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Research paper

Design, synthesis and evaluation of semi-synthetic triazole-containing caffeic acid analogues as 5-lipoxygenase inhibitors

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ABSTRACT

In this work the synthesis, structure–activity relationship (SAR) and biological evaluation of a novel series of triazole-containing 5-lipoxygenase (5-LO) inhibitors are described. The use of structure-guided drug design techniques provided compounds that demonstrated excellent 5-LO inhibition with IC_{50} of 0.2 and 3.2 μM in cell-based and cell-free assays, respectively. Optimization of binding and functional potencies resulted in the identification of compound **13d**, which showed an enhanced activity compared to the parent bioactive compound caffeic acid **5** and the clinically approved zileuton **3**. Compounds **15** and **16** were identified as lead compounds in inhibiting 5-LO products formation in neutrophils. Their interference with other targets on the arachidonic acid pathway was also assessed. Cytotoxicity tests were performed to exclude a relationship between cytotoxicity and the increased activity observed after structure optimization.

1. Introduction

5-Lipoxygenase (5-LO) is a crucial enzyme of the arachidonic acid (AA) cascade, involved in the first two catalytic reactions in the biosynthesis of leukotrienes (LTs) [1]. The role of LTs as inflammatory mediators is well established, with pathophysiological implications in different diseases like asthma, allergic rhinitis as well as cardiovascular diseases and certain types of cancer [2].

Some new 5-LO inhibitors of plant origin are currently under clinical development for the treatment of osteoarthritis, such as a combination of baicalein **1** and catechin **2**, named Flavocoxid (Limbrel) [3]. Nevertheless, the only 5-LO inhibitor to have reached the market so far is zileuton **3**, which has some major drawbacks such as liver toxicity, low potency, and a short half-life due to rapid metabolic breakdown (Fig. 1) [4].

With the rising number of indications for anti-LT therapy, 5-LO inhibitor drug development becomes increasingly important. A variety of polyphenols have been reported to be capable of preventing the formation of 5-LO products, among which prominent dietary compounds such as curcumin **4**, and caffeic acid **5** are worth mentioning (Fig. 2) [5].

The current interest in polyphenols has been driven primarily by their abundance in human diet and in plants [6]. Moreover, several polyphenols can be isolated as products from the waste streams of the food industry, thus providing a potentially sustainable source of these compounds. However, the health effects of polyphenols are

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Abbreviations: 5-LO, 5-Lipoxygenase; LT, Leukotriene; AA, Arachidonic Acid; SAR, Structure-Activity Relationship; CuAAC, Copper(I)-catalysed Azide Alkyne Cycloaddition; DCC, Dicyclohexylcarbodiimide; DCM, Dichloromethane; MTT, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide; FLAP, Five Lipoxygenase Activating Protein; FCC, Flash Column Chromatography; TLC, Thin Layer Chromatography.

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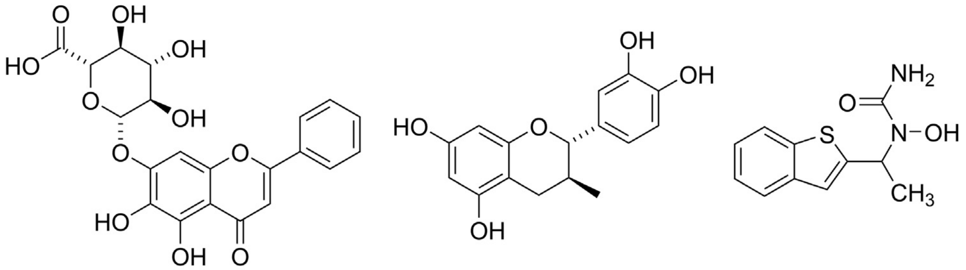
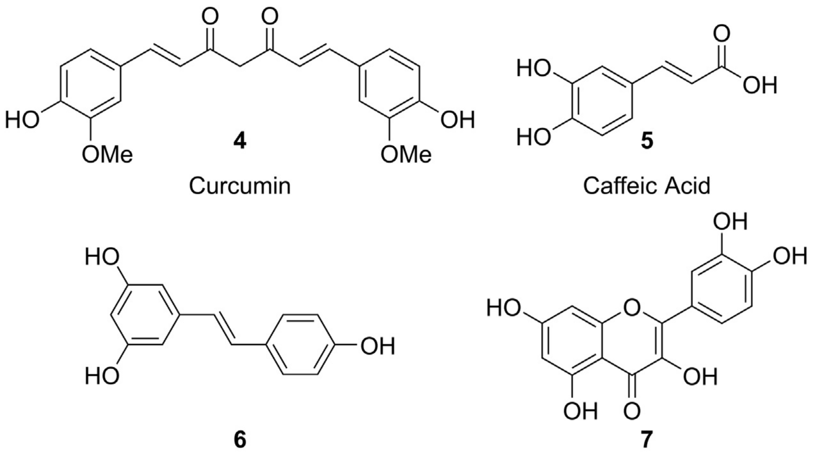


1
Baicalein

2
Catechin

3
Zileuton

Fig. 1. Zileuton and 5-LO inhibitors of plant origin.



4
Curcumin

5
Caffeic Acid

6
Resveratrol

7
Quercetin

Fig. 2. Naturally occurring bioactive polyphenols.

negatively influenced by the bioavailability and solubility issues often associated with these phytochemicals, which largely reduce their use in pharmaceuticals [7]. Recent attempts to overcome these issues include the pegylation of curcumin **4** [8] and the glycosylation of resveratrol **6** and curcumin **4** [9].

A new approach is explored in the present work with the incorporation of a triazole-containing linker into polyphenol molecules. Triazoles are, in fact, not only passive linkers capable of giving metabolic stability and increasing water solubility through hydrogen-bond formation, but are also important pharmacophores able to interact with different targets. They have been proven to be determinant for the activities in antifungal, antibacterial, and anticancer agents, HIV protease inhibitors [10] and anti-inflammatory compounds [11].

2. Results and discussion

2.1. Synthesis of analogues

In this study a new class of semi-synthetic polyphenols related to caffeic acid has been designed. Structural modifications have been made in order to evaluate the structure–activity relationship (SAR) on 5-LO. Particularly, the linker length (A), the linker nature (B), the substitution on the aromatic portion (C) and the functionalization of the hydroxyl group (D) have been considered (Fig. 3).

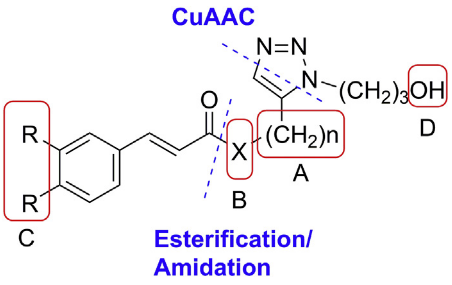


Fig. 3. Structural modifications on the triazolyl caffeic acid.

The retrosynthetic analysis of these compounds suggested an initial esterification or amidation of the caffeic acid followed by the formation of the triazole cycle, by means of a copper(I) catalysed azide-alkyne cycloaddition (CuAAC, Fig. 3) [12]. Carboxylic acids **8a** and **8b** were easily converted into **10a**–**10f** and **12** after refluxing in benzene in presence of DMF and an excess of thionyl chloride. Propargyl alcohols **9a**–**9d**, propargylamine **9e** or the 3-azidopropan-1-ol **11** were then added and reacted at room temperature to furnish **10a**–**10f** and **12** in good yields (Scheme 1).

Propargyl derivatives **10a**–**10f** were subjected to the classical CuAAC conditions, by reacting with the azidoalcohol **11** in presence of catalytic amounts of Cu(I)iodide (Scheme 2).

The molecular diversity achieved in this library of compounds could be further increased by preparing analogue **14**, which represents a regioisomer of **13a**, in order to evaluate a possible distinct activity against 5-LO. Compound **14** was smoothly obtained by