Interest of 3-arylcoumarins as xanthine oxidase inhibitors

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Abstract: In the current paper we studied the interest of a series of 3-arylcoumarin derivatives as xanthine oxidase inhibitors. For the best compound of the series, the 4'-methoxyphenyl-6-nitrocoumarin, it was determined the IC_{50} value and the type of inhibition. This work is a preliminary screening for further design and synthetize new non-purinergic derivatives as potential compounds involved in the inflammatory suppression, specially related to the gout.

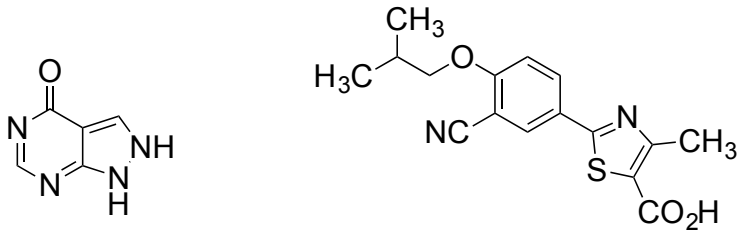
Keywords: 3-Arylcoumarins • Condensation reaction • Cross-coupling reaction • Xanthine oxidase inhibitors • Gout

1. Introduction

Xanthine oxidase (XO, EC 1.2.3.2) is a form of molybdoflavin protein that plays an important role at the end of the catabolic sequence of the purine nucleotide metabolism in humans and a few other uricotelic species.[1] It firstly catalyzes of hypoxanthine to xanthine, and then transforms it into uric acid, which is excreted in the urine. Overproduction or under excretion of uric acid in humans could lead to hyperuricemia and gout, which is caused by crystallization and deposition of uric acid in joints and surrounding tissue.[2]

Gout is a metabolic disorder associated with abnormal amounts of uric acid in the body, which causes inflammation, gouty arthritis, and uric acid nephrolithiasis.

Recent studies have indicated that asymptomatic hyperuricemia is associated with or may have a casual relationship with cardiovascular disease.[3] Allopurinol (1*H*-pyrazol[3,4-*d*]pyrimidin-4-ol, **Figure 1**) is a substrate and specific potent inhibitor for XO, and has been used for gout treatment for a number of years.[4] Several studies have indicated that allopurinol may induce hypersensitivity syndrome an Stevens-Johnson syndrome in patients.[5,6,7] More recently, febuxostat (2-(3-cyano-4-isobutoxyphenyl)-4-methyl-1,3-thiazole-5-carboxylic acid, **Figure 1**), a new non-purine XO inhibitor, has been approved for management of gout in the European Union and USA.[4,8] Many side effects of febuxostat have been reported.[9]



Allopurinol Febuxostat

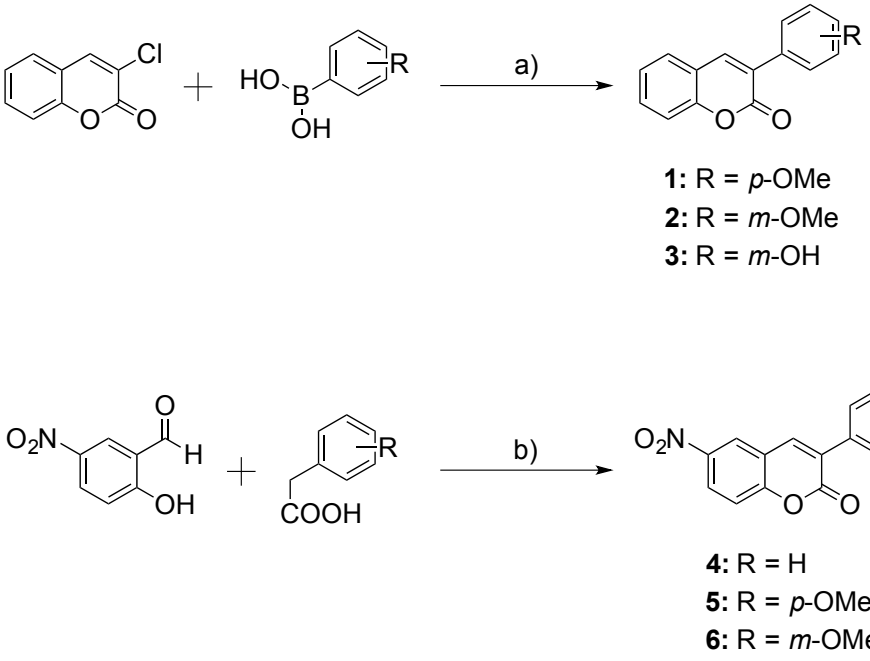
Figure 1. Chemical structure of allopurinol (reference compound) and febuxostat.

In this study, we intended to develop XO inhibitors with potent activity and low toxicity, using compounds with a chemical structure distinct from allopurinol and febuxostat. Therefore, XO inhibitory activity was evaluated and compared with allopurinol.

2. Results and discussion

Based on previous findings in the area, and in our experience with substituted 3-arylcoumarins, in the present work we proposed the synthesis (**Scheme 1**) and XO inhibition evaluation (**Table 1**) of a series of different substituted 3-

arylcoumarins.[10,11] With the aim of finding the structural features for the biological activity, we decided to explore the importance of the nature and position of small groups (methoxy, nitro and hydroxyl substituents) into both coumarin nucleus and 3-aryl ring (**Scheme 1**).



Scheme 1. Synthesis of 3-arylcoumarin derivatives. Reagents and conditions: a) sodium carbonate (2.0 equiv), palladium complex (0.5 mol %), DMF/H_2O (1:1), 110 ^oC, 120–180 min. b) NaH (1.0 equiv), acetic anhydride, r.t., 3 h.

The derivatives **1**-**3** were efficiently synthesized by a direct cross-coupling reaction, in presence of sodium carbonate, *N,N'*-bis(salicylidene)-ethylenediamino-palladium (salen-Pd), Na_2CO_3 in DMF/H_2O (1:1), at 110 ^oC, for 120–180 min. The reaction mixture was purified by flash chromatography, using hexane/ethyl acetate as eluent in a proportion of 9:1. Starting from the 3-chlorocoumarin and the respectively substituted arylboronic acids, we obtained three derivates in good yields (56–64%).

The derivatives **4**-**6** were efficiently synthesized by a condensation reaction in a dry Schlenk tube, in presence of sodium hydride and with acetic anhydride as solvent, at room temperature for three hours. The reaction mixture was purified by flash chromatography, using hexane/ethyl acetate as eluent in a proportion of 9:1. Starting from the commercially available nitro-substituted

salicylaldehyde and the respectively substituted arylacetic acids, we obtained three derivates in good yields (60–75%).

Table 1. XO inhibition for compounds **1**-**6**.

Compounds	% Inhibition at 100 µM	IC_{50} (µM)	Type of inhibition
1	22.5	-	-
2	40.38	-	-
3	14.5	-	-
4	19.2	-	-
5	85	9.0	Uncompetitive
6	76.6	-	-
Allopurinol[12]	-	0.1	Competitive

In general, the studied 3-arylcoumarins presented no significant inhibitory activity against XO (**Table 1**). Compound **5** (4'-methoxyphenyl-6-nitrocoumarin) proved to be the best candidate of the series. Therefore, the IC_{50} of this compound was calculated (9.0 µM) and the compound proved to be 90 times less active than allopurinol, used as reference compound. In addition, the type of inhibition was also determined (**Figure 2**). This compound is an uncompetitive inhibitor of XO, in contradiction to the reference compound.

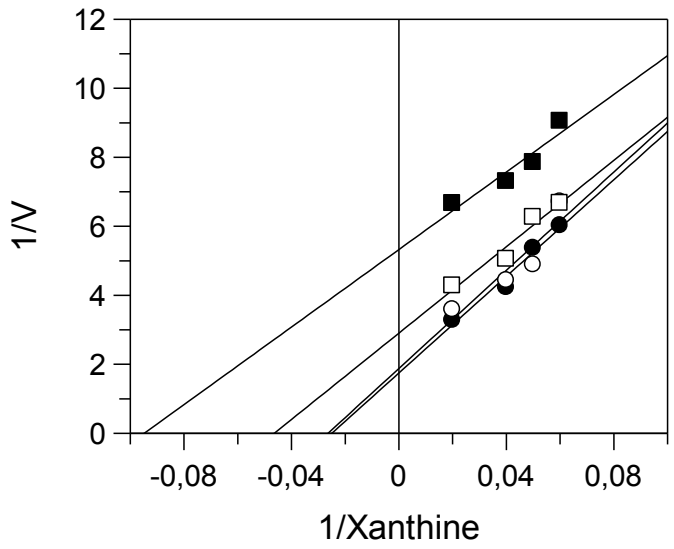


Figure 2. Lineweaver-Burk plots for inhibition of compound **5** on XO. Concentrations of compound **5** were 0 (●), 2.5 (○), 5.0 (□) and 7.5 (■) μM.

3. Conclusion

Compound **5** proved to be the best XO inhibitor of the studied series. It is an uncompetitive inhibitor that could be the inspiration for the design of further non-purinergic XO inhibitors.

4. Experimental methodologies

4.1. Synthesis

General procedure for the preparation of 3-arylcoumarins **1**-**3**. To a 20 mL two neck round bottomed flask was added a solution of 3-chlorocoumarin (0.83 mmol), arylboronic acid (1.04 mmol), Na_2CO_3 (1.66 mmol), and Pd-salen complex (0.5 mol %) in DMF/H_2O (1:1). The reaction mixture was heated at 110 ^oC for 120–180 min. The reaction was monitored by chromatography. After the completion of the reaction, the mixture was extracted with ethyl acetate (3 x 20 mL). The organic extracts were dried over anhydrous sodium sulphate, filtrated, and the solvent was evaporated under vacuum. The obtained crude product was purified by column chromatography (hexane/ethyl acetate 9:1) to give the coumarins **1**-**3**.

General procedure for the preparation of 3-arylcoumarins **4**-**6**. In a 20 mL dry Schlenk tube, to a solution of the conveniently substituted salicylaldehyde (2.46 mmol) and the arylacetic acid (2.46 mmol), in acetic anhydride (6 mL), NaH (2.46 mmol) was added in small portions, and the reaction mixture was stirred for 3 hours, at room temperature. The obtained crude was filtered and washed with diethyl ether. The solid was then purified by flash chromatography (hexane/ethyl acetate 9:1) to give the desired coumarins **4**-**6**.

4.2. Biological assays

The biological assays were carried out following the protocol described below. 0.1 M of phosphate buffer solution (pH 7.5), an aqueous solution of XO (0.5 U/mL, Sigma Chemical Co) and DMSO with or without the compound. Then, a 0.82 mM of xanthine solution was added and the activity of the XO was determined spectrophotometrically (Varian Cary 50) by measuring the formation of uric acid at 295 nm. The percent of XO activity inhibition was calculated as: inhibition (%) = (A – B) / A x 100, where A represents the difference in the absorbance of control sample between 0.5 and 1.0 min, and B represents the difference in absorbance of the test sample between 0.5 and 1.0 min. The IC_{50} value, a concentration giving 50 % inhibition of XO activity, was determinate by interpolation of dose-response curves. Allopurinol was used as a reference XO inhibitor.

Acknowledgements

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