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Ana Gomes, Eduarda Fernandes, Artur M.S. Silva, Clementina M.M. Santos,
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3 **Anti-inflammatory potential of 2-styrylchromones regarding their interference with**
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5 **arachidonic acid metabolic pathways**
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7 Ana Gomes^a, Eduarda Fernandes^{a*}, Artur M.S. Silva^b, Clementina M.M. Santos^{b,c}, Diana
8
9 C.G.A. Pinto^b, José A.S. Cavaleiro^b, José L.F.C. Lima^a
10

11
12 ^aREQUIMTE, Departamento de Química-Física, Faculdade de Farmácia, Universidade do
13
14 Porto, Rua Aníbal Cunha, 164, 4099-030 Porto, Portugal.
15

16
17 ^bDepartamento de Química & QOPNA, Universidade de Aveiro, 3810-193 Aveiro, Portugal.
18

19
20 ^cDepartamento de Agro-indústrias, Escola Superior Agrária de Bragança, Campus de Santa
21
22 Apolónia, 5301-855 Bragança, Portugal.
23
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25
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35
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39

40
41 *Address correspondence to:

42
43 Eduarda Fernandes, PhD,
44

45 REQUIMTE, Departamento de Química-Física Faculdade de Farmácia, Universidade do Porto
46

47 Rua Aníbal Cunha, 164 4099-030 Porto, Portugal.
48

49
50 Phone: 00351222078968; Fax: 00351222078961; E-mail: egracas@ff.up.pt.
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3 Abstract
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7 Cyclooxygenases (COXs) are the key enzymes in the biosynthesis of prostanoids. COX-1 is a
8 constitutive enzyme while the expression of COX-2 is highly stimulated in the event of
9 inflammatory processes, leading to the production of large amounts of prostaglandins (PGs), in
10 particular PGE₂ and PGI₂, which are pro-inflammatory mediators.
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16 Lipoxygenases (LOXs) are enzymes that produce hydroxy acids and leukotrienes (LTs). 5-
17 LOX metabolizes arachidonic acid to yield, among other products, LTB₄, a potent
18 chemoattractant mediator of inflammation.
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24 The aim of the present work was to evaluate the anti-inflammatory potential of 2-
25 styrylchromones (2-SC), a chemical family of oxygen heterocyclic compounds, vinyllogues of
26 flavones (2-phenylchromones), by studying their COX-1 and COX-2 inhibitory capacity as well
27 as their effects on the LTB₄ production by stimulated human polymorphonuclear leukocytes
28 (PMNL).
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36 Some of the tested 2-SC were able to inhibit both COX-1 activity and LTB₄ production which
37 makes them dual inhibitors of the COX and 5-LOX pathways. The most effective compounds in
38 this study were those having structural moieties with proved antioxidant activity (3',4'-catechol
39 and 4'-phenol substituted B-rings).
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45 This type of compounds may exhibit anti-inflammatory activity with a wider spectrum than
46 that of classical non-steroidal anti-inflammatory drugs (NSAIDs) by inhibiting 5-LOX product-
47 mediated inflammatory reactions, towards which NSAIDs are ineffective.
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3 Keywords: 2-Styrylchromones; Cyclooxygenase; 5-Lipoxygenase; Leukotriene B₄; Dual
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5 inhibitors; Inflammation
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10 1 Introduction
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14 Arachidonic acid is the most abundant polyunsaturated fatty acid in the phospholipid bilayer
15 of cell membranes. This second-messenger molecule is released by phospholipase A₂ in activated
16 cells and further metabolized in different eicosanoids by the cyclooxygenase and lipoxygenase
17 pathways (Fig. 1). Cyclooxygenases (COXs) are the key enzymes in the biosynthesis of
18 prostanoids. In mammalian cells, COXs exist in at least two isoforms (COX-1 and COX-2).
19 COX-1 is a constitutive enzyme, expressed in almost every cell type, responsible for the
20 physiological production of prostaglandins (PGs) i.e., PGI₂, PGE₂, PGF_{2α}, and PGD₂, in diverse
21 organs and thromboxane A₂ in platelets. COX-2, on the other hand, is usually absent in most
22 tissues (with the exception of kidney, parts of the brain, and gravid uterus where COX-2 is
23 constitutive) but its expression can be readily induced by numerous stimuli like such as growth
24 factors, tumor promoters, or cytokines [1,2]. The earliest expression of COX-2 is detected in
25 stromal cells, but it also can be found in multiple cells, like epithelial, endothelial, and stromal
26 cells in several types of tumors [3]. In addition, COX-2 is highly expressed by cells that are
27 involved in inflammation (e.g., neutrophils, macrophages, monocytes, mast cells, synoviocytes),
28 emerging as the isoform primarily responsible for the synthesis of the prostanoids involved in
29 pathological processes, such as acute and chronic inflammatory states. These inflammation-
30 related cell types can be stimulated by lipopolysaccharide, phorbol esters, cytokines, or growth
31 factors, producing large amounts of PGs, in particular PGE₂ and PGI₂, which are pro-
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3 inflammatory mediators that increase vascular permeability and promote edema at the sites of
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5 inflammation. Furthermore, these PGs are involved in the nociception of the inflammatory pain
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7 [4]. Thus, COX-2 isoform has been associated with acute and chronic inflammatory disorders [5]
8
9 as well as in the carcinogenesis and tumor progression [3].

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12 Lipoxygenases (LOXs) are enzymes that produce hydroxy acids and leukotrienes (LTs). From
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14 the LOXs existent in the mammalian tissues, 5-LOX, which is mainly found in cells of myeloid
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16 origin, i.e., polymorphonuclear leukocytes (PMNL), mast cells, macrophages, is the most
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18 implicated in inflammatory and allergic disorders [6]. 5-LOX metabolizes arachidonic acid to
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20 yield 5(*S*)-hydroxy-6,8,11,14-eicosatetraenoic acid (5-HETE), which is further metabolized to the
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22 bioactive 5-oxo-6,8,11,14-eicosatetraenoic acid (5-oxo-EETE) by PMNL 5-hydroxyeicosanoid
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24 dehydrogenase [7], and various LTs (LTA₄-LTE₄) [8]. 5-oxo-EETE and LTB₄, produced by
25
26 PMNL, are potent chemoattractant mediators of inflammation. LTB₄ stimulates neutrophil
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28 chemotaxis, enhances neutrophil-endothelial interactions, and stimulates neutrophil activation,
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30 leading to degranulation and the release of mediators, enzymes, and superoxide radicals [9],
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32 while 5-oxo-EETE is much more active than LTB₄ as an eosinophil chemoattractant [10]. LTC₄,
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34 LTD₄ and LTE₄, also known as Cys-leukotrienes, have shown to be essential mediators in asthma
35
36 pathophysiology [11].

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38 Although the major pathophysiological implication of LTs was considered to be the bronchial
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40 asthma, these eicosanoids contribute to the pathogenesis of other human inflammatory diseases
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42 such as rheumatoid arthritis, inflammatory bowel disease, Crohn's disease, and psoriasis (see
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44 [11-13] for reviews). Recently, the 5-LOX pathway has also been associated with atherosclerosis,
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46 osteoporosis and certain types of cancer like prostate cancer [12].
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3 Non-steroidal anti-inflammatory drugs (NSAIDs) are the most commonly used remedy in
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5 inflammatory disorders. However, they cause several adverse effects, the most important being
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7 gastric injury up to gastric ulceration, renal failure and asthma [14]. On the other hand, the COX-
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9 2 selective drugs, generically known as COXIBs, have recently been a cause of controversy due
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11 to the enhanced cardiovascular risk they carry [13]. Thus, alternative therapeutic solutions, with
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13 similar anti-inflammatory potency but with fewer side effects, are needed, especially for the
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15 control of chronic inflammatory diseases, which implicate longer therapies.
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19 2-Styrylchromones (2-SC) are a chemical family of oxygen heterocyclic compounds,
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21 vinylogues of flavones (2-phenylchromones), whose occurrence in nature has been reported
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23 [15,16]. Natural and synthetic 2-styrylchromones have been tested in different chemical and
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25 biological systems, showing activities with potential therapeutic applications [17-24]. The anti-
26
27 inflammatory potential of 2-styrylchromones, concerning their interference with the arachidonic
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29 acid metabolic pathways, has not been explored so far. However, this may represent a promising
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31 field of research considering the potent inhibition of eicosanoids production by certain flavonoids
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33 containing structural similarities with 2-SC (see [25] for review). Therefore, the purpose of this
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35 work was to evaluate the inhibition of COX-1 and COX-2 activities, in a cell-free system, as well
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37 as the inhibition of LTB₄ production, in human PMNL, by a group of 2-SC (Fig. 2).
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50 2 Material and methods 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65

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 [K₃Fe(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-Venkaraman 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-Venkaraman rearrangement [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

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3 A microplate reader (Synergy HT, BIO-TEK), was used to perform the spectrophotometric
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5 readings in all the assays.
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7 8 9 10 2.3 Determination of LTB₄ production by human PMNL 11

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14 Human PMNL were isolated from peripheral blood of healthy volunteers as previously
15 described [30]. Neutrophil suspensions (5×10^6 cells/mL) in HBSS were pre-incubated at 37°C
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17 for 10 min with the 2-SC (25 and 10 μ M) or with the lipoxygenase inhibitor, NDGA (1 μ M). The
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19 cells were subsequently incubated with A23187 (5 μ M) and arachidonic acid (10 μ g/mL) for 8
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21 min. The reactions were stopped by the addition of cold methanol. Samples were subsequently
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23 centrifuged at 13,000 x g for 1 min, and the supernatants were stored at -70°C until analysis. The
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25 amount of LTB₄ in the samples was measured using the above mentioned commercial EIA kit,
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27 according to the manufacturer's instructions. The results were expressed as the percent inhibition
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29 of control LTB₄ production. At least four determinations were done for each experiment.
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38 2.4 COX-1 and COX-2 inhibition assays 39

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42 The inhibition of COX-1 (ovine) and COX-2 (human recombinant) by 2-SC was determined
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44 in a cell-free system by quantifying the levels of PGF_{2 α} , produced by catalysis of arachidonic
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46 acid, using the above mentioned specific EIA kit according to the manufacturer's instructions.
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48 The COX inhibitors indomethacin and celecoxib were used as positive controls. The results were
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50 expressed as the percent inhibition of control COX-1 or COX-2 activity. At least four
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52 determinations were done for each experiment.
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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 1:4 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 3000g. 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

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3 The effect of the 2-SC on the cell viability was assessed the trypan blue exclusion method and
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5 by the release of LDH (as a measure of cell membrane integrity). The LDH activity was
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7 determined by following the rate of oxidation of NADH at 340 nm.
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10 11 12 2.8 Statistical analysis 13 14 15 16

17 Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by
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19 Dunnett's multicomparison test using GraphPad Prism version 5.00 for Windows, GraphPad
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21 Software, San Diego California USA, www.graphpad.com". When comparing with control
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23 group, values of P less than 0.05 were considered significant.
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26 Pearson correlation tests were performed using GraphPad Prism version 5.00 for Windows,
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28 GraphPad Software, San Diego California USA, www.graphpad.com".
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32 33 3 Results 34 35 36 37

38 3.1 Inhibition of LTB₄ production by human PMNL 39 40 41 42

43 All the tested 2-SC showed to have inhibitory effects on the LTB₄ production by human
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45 PMNL. 2-SC from group 1 were more effective inhibitors of LTB₄ production than the
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47 correspondent compounds from the other groups. Compounds 1A, 1C, and 1D, at the
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49 concentration of 25 μ M, were able to inhibit the LTB₄ production with a \approx 90% effect, while the
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51 compound 1B showed a 66.0 \pm 12.4% effect at the same concentration (Fig. 3). These compounds
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53 were still very effective at the lowest tested concentration (10 μ M), 1C being the strongest
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2 inhibitor (80.9±10.1% effect, P<0.001) and 1B the weakest inhibitor (38.5±11.8% effect,
3 P<0.01). 1A showed a 63.9±9.2% effect (P<0.001) and 1D showed a 62.3±7.9% effect
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5 (P<0.001). The compounds from group 2 were more effective inhibitors than the correspondent
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7 compounds from group 3. 2C was the most effective compound from group 2, reaching a
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9 78.0±9.0% effect at the concentration of 25 µM, while 3D was the most effective compound from
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11 group 3, showing a 48.9±10.3% effect at the same concentration (Fig. 3). These compounds were
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13 still significantly effective at the concentration of 10 µM, where 2C showed an effect of
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15 50.6±11.9% (P<0.001) and 3D showed an effect of 42.2±9.6% (P<0.01). The compounds 1B, 2B,
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17 and 3B were the weakest inhibitors in the respective groups. The 5-LOX inhibitor NDGA (1 µM)
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19 reached a 76.1±11.0% inhibitory effect (Fig. 3).
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33 3.2 Inhibition of COX-1 and COX-2 in a cell-free system 34 35 36 37

38 All 2-SC from group 1 where able to inhibit COX-1. 1A significantly inhibited the enzyme's
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40 activity at the concentrations of 100 µM (46.0±5.6%) and 250 µM (74.4±12.8%). 1B inhibition
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42 was only significant at 250 µM (54.2±12.6%). Due to solubility issues, compounds 1C and 1D
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44 could only be tested at a maximum concentration of 100 µM, with 1D showing a significant
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46 inhibition (24.6±6.4%). From group 2, only 2A and 2B where shown to inhibit COX-1, when
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48 tested at the concentration of 250 µM, 2A being a significant inhibitor (66.5±11.4%) (Fig. 4). No
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50 other 2-SC was able to inhibit COX-1. None of the studied 2-SC inhibited COX-2 at the tested
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52 concentrations (10 and 25 µM). Indomethacin (1 µM) inhibited COX-1 (26.6±6.6%) and COX-2
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3 (92.6±2.1%) and the selective COX-2 inhibitor celecoxib (10 µM) could only inhibit this
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5 isoenzyme (68.2±3.0%).
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10 **-Please place fig. 4 here-**
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13 14 3.3 Reducing power 15 16 17 18

19 From the tested 2-SC, compounds 1A-1D and 2B were able to reduce, significantly, ferric ion.
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21 Compounds from group 1 were shown to be more efficient reducers than ascorbic acid (Fig. 5).
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26 **-Please place fig. 5 here-**
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31 3.4 Metal chelating activity 32 33 34 35

36 The UV-Vis absorption spectrum of 2-SC 1A-1D shown a common absorption peak at 380 nm
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38 which presented bathochromic shift upon addition of growing amounts of Fe(II), increasing
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40 gradually until the 400 nm (Fig. 6).
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43 No relevant changes were observed in the UV-Vis spectrum of 2-SC from groups 2 and 3
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45 upon addition of Fe(II).
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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 3.1, and the scavenging activity for singlet oxygen (¹O₂), and peroxynitrite anion (ONOO⁻) (these assays were performed in a previous work [24]).

Please place Table 1 here

4 Discussion

In this work, 2-SC were tested for their anti-inflammatory potential, through their capacity to interfere with the arachidonic 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 LTB₄ 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

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3 [34], a correlation was found between the lipoxygenase inhibitory activity of flavonoid molecules
4 and their planar character, which probably facilitates the access, through a hydrophobic cavity, to
5 the catalytic site of the enzyme. Considering this rationale, the planar character of 2-SC
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7 [16,29,35], conferred by the styryl link between the B-ring and the C-ring, may contribute to their
8 effectiveness as 5-LOX inhibitors.
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14 Several mechanisms, other than a direct inhibition of 5-LOX, can explain the observed
15 inhibition of LTB₄ production by 2-SC. These include the inhibition of phospholipase A₂, and
16 thus of the arachidonic acid release, the inhibition of LTA₄ hydrolase, or the blockade of 5-
17 lipoxygenase activating protein. Nevertheless, it is conceivable that 2-SC, in conformity with
18 flavonoids and other phenolic compounds, act as redox 5-LOX inhibitors. Catalytically active 5-
19 LOX requires the conversion of Fe(II) to Fe(III), conferred by certain lipid hydroperoxides
20 (LOOH). Most 5-LOX inhibitors act at the catalytic domain by reducing or chelating the active-
21 site iron or by scavenging radical intermediates in the redox cycle of the iron [6,25]. Accordingly,
22 the reduction of ferric ion is a possible mechanism of inhibition of LTB₄ production by 2-SC,
23 since the most effective compounds (1A-1D) also behaved as strong reducers (Fig. 3 vs. Fig. 5).
24 Indeed, significant correlation was found between the inhibition of LTB₄ production and the
25 reducing power of the tested compounds (Table 1). On the other hand, the spectral changes of
26 those compounds upon addition of Fe(II) indicate the formation of iron complexes. The similar
27 changes in all the compounds indicate that the Fe(II)-binding site is between the *ortho*-hydroxyl
28 groups in the B-ring, considered to be one “iron binding motif” in flavonoid molecules [32,36].
29 Furthermore, the Fe(II) titration curves reveal the formation of 1:2 Fe (II):compound complexes
30 (Fig. 6). Besides indicating a possible mechanism of inhibition of LTB₄ production, the metal
31 chelating capacity of the 2-SC from group 1, shown here for the first time, is also a very
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3 important indicator of their antioxidant activity. Finally, the capacity of some 2-SC to scavenge
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5 reactive oxygen species (ROS) and reactive nitrogen species (RNS), demonstrated in a previous
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7 study [24], is also likely to contribute to their LTB₄ production inhibitory effect. Noteworthy,
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9 significant correlations were found between the inhibition of LTB₄ production observed in the
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11 present study and the scavenging activity for singlet oxygen (¹O₂), and peroxynitrite anion
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13 (ONOO⁻) reported before (Table 1).
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17 2-SC 1A, 1B, 1D and 2A were shown to significantly inhibit COX-1. This is also the first
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19 time that such biological activity of 2-SC is disclosed. On the other hand, none of the tested
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21 compounds was able to inhibit COX-2. Still, it is conceivable that this kind of effect might be
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23 seen at higher concentrations in accordance to what has been previously observed with a
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25 structural-similar compound (7,3',4'-trihydroxyflavone) [37]. However, due to solubility issues,
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27 the studied 2-SC could not be tested at higher concentrations. On the other hand, several
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29 flavonoids have previously shown to inhibit COX-2 expression (see [38] for review). The
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31 flavonoid's structural features considered by others [39] as relevant for this kind of activity are
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33 shared by some of the tested 2-SC, specifically compounds 1A and 2A. Thus, 2-SC, particularly
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35 the two referred compounds, may still be COX-2 inhibitors *in vivo* despite the negative results
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37 obtained in the present study.
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43 The mechanism through which 2-SC inhibit COX-1 is likely to consist in the scavenging of
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45 the radical intermediates involved in COX enzyme catalysis, especially the phenoxy radical
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47 formed on a tyrosine residue, according to what has been previously suggested for other
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49 compounds with antioxidant moieties [40]. This mechanism is consistent with the fact that the
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51 only effective 2-SC were those which have shown higher ROS and RNS scavenging effects in
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53 our previous study [24].
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3 The capacity of some of the tested 2-SC to inhibit both COX-1 and LOX pathways plays in
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5 favour of their potential use as effective and secure anti-inflammatory drugs, when compared to
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7 the commonly used NSAIDs and COXIBs. It is well known that NSAIDs may cause several
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9 adverse effects such as gastric ulceration, renal failure and asthma. On the other hand, the safety
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11 of selective COXIBs has been recently questioned due to the apparent association of these drugs
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13 with an increased risk of cardiovascular events. The cardiovascular adverse effects are probably
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15 due to a reduction on the levels of PGI₂, an important anti-thrombotic eicosanoid, in
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17 concomitance to an excess of thromboxane A₂, which has the opposite effect [41-43].
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19 Considering gastric effects, COX-2 selective inhibitors can delay the repair of existing
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21 gastrointestinal damage caused by ulcers, most likely by reducing the synthesis of PGs that are
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23 thought to play an important role in the healing process [44]. In fact, the inhibition of COX
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25 pathway has to be interpreted with careful because of the diversity of COX-derived mediators
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27 with different properties. Indeed, the PGE₂, which has been mostly considered a pro-
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29 inflammatory mediator, has, on the other hand, given proves of involvement in anti-inflammatory
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31 and pro-resolving responses [45-47]. Furthermore, the COX inhibition shunts the arachidonic
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33 acid metabolism toward the 5-LOX pathway enhancing the gastric mucosal damages due to the
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35 augmented production of LTB₄ [44,48] and inducing adverse reactions in patients with asthma as
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37 a result of the cys-LTs overgeneration [44].
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45 According to Bertolini *et al.* [14], dual acting anti-inflammatory drugs may represent a
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47 breakthrough in the treatment of rheumatic diseases, in view of several important arguments: (i)
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49 the same molecule (i.e., one drug alone) inhibits both COXs and 5-LOX; (ii) the inhibition of
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51 both COX isoforms ensures a high anti-inflammatory efficacy and the concurrent preservation of
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53 the cardiovascular protective effects; (iii) the simultaneous inhibition of 5-LOX prevents pro-
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3 inflammatory and gastrointestinal damaging effects of leukotrienes. From our point of view, the
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5 use of dual inhibitors of the COX/5-LOX pathways seems advantageous to the treatment of other
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7 inflammatory disorders beyond rheumatic diseases. In fact, this type of compounds may exhibit
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9 anti-inflammatory activity with a wider spectrum than that of classical NSAIDs by inhibiting 5-
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11 LOX product-mediated inflammatory reactions towards which NSAIDs are ineffective [44].
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15 Importantly, it has been demonstrated that the antioxidant effect of many NSAIDs may
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17 contribute to their therapeutic effectiveness [30,49-51]. Thus, the strong antioxidant effects of 2-
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19 SC will certainly contribute to their anti-inflammatory efficacy *in vivo*.
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23 In conclusion, in the present study, several of the tested 2-SC were shown, for the first time, to
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25 prevent LTB₄ production by human leukocytes and to inhibit COX-1 activity. These effects,
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27 together with the well known antioxidant properties of these compounds may become a new
28
29 therapeutic option in the treatment of inflammatory processes.
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34
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36
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7 Fig. 1 Metabolism of arachidonic acid by the cyclooxygenase and 5-lipoxygenase pathways.
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10 *5-Lipoxygenase-activating protein
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14 Fig. 2 Chemical structures of the tested 2-SC.
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19 Fig. 3 Inhibition of human PMNL production of LTB₄ by 2-SC (25 μM) and NDGA (1 μM)
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21 determined by EIA. Each value represents mean±SEM of at least 4 experiments performed in
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23 duplicate. ***P<0.001, **P<0.01, *P<0.05, significantly different from control.
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29 Fig. 4 Inhibition of COX-1 activity by 2-SC, determined by EIA. Each value represents
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31 mean±SEM at least 4 experiments performed in duplicate. ***P<0.001, *P<0.05, significantly
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33 different from control.
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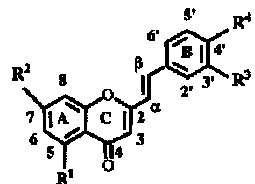
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38 Fig. 5 Reducing activity of 2-SC and ascorbic acid (AA). All the compounds were tested at the
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40 final concentration of 25 μM. Each value represents mean ± SEM of triplicate measurements.
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42 ***P<0.001, *P<0.05, significantly different from control.
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47 Fig. 6 UV-Vis absorption spectrum of 10 μM of compounds 1A, 1B, 1C, and 1D in the
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49 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
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51 direction of the arrows indicates crescent amounts of Fe(II). Insets: titration curves.
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7 Table 1 – Pearson correlations between the inhibition of LTB₄ production and the reducing
8 activity and between the LTB₄ production and the scavenging activity against ¹O₂ (IC₃₀) and
9 ONOO⁻ (with and without bicarbonate) (IC₅₀) [24] of the tested 2-SC.
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14 *significant at p < 0.05; **significant at p < 0.01. a) without bicarbonate; b) with bicarbonate.
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- 1A: $R^1=R^2=R^3=R^4=OH$
 1B: $R^1=H, R^2=R^3=R^4=OH$
 1C: $R^2=H, R^1=R^3=R^4=OH$
 1D: $R^1=R^2=H, R^3=R^4=OH$
 2A: $R^2=H, R^1=R^3=R^4=OH$
 2B: $R^1=R^2=H, R^3=R^4=OH$
 2C: $R^2=R^3=H, R^1=R^4=OH$
 2D: $R^1=R^2=R^3=H, R^4=OH$
 3A: $R^3=R^4=H, R^1=R^2=OH$
 3B: $R^1=R^2=R^3=H, R^4=OH$
 3C: $R^2=R^3=R^4=H, R^1=OH$
 3D: $R^1=R^2=R^3=R^4=H$

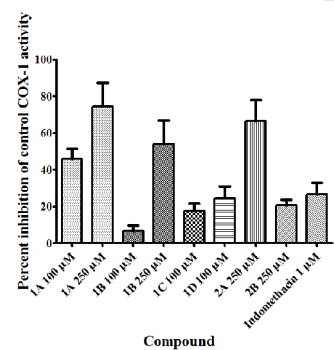
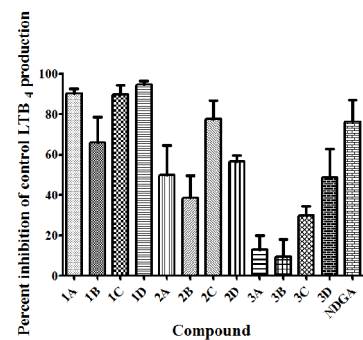
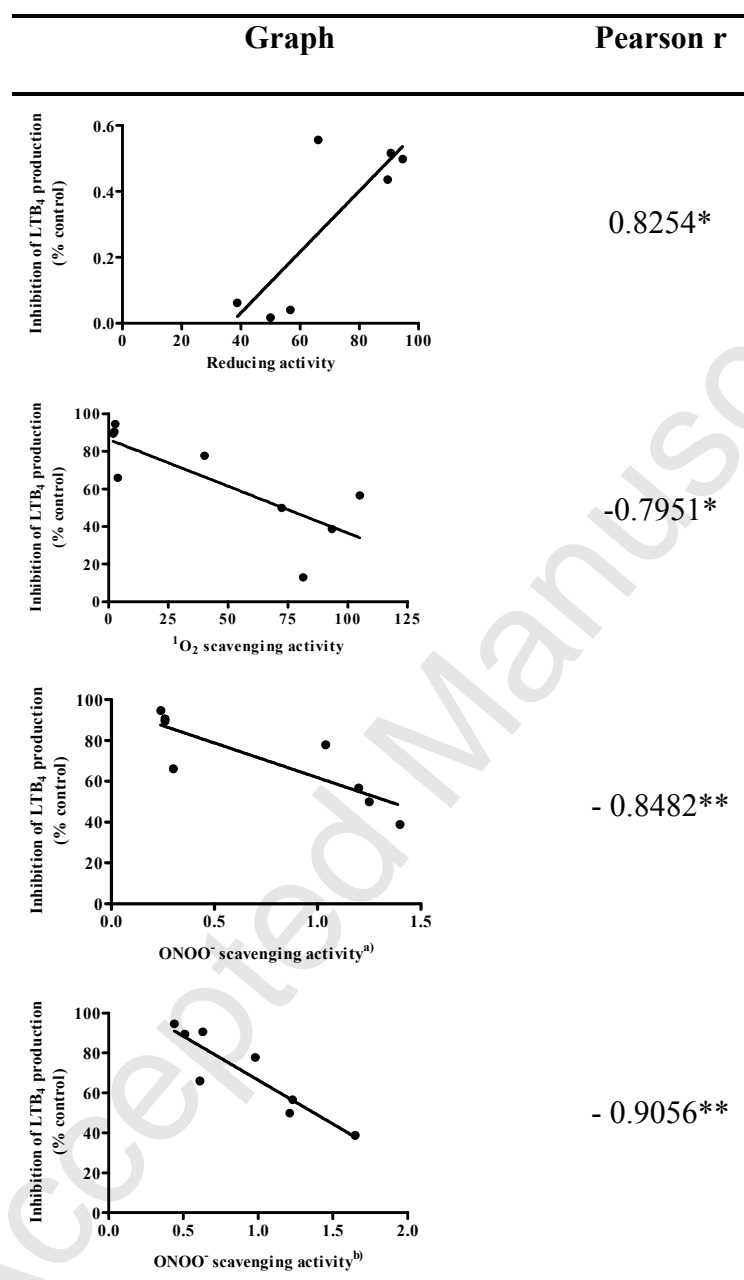
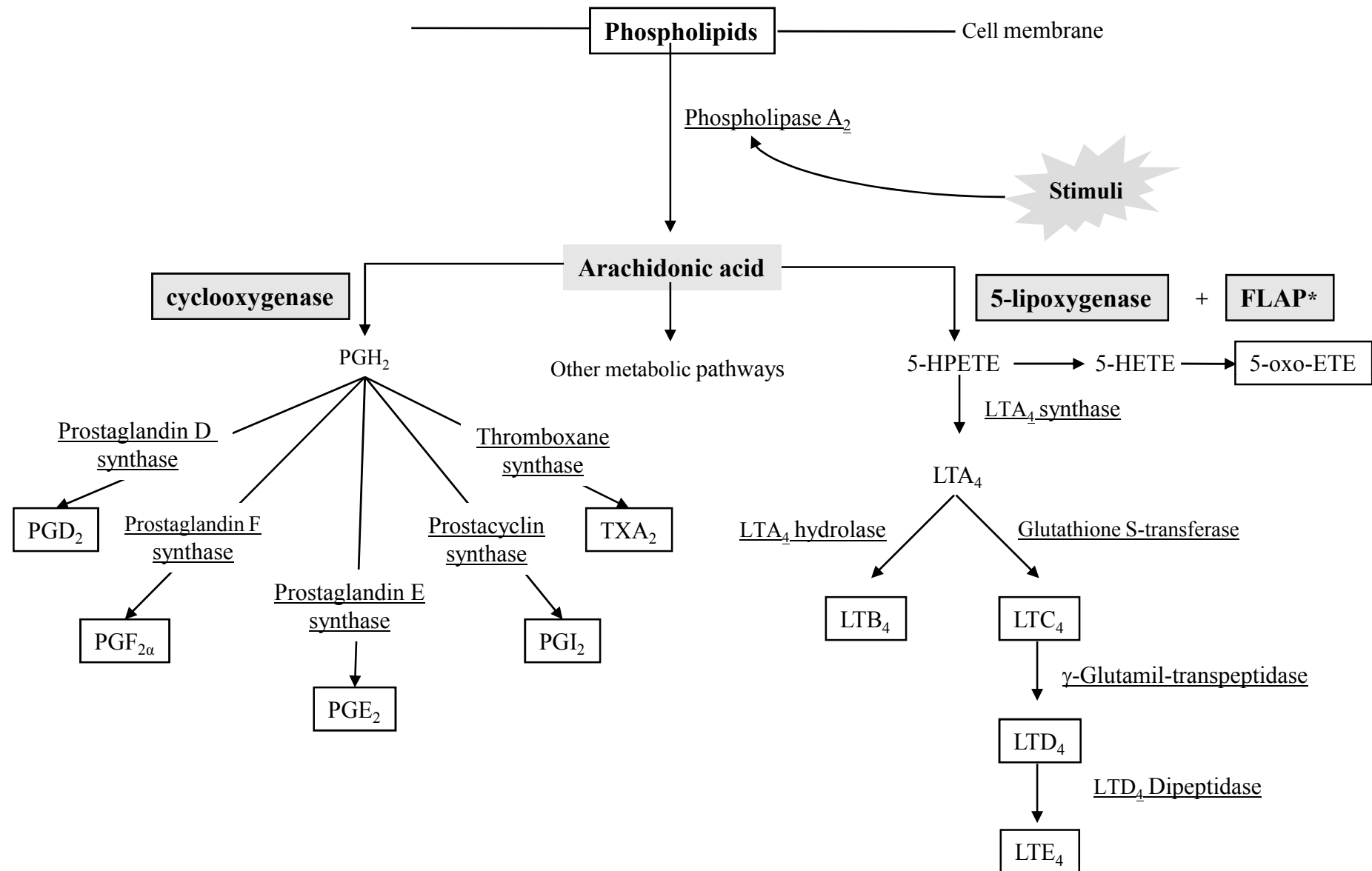
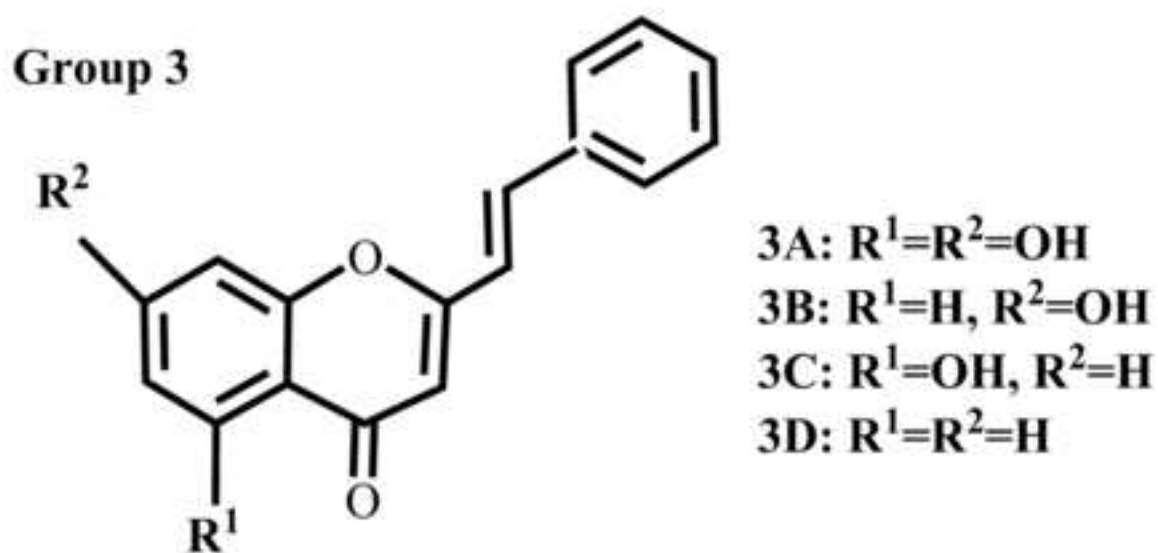
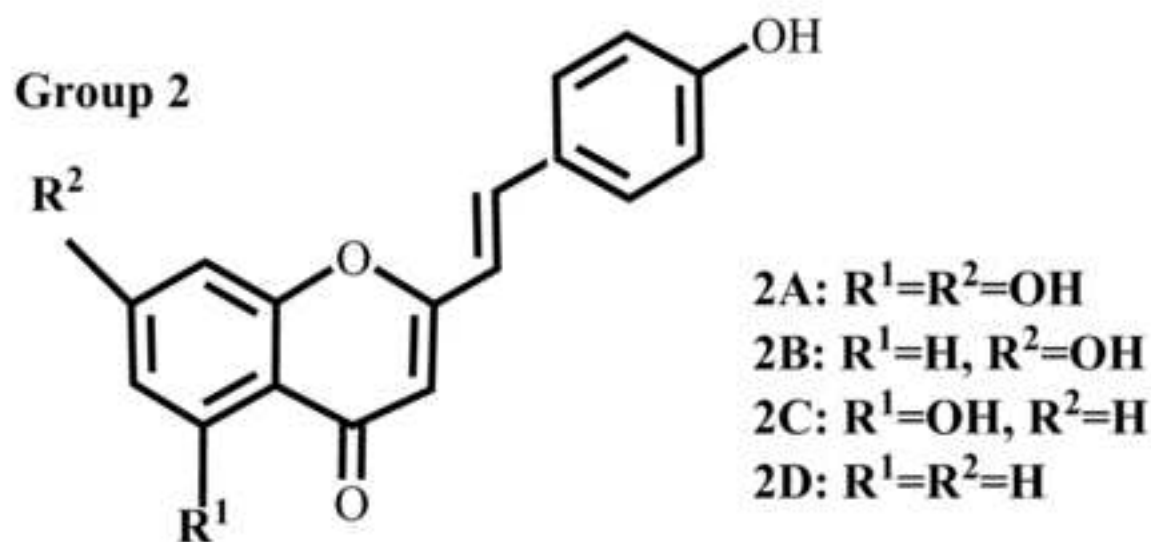
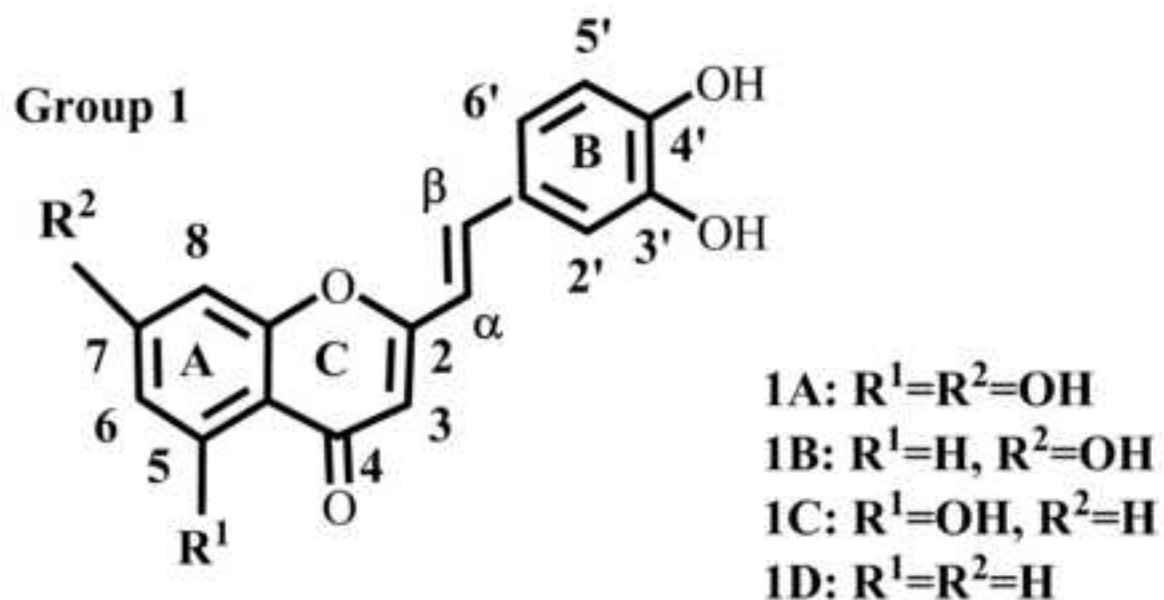
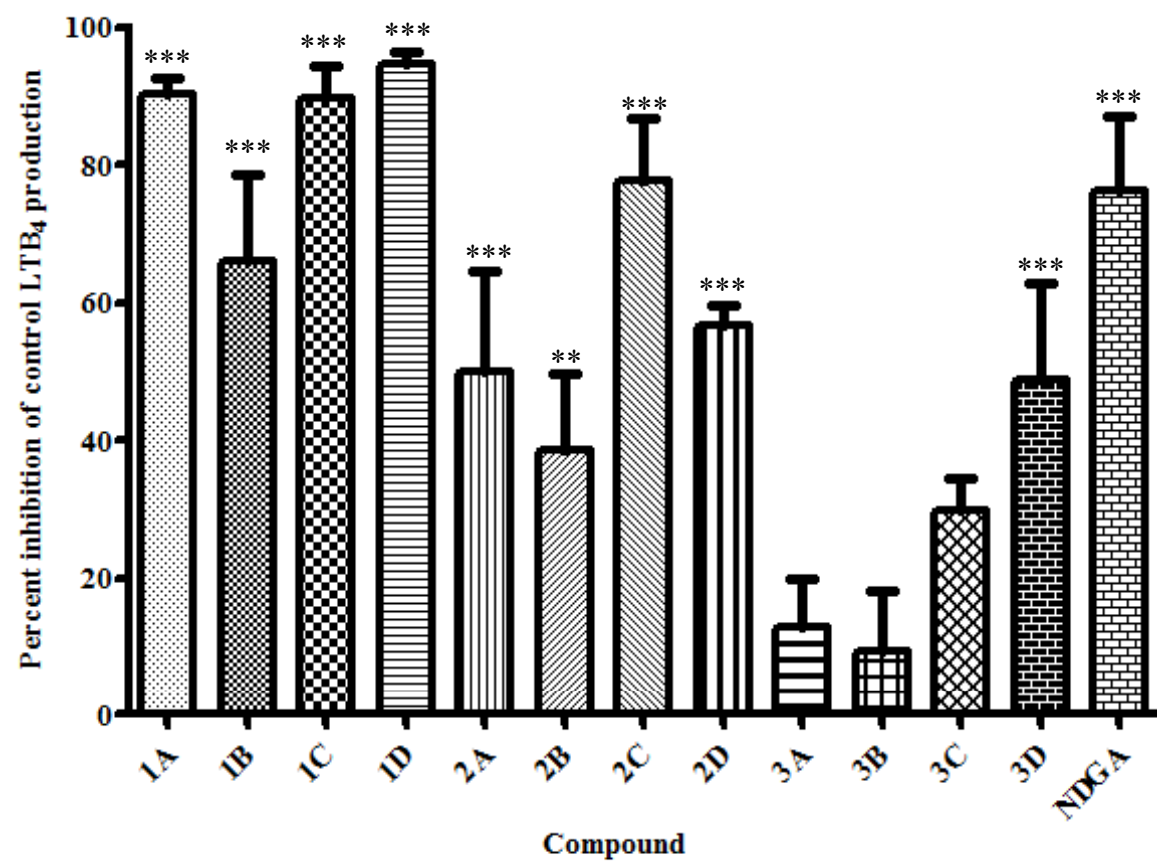


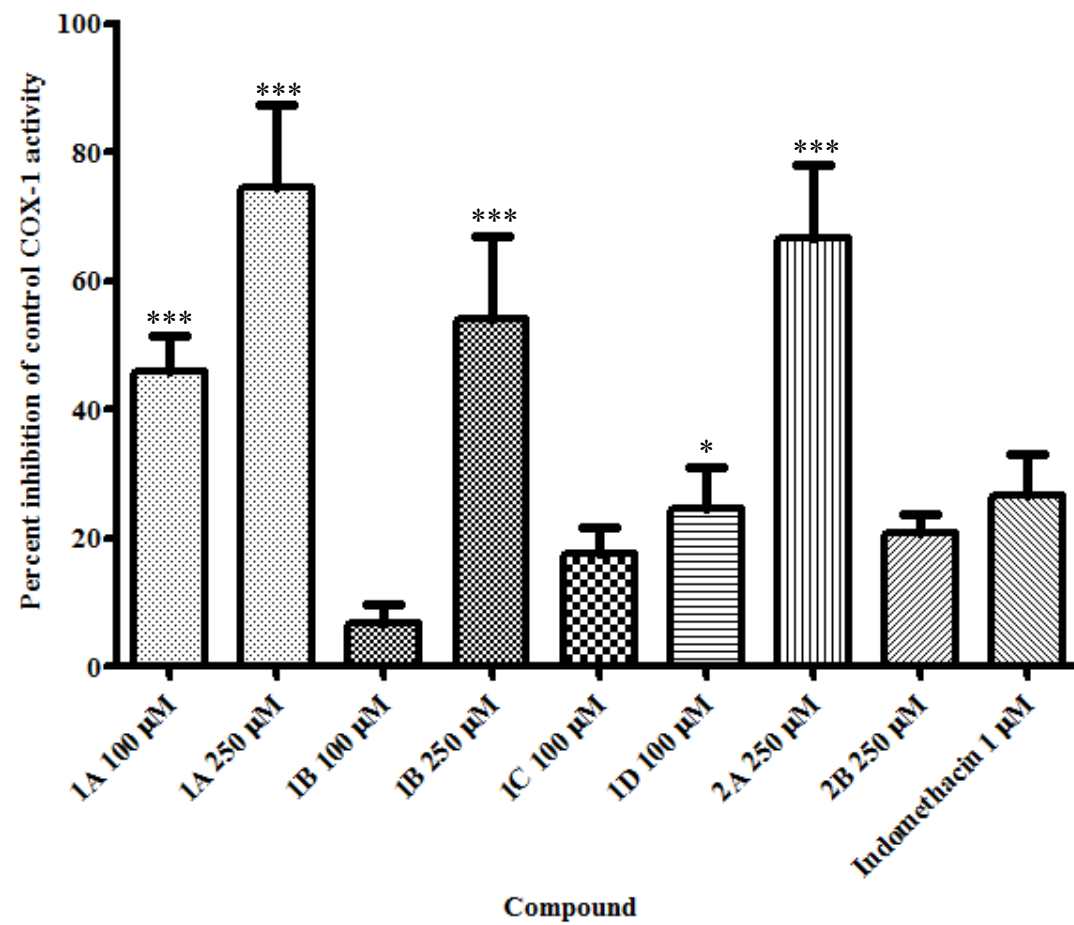
Table 1

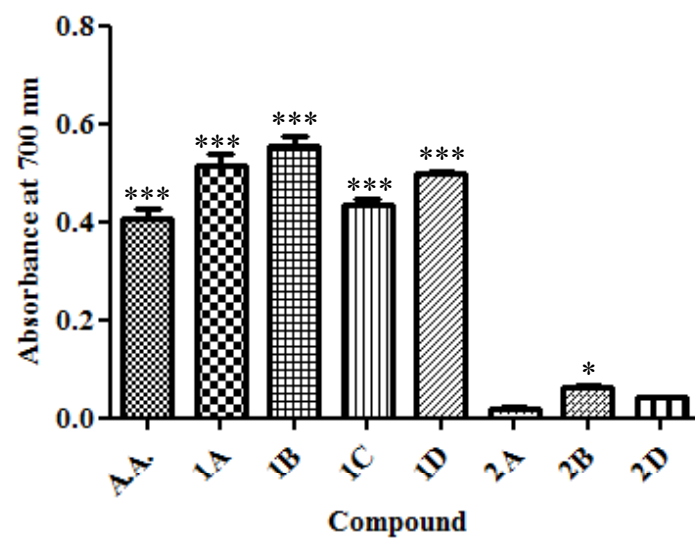


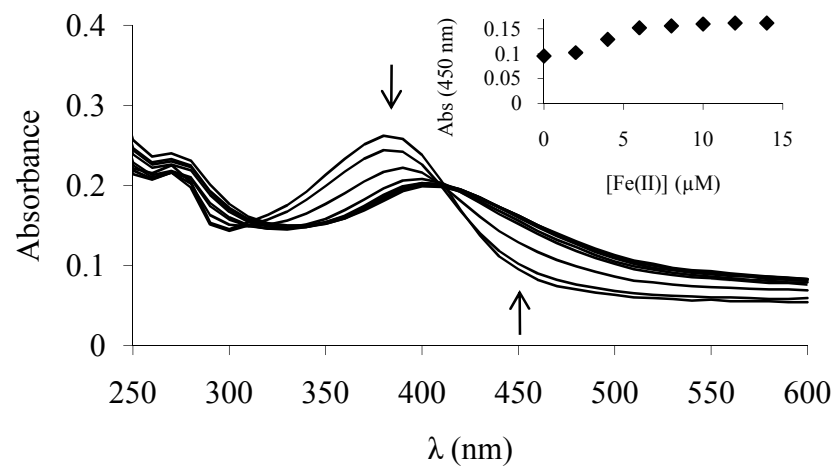
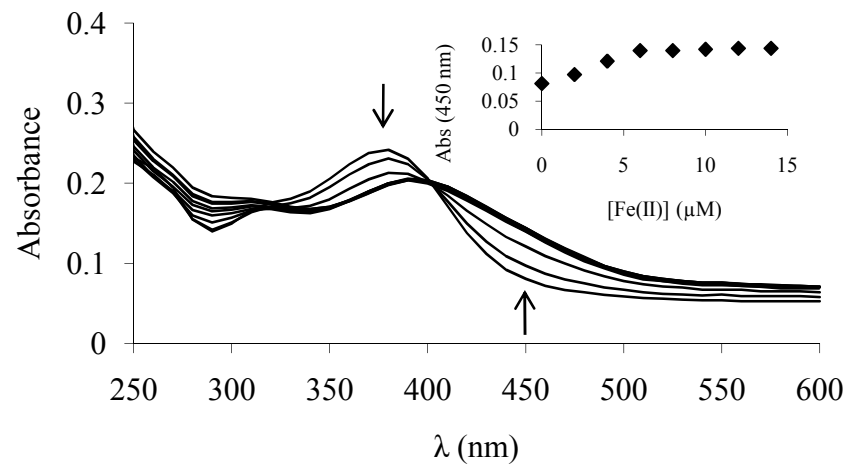
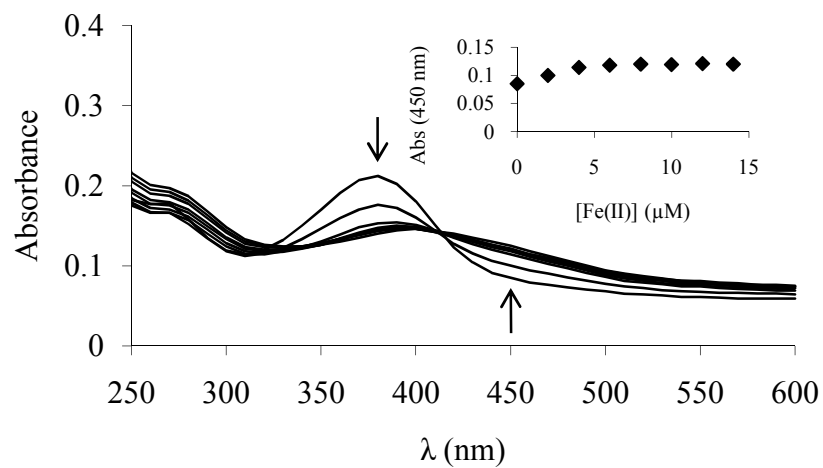
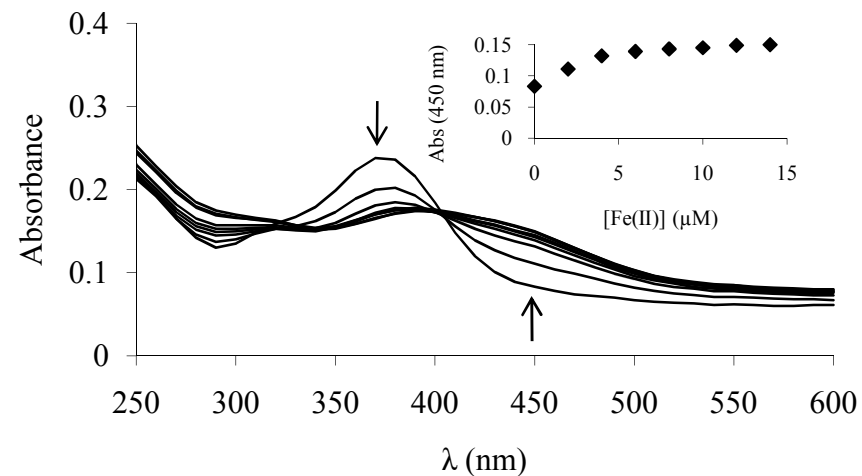










**1A****1B****1C****1D**