoto et al. [33] had previously found that the flavone structure represents a pharmacophore for 5-LOX inhibitory activity. In a recent theoretical study [34], a correlation was found between the lipoxygenase inhibitory activity of flavonoid molecules and their planar character, which probably facilitates the access, through a hydrophobic cavity, to the catalytic site of the enzyme. Considering this rationale, the planar character of 2-SC [16,29,35], conferred by the styryl link between the B-ring and the C-ring, may contribute to their effectiveness as 5-LOX inhibitors.

Several mechanisms, other than a direct inhibition of 5-LOX, can explain the observed inhibition of LTB_4 production by 2-SC. These include the inhibition of phospholipase A_2 , and thus of the arachidonic acid release, the inhibition of LTA_4 hydrolase, or the blockade of 5-lipoxygenase activating protein. Nevertheless, it is conceivable that 2-SC, in conformity with flavonoids and other phenolic compounds, act as redox 5-LOX inhibitors. Catalytically active 5-LOX requires the conversion of Fe(II) to Fe(III), conferred by certain lipid hydroperoxides (LOOH). Most 5-LOX inhibitors act at the catalytic domain by reducing or chelating the active-site iron or by scavenging radical intermediates in the redox cycle of the iron [6,25]. Accordingly, the reduction of ferric ion is a possible mechanism of inhibition of LTB_4 production by 2-SC, since the most effective compounds (1A–1D) also behaved as strong reducers (Fig. 3 vs. Fig. 5). Indeed, significant correlation was found between the inhibition of LTB_4 production and the reducing power of the tested compounds (Table 1). On the other hand, the spectral changes of those compounds upon addition of Fe(II) indicate the formation of iron complexes. The similar changes in all the compounds indicate that the Fe(II)-binding site is between the *ortho*-hydroxyl groups in the B-ring, considered to be one “iron binding motif” in flavonoid molecules [32,36]. Furthermore, the Fe(II) titration curves reveal the formation of 1:2 Fe(II):compound complexes (Fig. 6). Besides indicating a possible mechanism of inhibition of LTB_4 production, the metal chelating capacity of the 2-

SC from group 1, shown here for the first time, is also a very important indicator of their antioxidant activity. Finally, the capacity of some 2-SC to scavenge reactive oxygen species (ROS) and reactive nitrogen species (RNS), demonstrated in a previous study [24], is also likely to contribute to their LTB_4 production inhibitory effect. Noteworthy, significant correlations were found between the inhibition of LTB_4 production observed in the present study and the scavenging activity for singlet oxygen ($^1\text{O}_2$), and peroxytrite anion (ONOO^-) reported before (Table 1).

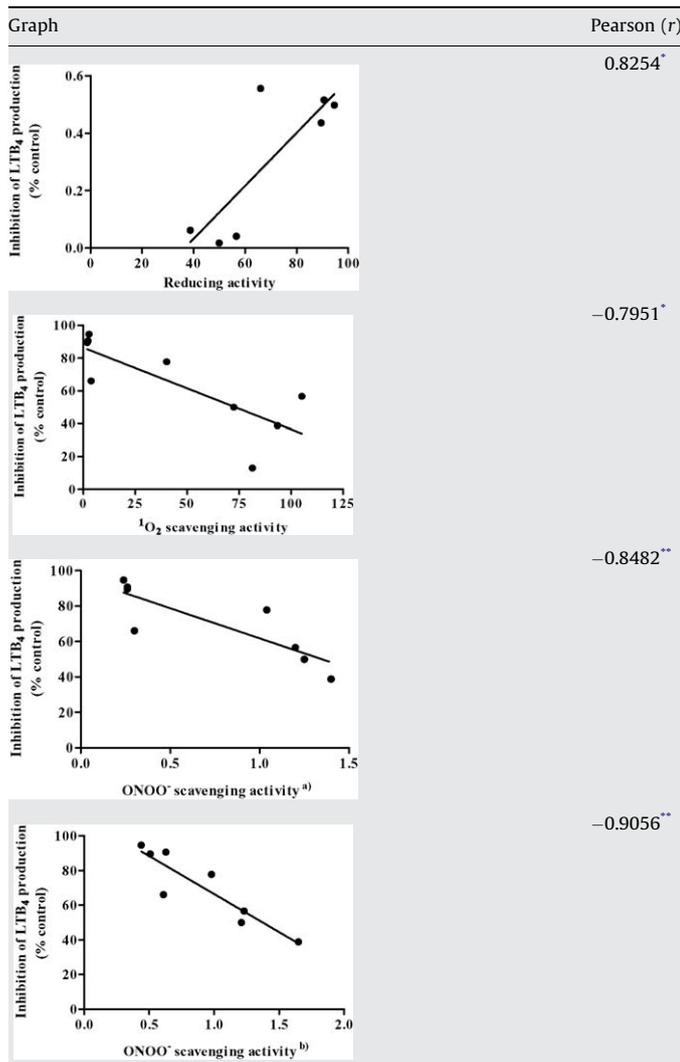
2-SC 1A, 1B, 1D and 2A were shown to significantly inhibit COX-1. This is also the first time that such biological activity of 2-SC is disclosed. On the other hand, none of the tested compounds was able to inhibit COX-2. Still, it is conceivable that this kind of effect might be seen at higher concentrations in accordance to what has been previously observed with a structural-similar compound (7,3',4'-trihydroxyflavone) [37]. However, due to solubility issues, the studied 2-SC could not be tested at higher concentrations. On the other hand, several flavonoids have previously shown to inhibit COX-2 expression (see [38] for review). The flavonoid's structural features considered by others [39] as relevant for this kind of activity are shared by some of the tested 2-SC, specifically compounds 1A and 2A. Thus, 2-SC, particularly the two referred compounds, may still be COX-2 inhibitors *in vivo* despite the negative results obtained in the present study.

The mechanism through which 2-SC inhibit COX-1 is likely to consist in the scavenging of the radical intermediates involved in COX enzyme catalysis, especially the phenoxy radical formed on a tyrosine residue, according to what has been previously suggested for other compounds with antioxidant moieties [40]. This mechanism is consistent with the fact that the only effective 2-SC were those which have shown higher ROS and RNS scavenging effects in our previous study [24].

The capacity of some of the tested 2-SC to inhibit both COX-1 and LOX pathways plays in favour of their potential use as effective and secure anti-inflammatory drugs, when compared to the commonly used NSAIDs and COXIBs. It is well known that NSAIDs may cause several adverse effects such as gastric ulceration, renal failure and asthma. On the other hand, the safety of selective COXIBs has been recently questioned due to the apparent association of these drugs with an increased risk of cardiovascular events. The cardiovascular adverse effects are probably due to a reduction on the levels of PGI_2 , an important anti-thrombotic

Table 1

Pearson correlations between the inhibition of LTB₄ production and the reducing activity and between the LTB₄ production and the scavenging activity against ¹O₂ (IC₅₀) and ONOO⁻ (with and without bicarbonate) (IC₅₀) [24] of the tested 2-SC.



(a) Without bicarbonate; (b) with bicarbonate.

^{*} Significant at $P < 0.05$.
^{**} Significant at $P < 0.01$.

eicosanoid, in concomitance to an excess of thromboxane A₂, which has the opposite effect [41–43]. Considering gastric effects, COX-2 selective inhibitors can delay the repair of existing gastrointestinal damage caused by ulcers, most likely by reducing the synthesis of PGs that are thought to play an important role in the healing process [44]. In fact, the inhibition of COX pathway has to be interpreted with care because of the diversity of COX-derived mediators with different properties. Indeed, the PGE₂, which has been mostly considered a pro-inflammatory mediator, has, on the other hand, given proof of involvement in anti-inflammatory and pro-resolving responses [45–47]. Furthermore, the COX inhibition shunts the arachidonic acid metabolism toward the 5-LOX pathway enhancing the gastric mucosal damages due to the augmented production of LTB₄ [44,48] and inducing adverse reactions in patients with asthma as a result of the cys-LTs overgeneration [44].

According to Bertolini et al. [14], dual acting anti-inflammatory drugs may represent a breakthrough in the treatment of rheumatic diseases, in view of several important arguments: (i) the same molecule (i.e., one drug alone) inhibits both COXs and 5-LOX; (ii)

the inhibition of both COX isoforms ensures a high anti-inflammatory efficacy and the concurrent preservation of the cardiovascular protective effects; (iii) the simultaneous inhibition of 5-LOX prevents pro-inflammatory and gastrointestinal damaging effects of leukotrienes. From our point of view, the use of dual inhibitors of the COX/5-LOX pathways seems advantageous to the treatment of other inflammatory disorders beyond rheumatic diseases. In fact, this type of compounds may exhibit anti-inflammatory activity with a wider spectrum than that of classical NSAIDs by inhibiting 5-LOX product-mediated inflammatory reactions towards which NSAIDs are ineffective [44].

Importantly, it has been demonstrated that the antioxidant effect of many NSAIDs may contribute to their therapeutic effectiveness [30,49–51]. Thus, the strong antioxidant effects of 2-SC will certainly contribute to their anti-inflammatory efficacy *in vivo*.

In conclusion, in the present study, several of the tested 2-SC were shown, for the first time, to prevent LTB₄ production by human leukocytes and to inhibit COX-1 activity. These effects, together with the well known antioxidant properties of these compounds may become a new therapeutic option in the treatment of inflammatory processes.

Acknowledgements

The authors acknowledge FCT and FEDER financial support for the project POCI/QUI/59284/2004 and the Organic Chemistry Research Unit (no. 62; Univ. Aveiro). Ana Gomes acknowledges FCT and FSE her PhD grant (SFRH/BD/23299/2005).

References

- [1] Kam PCA. See AUL. Cyclo-oxygenase isoenzymes: physiological and pharmacological role. *Anaesthesia* 2000;55:442–9.
- [2] Cipollone F, Cicolini G, Bucci M. Cyclooxygenase and prostaglandin synthases in atherosclerosis: recent insights and future perspectives. *Pharmacol Ther* 2008;118:161–80.
- [3] Wang MT, Honn KV, Nie D. Cyclooxygenases, prostanooids, and tumor progression. *Cancer Metastasis Rev* 2007;26:525–34.
- [4] Davies P, Bailey PJ, Goldenberg MM, Fordhutchinson AW. The role of Arachidonic-acid oxygenation products in pain and inflammation. *Annu Rev Immunol* 1984;2:335–57.
- [5] Vane JR, Botting RM. Anti-inflammatory drugs and their mechanism of action. *Inflamm Res* 1998;47(Suppl. 2):S78–87.
- [6] Jampilek J, Dolezal M, Opletalova V, Hartl J. 5-lipoxygenase, leukotrienes biosynthesis and potential antileukotrienic agents. *Curr Med Chem* 2006; 13:117–29.
- [7] Powell WS, Gravelle F, Gravel S. Metabolism of 5(S)-Hydroxy-6,8,11,14-Eicosatetraenoic acid and other 5(S)-hydroxyeicosanoids by a specific dehydrogenase in human polymorphonuclear leukocytes. *J Biol Chem* 1992;267: 19233–41.
- [8] Samuelsson B, Dahlen SE, Lindgren JA, Rouzer CA, Serhan CN. Leukotrienes and Lipoxins – structures, biosynthesis, and biological effects. *Science* 1987; 237:1171–6.
- [9] Busse WW. Leukotrienes and inflammation. *Am J Respir Crit Care Med* 1998;157:S210–3.
- [10] Stamatou P, Hamid Q, Taha R, Yu WG, Issekutz TB, Rokach J, et al. 5-oxo-ETE induces pulmonary eosinophilia in an integrin-dependent manner in brown Norway rats. *J Clin Invest* 1998;102:2165–72.
- [11] Sharma JN, Mohammed LA. The role of leukotrienes in the pathophysiology of inflammatory disorders: is there a case for revisiting leukotrienes as therapeutic targets? *Inflammopharmacology* 2006;14:10–6.
- [12] Werz O, Steinhilber D. Therapeutic options for 5-lipoxygenase inhibitors. *Pharmacol Ther* 2006;112:701–18.
- [13] Khanapure SP, Garvey DS, Janero DR, Letts LG. Eicosanoids in inflammation: biosynthesis, pharmacology, and therapeutic frontiers. *Curr Top Med Chem* 2007;7:311–40.
- [14] Bertolini A, Ottani A, Sandrini M. Selective COX-2 inhibitors and dual acting anti-inflammatory drugs: critical remarks. *Curr Med Chem* 2002;9:1033–43.
- [15] Gerwick WH, Lopez A, Van Dyne GD, Clardy J, Ortiz W, Baez A. Hormothamnione, a novel cytotoxic styrylchromone from the marine cyanophyte *Hormothamnion enteromorphoides* grunow. *Tetrahedron Lett* 1986;27:1979–82.
- [16] Gerwick WH. 6-Desmethoxyhormothamnione, a new cytotoxic styrylchromone from the marine cryptophyte *Chrysothamnium taylori*. *J Nat Prod* 1989;52:252–6.
- [17] Doria G, Romeo C, Forgiione A, Sberze P, Tibolla N, Corno ML, et al. Antiallergic agents. III. Substituted *trans*-2-ethenyl-4-oxo-4H-1-benzopyran-6-carboxylic acids. *Eur J Med Chem* 1979;14:347–51.

- [18] Desideri N, Conti C, Mastromarino P, Mastropaolo F. Synthesis and anti-rhinovirus activity of 2-styrylchromones. *Antivir Chem Chemother* 2000;11:373–81.
- [19] Brion D, Le Baut G, Zammatio F, Pierre A, Atassi G, Belachm L. Preparation of 2-styryl-4-chromanones as anticancer agents. European patent application EP 454:587. *Chem Abstr* 1991;116:106092k.
- [20] Karton Y, Jiang JL, Ji XD, Melman N, Olah ME, Stiles GL, et al. Synthesis and biological activities of flavonoid derivatives as A3 adenosine receptor antagonists. *J Med Chem* 1996;39:2293–301.
- [21] Fernandes E, Carvalho F, Silva AMS, Santos CMM, Pinto DCGA, Cavaleiro JAS, et al. 2-Styrylchromones as novel inhibitors of xanthine oxidase. A structure-activity study. *J Enzyme Inhib Med Chem* 2002;17:45–8.
- [22] Fernandes E, Carvalho M, Carvalho F, Silva AMS, Santos CMM, Pinto DCGA, et al. Hepatoprotective activity of polyhydroxylated 2-styrylchromones against *tert*-butylhydroperoxide induced toxicity in freshly isolated rat hepatocytes. *Arch Toxicol* 2003;77:500–5.
- [23] Filipe P, Silva AM, Morliere P, Brito CM, Patterson LK, Hug GL, et al. Polyhydroxylated 2-styrylchromones as potent antioxidants. *Biochem Pharmacol* 2004;67:2207–18.
- [24] Gomes A, Fernandes E, Silva AM, Santos CM, Pinto DC, Cavaleiro JA, et al. 2-Styrylchromones: novel strong scavengers of reactive oxygen and nitrogen species. *Bioorg Med Chem* 2007;15:6027–36.
- [25] Werz O. Inhibition of 5-lipoxygenase product synthesis by natural compounds of plant origin. *Planta Med* 2007;73:1331–57.
- [26] Santos CMM, Silva AMS, Cavaleiro JAS. Synthesis of new hydroxy-2-styrylchromones. *Eur J Org Chem* 2003;2003:4575–85.
- [27] Baker W. Molecular rearrangement of some o-acyloxyacetophenones and the mechanism of the production of 3-acylchromones. *J Chem Soc* 1933;1381–9.
- [28] Mahal HS, Venkataraman K. Synthetical experiments in the chromone group. Part XIV. The action of sodamide on 1-acyloxy-2-acetonaphthones. *J Chem Soc* 1934;1767–9.
- [29] Price WA, Silva AMS, Cavaleiro JAS. 2-Styrylchromones – biological action, synthesis and Reactivity. *Heterocycles* 1993;36:2601–12.
- [30] Costa D, Marques AP, Reis RL, Lima JLFC, Fernandes E. Inhibition of human neutrophil oxidative burst by pyrazolone derivatives. *Free Radic Biol Med* 2006;40:632–40.
- [31] Mathew S, Abraham TE. In vitro antioxidant activity and scavenging effects of *Cinnamomum verum* leaf extract assayed by different methodologies. *Food Chem Toxicol* 2006;44:198–206.
- [32] Guo M, Perez C, Wei Y, Rapoza E, Su G, Bou-Abdallah F, et al. Iron-binding properties of plant phenolics and cranberry's bio-effects. *Dalton Trans* 2007;4951–61.
- [33] Yoshimoto T, Furukawa M, Yamamoto S, Horie T, Watanabe-Kohno S. Flavonoids: potent inhibitors of arachidonate 5-lipoxygenase. *Biochem Biophys Res Commun* 1983;116:612–8.
- [34] Redrejo-Rodriguez M, Tejada-Cano A, Pinto MD, Macias P. Lipoxygenase inhibition by flavonoids: semiempirical study of the structure-activity relation. *J Mol Struct-Theochem* 2004;674:121–4.
- [35] Silva AMS, Pinto D, Tavares HR, Cavaleiro JAS, Jimeno ML, Elguero J. Novel (*E*)- and (*Z*)-2-styrylchromones from (*E,E*)-2'-hydroxycinnamylideneacetophenones – xanthenes from daylight photooxidative cyclization of (*E*)-2-styrylchromones. *Eur J Org Chem* 1998;1998:2031–8.
- [36] Mira L, Fernandez MT, Santos M, Rocha R, Florencio MH, Jennings KR. Interactions of flavonoids with iron and copper ions: a mechanism for their antioxidant activity. *Free Radic Res* 2002;36:1199–208.
- [37] Selvam C, Jachak SM, Bhutani KK. Cyclooxygenase inhibitory flavonoids from the stem bark of *Semecarpus anacardium* Linn. *Phytother Res* 2004;18:582–4.
- [38] Gomes A, Fernandes E, Lima JL, Mira L, Corvo ML. Molecular mechanisms of anti-inflammatory activity mediated by flavonoids. *Curr Med Chem* 2008;15:1586–605.
- [39] Rosenkranz HS, Thampatty BP. SAR: flavonoids and COX-2 inhibition. *Oncol Res* 2003;13:529–35.
- [40] Dannhardt G, Laufer S. Structural approaches to explain the selectivity of COX-2 inhibitors: is there a common pharmacophore. *Curr Med Chem* 2000;7:1101–12.
- [41] Antman EM, DeMets D, Loscalzo J. Cyclooxygenase inhibition and cardiovascular risk. *Circulation* 2005;112:759–70.
- [42] Solomon DH. Selective cyclooxygenase 2 inhibitors and cardiovascular events. *Arthritis Rheum* 2005;52:1968–78.
- [43] Grosser T, Fries S, FitzGerald GA. Biological basis for the cardiovascular consequences of COX-2 inhibition: therapeutic challenges and opportunities. *J Clin Invest* 2006;116:4–15.
- [44] Fiorucci S, Meli R, Bucci M, Cirino G. Dual inhibitors of cyclooxygenase and 5-lipoxygenase. A new avenue in anti-inflammatory therapy? *Biochem Pharmacol* 2001;62:1433–8.
- [45] Vancheri C, Mastruzzo C, Sortino MA, Crimi N. The lung as a privileged site for the beneficial actions of PGE(2). *Trends Immunol* 2004;25:40–6.
- [46] Harizi H, Juzan M, Pitard V, Moreau JF, Gualde N. Cyclooxygenase-2-issued prostaglandin E-2 enhances the production of endogenous IL-10, which down-regulates dendritic cell functions. *J Immunol* 2002;168:2255–63.
- [47] Serhan CN. Resolution phase of inflammation: Novel endogenous anti-inflammatory and proresolving lipid mediators and pathways. *Annu Rev Immunol* 2007;25:101–37.
- [48] Hudson N, Balsitis M, Everitt S, Hawkey CJ. Enhanced gastric-mucosal leukotriene-B(4) synthesis in patients taking nonsteroidal antiinflammatory drugs. *Gut* 1993;34:742–7.
- [49] Fernandes E, Toste SA, Lima J, Reis S. The metabolism of sulindac enhances its scavenging activity against reactive oxygen and nitrogen species. *Free Radic Biol Med* 2003;35:1008–17.
- [50] Fernandes E, Costa D, Toste SA, Lima JLFC, Reis S. In vitro scavenging activity for reactive oxygen and nitrogen species by nonsteroidal anti-inflammatory indole, pyrrole, and oxazole derivative drugs. *Free Radic Biol Med* 2004;37:1895–905.
- [51] Costa D, Vieira A, Fernandes E. Dipyrone and aminopyrine are effective scavengers of reactive nitrogen species. *Redox Rep* 2006;11:136–42.