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2-Styrylchromones As Novel Inhibitors of Xanthine Oxidase. A Structure–activity Study

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The purpose of this study was the evaluation of the xanthine oxidase (XO) inhibition produced by some synthetic 2-styrylchromones. Ten polyhydroxylated derivatives with several substitution patterns were synthesised, and these and a positive control, allopurinol, were tested for their effects on XO activity by measuring the formation of uric acid from xanthine. The synthesised 2-styrylchromones inhibited xanthine oxidase in a concentration-dependent and non-competitive manner. Some IC_{50} values found were as low as 0.55 μM, which, by comparison with the IC_{50} found for allopurinol (5.43 μM), indicates promising new inhibitors. Those 2-styrylchromones found to be potent XO inhibitors should be further evaluated as potential agents for the treatment of pathologies related to the enzyme's activity, as is the case of gout, ischaemia/reperfusion damage, hypertension, hepatitis and cancer.

Keywords: 2-styrylchromones; Benzopyrone; Allopurinol; Xanthine oxidase inhibition

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INTRODUCTION

2-Styrylchromones are a small group of natural heterocyclic compounds. Only two natural 2-styrylchromones are known and they were extracted from the blue-green algae *Chrysophaem taylori* in the 1980s.[1,2] The synthesis of this class of compounds commenced about six decades prior to the isolation of the first natural product.[3] Natural derivatives have demonstrated cytotoxic activity against leukaemia cells, while those obtained by synthesis have exhibited anti-allergic, antitumour and anticancer properties.[1,2,4,5]

2-Styrylchromones (Figure 1) have a common structural feature with flavones in containing the benzopyrone moiety. Taking into account that flavones are known to be xanthine oxidase (XO) inhibitors,[6–9] the evaluation of this activity for 2-styrylchromones was thought to be of prime importance, since little is known about the biochemical mechanisms responsible for the effects of this group of compounds.

XO is a highly versatile enzyme, which is widely distributed among species and within the various tissues of mammals.[10] XO exhibits a broad activity toward reducing substrates. It can hydroxylate a wide variety of purines (notably xanthine and hypoxanthine), pteridines, related aromatic heterocycles, and also a range of aliphatic and aromatic aldehydes, taking these to the corresponding carboxylic acids, with concomitant reduction of molecular oxygen.[11–13] In XO-catalysed reactions, oxygen is reduced by one or two electrons giving rise to superoxide radical ($O_2^{\cdot-}$) or hydrogen peroxide (H_2O_2).[14,15] Consequently, xanthine oxidase is considered to be an important biological source of reactive oxygen species (ROS), which induce oxidative stress and are involved in many pathological processes such as inflammation, atherosclerosis, cancer and aging.[16] It has also been shown that xanthine oxidase decomposes low molecular weight S-nitrosothiols (*e.g.* S-nitrosoglutathione and S-nitrosocysteine) by superoxide-dependent and

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-independent mechanisms, and, according to the availability of oxygen in the system, secondarily leads to peroxynitrite formation.[17] This may alter the transport and storage of nitric oxide (NO) by S-nitrosothiols and the activity of proteins that are regulated by S-nitrosylation. Thus, the superoxide radical destroys the endothelium-derived vascular relaxing factor (nitric oxide) causing vascular constriction.[18] It is of note that XO tissue levels are increased after ischaemia reperfusion[19] with serum levels increased in hepatitis[20] and brain tumours.[21] It is also known that an extensive metabolism of xanthine by XO will increase body uric acid levels. Due to the low solubility of uric acid, there is a tendency for urate crystals to be deposited in the urinary tract and in the synovial fluid of joints, a process associated with painful inflammation, designated gout.[22] Therefore, XO inhibitors are expected to be therapeutically useful for the treatment of the aforementioned pathological states.

The aim of this study was to evaluate the activity profile of some synthetic 2-styrylchromone derivatives as inhibitors of XO. Structure–activity relationship data was obtained by comparing ten synthetic 2-styrylchromones **1–3** (Figure 1) and a positive control, allopurinol.

MATERIALS AND METHODS

Reagents

Xanthine and xanthine oxidase (XO) grade I from buttermilk (EC 1.1.3.22), were purchased from Sigma. The other chemicals were obtained from Merck. All reagents were of analytical grade.

2-Styrylchromones **1–3** were obtained from 2′-hydroxyacetophenones and cinnamic acid derivatives.[23–25]

Xanthine Oxidase Activity

The effect of the tested compounds on XO activity was evaluated by measuring the formation of uric acid from xanthine in a double beam spectrophotometer (Shimadzu 2600), at room temperature. The reaction mixtures in the sample wells consisted of xanthine (400 μL, 44 μM final concentration), XO in EDTA 0.1 mM (100 μL, 0.29 U/ml final concentration), and test compounds (100 μL, in various concentrations). The test compounds were dissolved in DMSO. Xanthine was dissolved in NaOH 1 μM, and subsequently in phosphate buffer 50 mM with EDTA 0.1 mM, pH 7.8. The absorbance was measured at 295 nm for 2 min.

Additionally, this procedure was repeated for some compounds with several concentrations of xanthine (5.5, 11, 22, and 44 μM), in order to evaluate the type of inhibition using the Lineweaver–Burk plot.

Statistical Analysis

Each separate experiment for XO inhibition was conducted in duplicate, in a minimum of 5 assays per tested compound. The results are expressed as mean ± SE. IC_{50} values were determined from plots of concentration vs percentage inhibition curves.

RESULTS

All the tested 2-styrylchromone derivatives **1–3** were found to be inhibitors of the XO-mediated oxidation of xanthine to uric acid in a concentration-dependent manner (Table I). IC_{50} values of the tested compounds are also listed in Table I. Allopurinol, a known xanthine oxidase inhibitor clinically used in the treatment of gout,[26] was also very effective in the present assay, giving an IC_{50} of 5.43 ± 0.80 μM (Table I). Four of the tested 2-styrylchromones were found to be more potent than this drug and the rank order of xanthine oxidase inhibition was **3c** > **3b** > **1d** > **3a** > allopurinol > **2c** > **1c** > **2b** > **1b** > **2a** > **1a** (Table I).

Kinetic studies were performed in order to determine the type of inhibition of these compounds. Lineweaver–Burk plots (Figure 2) indicate that the

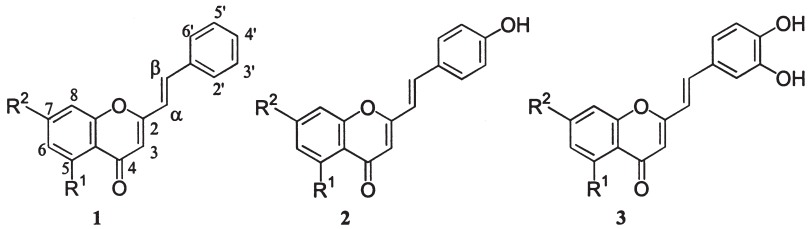

FIGURE 1 Structure of the tested 2-styrylchromones **1–3**.

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TABLE I The inhibitory effects of 2-styrylchromones **1–3** and allopurinol on xanthine oxidase activity

	Concentration (μM)/% inhibition ± SE*						
Compound	0.625	2.5	10	25	50	100	IC_{50} (μM)
1a				4.34 ± 0.80	12.04 ± 1.83	32.99 ± 2.65	>100
1b			6.24 ± 0.95	18.77 ± 2.18	52.03 ± 5.83	86.45 ± 3.15	52.06 ± 4.38
1c		15.93 ± 0.58	35.48 ± 1.58	61.17 ± 1.01	81.56 ± 1.48		16.96 ± 0.85
1d	21.1 ± 1.51	48.28 ± 0.70	76.99 ± 1.19	90.26 ± 0.77			2.52 ± 0.08
2a			13.6 ± 1.14	25.51 ± 1.72	37.44 ± 1.51	62.37 ± 2.53	77.35 ± 3.38
2b		17.77 ± 1.43	38.45 ± 1.98	57.12 ± 2.64	74.76 ± 2.39		19.64 ± 2.97
2c		25.2 ± 1.63	52.79 ± 1.84	71.51 ± 1.8	81.87 ± 1.42		9.46 ± 1.08
3a	14.33 ± 0.98	38.89 ± 2.26	69.83 ± 1.28				4.36 ± 0.57
3b	26.42 ± 1.11	54.32 ± 1.68	81.74 ± 1.22				2.03 ± 0.19
3c	54.31 ± 2.03	76.74 ± 1.42	92.19 ± 0.98				0.55 ± 0.03
Allopurinol		31.41 ± 2.32	64.73 ± 1.18	85.06 ± 0.61	92.87 ± 0.45		5.43 ± 0.80

*n = 5.

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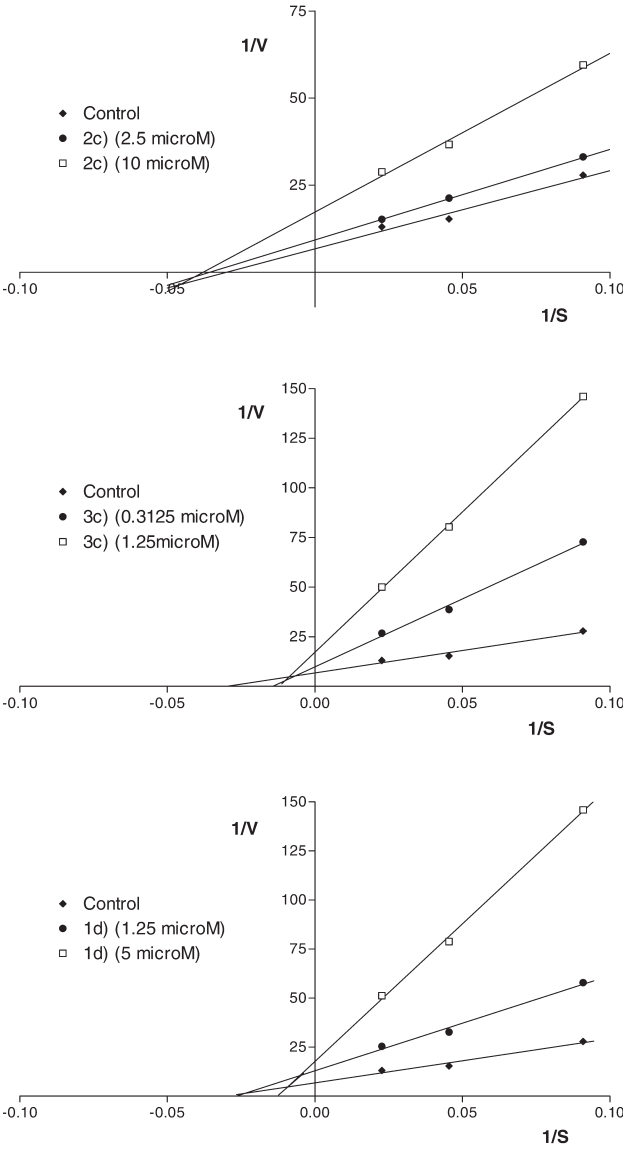

FIGURE 2 Lineweaver–Burk plots in the absence (control) and in the presence of 2-styrylchromones **2c**, **3c**, and **1d** with xanthine as the substrate. V = ΔA/min; S = xanthine (μM).

inhibition by the most potent compound **3c**, as well as two other representative compounds **1d** and **2c**, was of a non-competitive inhibition type.

DISCUSSION

Although the chemical structure of 2-styrylchromones gives a good indication of its potential as a XO inhibitor, to our knowledge the present study clearly demonstrates this activity for the first time. From the results obtained in the present study, some considerations about the structure–activity relationships can be made. The catechol group linked to the styryl moiety of the molecule (compounds **3a–c**) strongly contributes to the inhibition of xanthine oxidase. In fact, the absence of hydroxyl groups in the aromatic ring linked to the styryl moiety (compounds **1a–d**), or the presence of only one *p*-phenolic group (compounds **2a–c**) substantially decreased the inhibition. Interestingly, caffeic acid, also a styryl bearing catechol compound, is a strong inhibitor of XO, with an IC_{50} of around 20–70 μM, depending on the experimental conditions.[27,28] The α,β-unsaturated double bond plays a very important role in the XO inhibition of caffeic acid.[28] The low IC_{50} found for **3c** is probably the result of a potentiation of effects by the catechol group linked to the styryl moiety of the 5,7-dihydroxylated benzopyrone.

The hydroxylation pattern in the benzopyrone moiety was important for the potency of XO inhibition. Indeed, the unsubstituted 2-styrylchromone **1a** was practically inactive. This finding is in agreement with previous studies with flavones[8] and coumarins.[7] The presence of hydroxyl groups at the C-5 and C-7 positions of the benzopyrone lead to an observed increase in activity, when compared with the presence of only one substitution, which is also in

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agreement with the similar activity profile for flavones.[29] It was found that the 7-hydroxylated derivatives were more potent than the respective 5-hydroxylated derivatives. Again, the importance of the C-7 hydroxylation has also been observed for the XO inhibitory activity of flavone derivatives[8] and coumarin derivatives.[30] Some other interesting structural features of flavones including the presence of the double bond between C-2 and C-3 (essential for planarity of the molecule) as well as the absence of a hydroxyl at C-3 enhances XO inhibitory activity.[29] Furthermore, the presence of an additional hydroxyl at C-6 has been shown to considerably increase the activity of some flavones[7] and decrease it in others.[29] Although such types of styrylchromones have not been obtained to date, it seems to be of interest to evaluate their activity further.

The mode of inhibition of the studied compounds was of a mixed non-competitive inhibition type. This means that the binding site of these compounds to XO is not the molybdenum site but more probably the iron–sulfur group of the enzyme. However, this type of inhibition is not the rule for benzopyrone-bearing compounds, as can be inferred from the competitive or uncompetitive mode of inhibition reported for various flavonoids,[6,31] 7-hydroxycoumarin and esculetin.[30,32]

In conclusion, various 2-styrylchromones were found to be potent XO inhibitors and should be further evaluated as potential agents for the treatment of pathologies related with its activity, *e.g.* gout, ischaemia/reperfusion damage, hypertension, hepatitis and cancer.

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