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Research paper

Discovery of novel hybrids of diaryl-1,2,4-triazoles and caffeic acid as dual inhibitors of cyclooxygenase-2 and 5-lipoxygenase for cancer therapy

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ABSTRACT

Inflammation plays a key role in cancer initiation and propagation. Cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX), two important enzymes in inflammatory responses are up-regulated in various tumor types. Dual inhibition of COX-2 and 5-LOX constitutes a rational concept for the design of more efficacious anti-tumor agents with an improved safety profile. We have previously reported a series of diaryl-1,2,4-triazole derivatives as selective COX-2 inhibitors. Herein, we hybridized the diaryl-1,2,4-triazoles with caffeic acid (CA) which was reported to display 5-LOX inhibitory and anti-tumor activities, affording a novel class of COX-2/5-LOX dual inhibitors as anti-tumor drug candidates. Most of these compounds exhibited potent COX-2/5-LOX inhibitory and antiproliferative activities *in vitro*. And the most potent compound **22b** could significantly inhibit tumor growth *in vivo*. Furthermore, mechanistic investigation showed that the representative compound **15c** blocked cell cycle in G2 phase and induced apoptosis in human non-small cell lung cancer A549 cells in a dose-dependent manner. Our preliminary investigation results would provide new clues for the cancer theatment with COX-2/5-LOX dual inhibitors.

1. Introduction

Inflammation is a complex process that involves widespread changes in cellar and molecular components of physiology. Controlled inflammation is necessary for a series of protective process including wound healing, tissue repair and defense against pathogens. However, prolonged chronic inflammation is mostly detrimental and has been linked to a number of diseases, including cancer [1–3]. Recent studies have validated the postulation that within the tumor microenvironment, a network of various pro-inflammatory mediators participate in complex signaling process that facilitates extravasations of tumor cells through the stroma, promoting the development of carcinogenesis [4].

Rarchidonic acid (AA), an essential polyunsaturated fatty acid with 20 carbons, is the major precursor of several classes of signal molecules, which are important mediators in inflammatory responses. The two main metabolic pathways of AA are cyclooxygenase (COX) and lipoxygenase (LOX) pathways. AA is converted to prostaglandins, prostacyclins and thromboxanes by COX and to hydroxyeicosatetraenioc acids (HETEs) or leukotrienes (LTs) by LOX. Recent studies regarding the relationship between AA metabolic process and carcinogenesis reveal novel molecular target for cancer treatment [5].

It has been reported that COX-2 is up-regulated in various tumor types, such as pancreatic, prostate and colorectal cancers [6–8], resulting in elevation of downstream prostaglandin E_2 (PGE_2) levels

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[9]. Lots of results indicate that PGE_2 could increase the motility and metastic potential of tumor cells, promote tumor angiogenesis, induce local immunosuppression and inhibit apoptosis [5,10]. Besides PGE_2 mechanism, COX-2 itself could also conduces to carcinogens. COX-2 peroxidase activity is able to transform many procarciogens into ultimate carcinogens, which can active many genes involved in cell proliferation [5,11]. Furthermore, COX-2 could also promote cancer cell survival through lowering the levels of unesterified AA [12]. Inhibition of COX-2 activity has been shown to induce apoptosis and inhibit proliferation and angiogenesis [13,14].

Similar to COX-2, the expression and activity of 5-LOX have been found to be up-regulated in many cancer cell lines [15–19], and closely related to tumor size, depth and vessel invasion [20]. It is evident from recent studies that 5-LOX and its downstream products leukotriene B_4 (LTB_4) and 5-hydroxyeicosatetranoic acid (5-HETE) could enhance cell proliferation and suppress apoptosis, thereby promoting the development of carcinogenesis [15,18,19,21–24]. It seems likely that COX-2 and 5-LOX may represent an integrated system that regulates the proliferation, metastatic and proangiogenic potential of cancer cells [5]. Considering the frequent co-expression of these two enzymes and the striking analogy of their biological functions, dual inhibitors of COX-2 and 5-LOX may present a superior anticancer profile in carcinogenesis. And notably, there is a cross-talk between COX-2 and 5-LOX pathways, inhibition of only one of them would shunt AA metabolism to the other pathway, thereby inducing potential side effects [25]. Hence the dual COX-2/5-LOX inhibitors would also be safer. Overall, dual inhibition of COX-2 and 5-LOX constitutes a rational concept for the design of more efficacious anti-tumor agents with an improved safety profile.

Triazole heterocycle is a building block of great value in drug candidates [26,27]. Recently, we have identified a series of 1,5-diaryl-1,2,4-trizole derivatives as selective COX-2 inhibitors, among which compound **1** (Fig. 1) displayed potent and selective COX-2 inhibitory activity ($IC_{50} = 0.37$ μM, SI = 0.018), equipotent to that of celecoxib ($IC_{50} = 0.26$ μM, SI = 0.015) [28]. Successively, we developed a series of diaryl-1,2,4-triazoles bearing N-hydroxyurea moiety as COX-2/5-LOX dual inhibitors for anti-inflammation [29]. Inspired by the obtained interesting results of our previous studies and in continuation of our endeavor for novel anticancer drugs, we became interest in exploring dual COX-2/5-LOX inhibitors as anti-tumor candidates with novel structural characteristics, better anticancer effects and improved safety profiles. Meanwhile, we noticed that the caffeic acid (CA), a widespread phenolic compound from the group of hydroxycinnamates, selectively inhibits 5-LOX [30] and a novel series of triazole-containing caffeic acid analogues was reported as 5-LOX inhibitors recently [31]. Moreover, CA and its derivatives, for instance, phenethyl caffeate (CAPE) could also inhibit the growth and differentiation of various cancer cells, promote apoptosis and prevent chemical carcinogenesis [32–36]. However, it has been found that CA has a stimulatory effect on prostaglandin synthase, which may result from its stimulation on COX pathway [30]. Accordingly, we hybridized the CA scaffold with the selective COX-2 inhibitory moiety of compound **1** (Fig. 2) and designed a series of 1,5-diarylsubstituted-1,2,4-triazole derivatives as COX-2/5-LOX dual inhibitors for anticancer candidates. Herein we report the synthesis, *in vitro* and *in vivo* biological evaluation and docking studies of these novel hybrids.

2. Results and discussion

2.1. Chemistry

The 1,5-diphenyl-1,2,4-triazole-3-thiol (**6a-i**) were prepared from corresponding phenyl hydrazine and benzoyl chloride as described in our previous report [28] (Scheme 1). Subsequent treatment of the intermediate **6a-g** with ethyl bromoacetate or ethyl 3-bromopropionate in the presence of K_2CO_3, followed by reduction using $LiAlH_4$, afforded the respective 1,5-diphenyl-1,2,4-triazol-3-thioalkylol (**8a-f**). Then the hydroxyl group of 8**a-e** was transformed into triazo group. And the triazo group could be reduced to amino group through Staudinger reaction, yielding the intermediates **11a-e** (Scheme 2).

The synthesis of CA esters is outlined in Scheme 3. Acetylation of hydroxyl groups of CA provided corresponding ester **12**, which was subsequently reacted with 1,2-dibromoethane or 1,3-dibromopropane, respectively, in presence of Et_3N in acetone, to produce intermediates **13a-b**. Condensation of **13a-b** with 1,2,4-triazole-3-thiol **6a-b**, **6d-e**, **6g-i** in the presence of K_2CO_3 afforded compounds **14a-n**. Deprotection of **14b**,**d**,**e**,**k**,**o** using 30% MeONa in MeOH yielded target compounds **15a-e**. The target compounds bearing *p*-NH_2SO_2 moiety at C-5 phenyl ring were obtained by deprotection of *tert*-butyl in CF_3COOH—$PhOCH_3$ solution.

And the synthesis route of CA amide derivatives is shown in Scheme 4. Protection of the hydroxyl groups of CA as an acetate followed by reaction with thionyl chloride afforded the acyl chloride **13**. Condensation of **13** with 1,2,4-triazole-3-thioalkylamine (**11a-e**) in the presence of TEA given corresponding amides **17a-g**. Subsequently, **17c** was deprotected in condition of MeONa providing the target product **18**.

Besides, we substituted the *meta*-hydroxyl group of CA phenyl ring with methoxyl group and obtained a series of ferulaic acid esters. Different from CA, the ferulaic acid was protected with Ac_2O in the presence of concentrated H_2SO_4 (Scheme 5) and the other operation steps were similar to those in Scheme 3.

At last, both of the hydroxyl groups of CA were removed resulting in cinnamic acid ester derivatives. These compounds (**23a, b**) could be directly prepared by treating 1,2,4-triazol-3-thioalkylol (**8d** of **8f**) with cinnamic acid in the presence of DCC and DMAP in

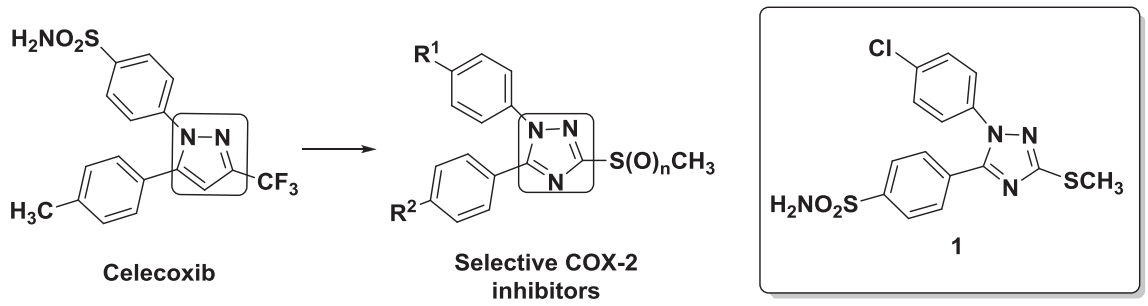

Fig. 1. 1,5-diaryl-1,2,4-triazole derivatives as selective COX-2 inhibitors.