European Journal of Medicinal Chemistry 157 (2018) 909–924

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Design, synthesis and biological evaluation of novel ferrocene-pyrazole derivatives containing nitric oxide donors as COX-2 inhibitors for cancer therapy

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ARTICLE INFO

Article history:
Received 24 April 2018
Received in revised form
20 July 2018
Accepted 17 August 2018
Available online 22 August 2018

Keywords:
Pyrazole
Ferrocene
COX-2 inhibitor
NO-Releasing
Anticancer

ABSTRACT

A series of novel ferrocene-pyrazole derivatives containing nitric oxide donors as COX-2 inhibitors for cancer therapy were designed, synthesized and biologically evaluated. Among them, compound **7l** displayed the most potent inhibitory against COX-2 ($IC_{50} = 0.82\ \mu M$) and antiproliferative activities against Hela cells ($IC_{50} = 0.34\ \mu M$) compared with Celecoxib ($IC_{50} = 0.38$ and $7.91\ \mu M$). The further mechanistic studies revealed that **7l** could induce apoptosis of Hela cells by mitochondrial depolarization and the antiproliferative activities of **7l** were positively correlated with the levels of intracellular NO release in Hela cells. Most notably, **7l** could dramatically suppress tumor growth in Hela cells xenografted mouse model. In summary, compound **7l** may be promising candidates for cancer therapy.

1. Introduction

Cyclooxygenase (COX) is a rate-limiting enzymes in the biosynthesis of prostaglandins (PGs), which are involved in various physiological and pathophysiological processes such as inflammation, gastrointestinal cytoprotection and ulceration, as well as tumor angiogenesis [1–3]. In general, there exist mainly two isoforms: a constitutive form (COX-1) and an inducible form (COX-2), and recently a third isoform named COX-3 is also discovered [4,5]. COX-1 is constitutively expressed in most normal tissues such as the gastrointestinal tract, kidneys, platelets and other organisms and plays an important role in the synthesis of PGs which are involved in the regulation of physiological functions [6]. In contrast, COX-2 is inducible and undetectable in most normal tissues, but is induced by stimuli such as pro-inflammatory cytokines, growth factors and oncogenes [7]. Moreover, multiple lines evidences have confirmed that the overexpression of COX-2 in several solid malignancies is associated with the formation of carcinogens, tumor proliferation, angiogenesis, invasiveness and prevention of apoptosis [2,8]. Numerous studies have demonstrated the inhibition of COX-2 can prevent the formation of tumor and COX-2 inhibitors could act as a chemotherapy agents to diminishing the occurrence and growth of malignancies. Therefore, the development of novel COX-2 inhibitors seems be a rational strategy for cancer treatment [9].

At present, a large number of selective COX-2 inhibitors, named coxibs, have been discovered. As illustrated in Fig. 1, these COX-2 inhibitors (**a ~ c**) show efficacy in a great deal of cancer studies, such as antiproliferation, apoptosis, migration, and invasion [10]. Unfortunately, their clinical use has been limited by numerous side effects, especially cardiovascular hazard and myocardial infarction, in long term use and high dosage [11,12]. Due to increased risk of cardiovascular events, Rofecoxib and Valdecoxib were withdrawn from the market in 2004 and 2005 respectively.

Recently, a novel class of agents, named COX-inhibiting nitric oxide (NO) donors (CINODs), have been developed by conjugating a COX inhibiting feature with a NO donor [13]. Some CINODs, such as Naproxcinod (**d**, Fig. 1), have underwent clinical development studies and shown a more favorable clinical profile than the parent

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https://doi.org/10.1016/j.ejmech.2018.08.048
0223-5234/

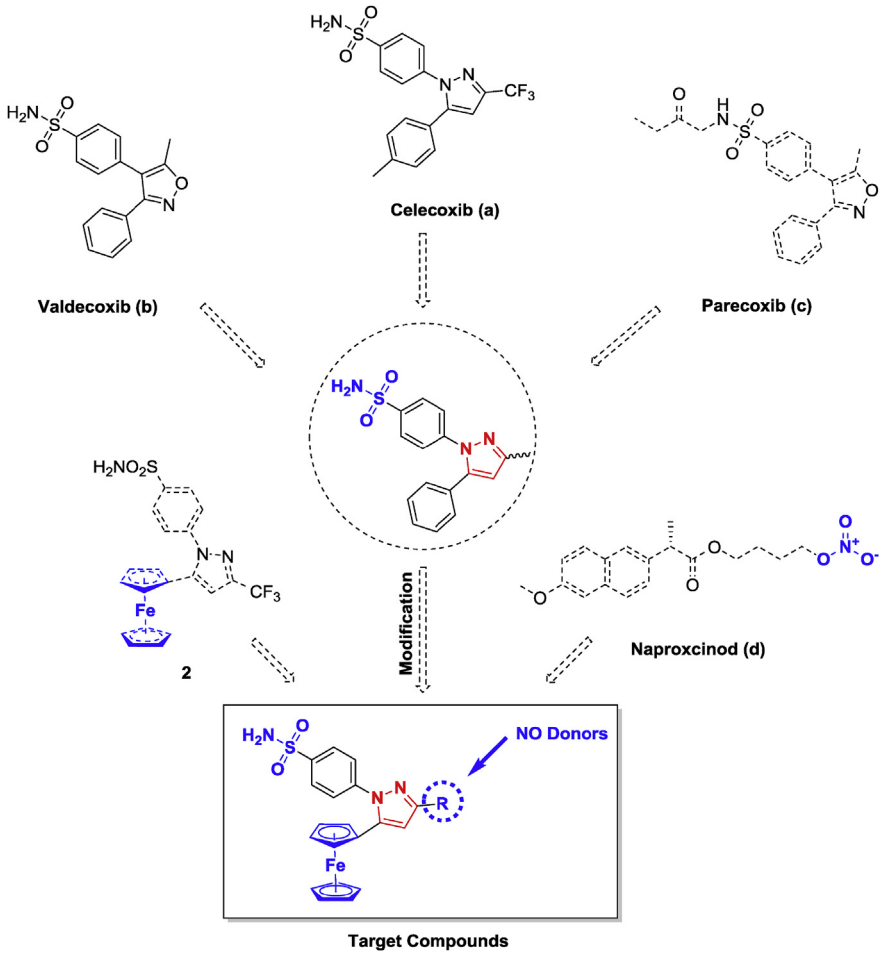

Fig. 1. Structures of Reference Compounds and design strategy of the target compounds.

Naproxen [14]. As an important signal molecule, NO plays an essential and far-reaching role in regulating various physiological and pathological process, such as vasodilation, thrombosis, and immune system [15,16]. Besides, numerous literature provide evidence that the high concentration of NO was effective on some malignant tumors, including breast, ovarian, prostate and pancreatic cancer, by inhibiting the proliferation and promoting their apoptosis [17,18]. Considering its specific character of NO, researchers are increasingly interested in the development of a new class of coxibs containing NO-releasing moiety.

In general, diarylheterocycle is a known pharmacophore and has been widely explored for the development of selective COX-2 inhibitors [19,20]. Additionally, sulfonamides moiety plays a crucial role in COX-2 selectivity and many COX-2 inhibitors were substituted by this moiety at para-position of one aryl ring, such as Celecoxib, Valdecoxib and Parecoxib (Fig. 1) [21,22]. Among them, Celecoxib exhibited the most potent COX-2 inhibitory activities and recent study revealed that this famous COX-2 inhibitor possess promising anticancer properties [23]. Based on the structure of these coxibs, diarylpyrazole sulfonamides scaffold was extracted and a series of diarylpyrazole sulfonamides derivatives have been designed by our group. Meanwhile, we noticed that ferrocene, one of the most promising organometallic compound, has attracted active interest in medicinal chemistry due to its excellent biological activities, such as non-toxicity, antimicrobial and anticancer, and several ferrocene derivatives with excellent biological activities have been reported [24,25]. Various studies have revealed that substitution of a phenyl ring of tested biomolecular with a ferrocene fragment always can result in unexpected biological activity [26–29]. Thus, we substituted a phenyl group of Celecoxib with ferrocene and obtained compound **2**. The result revealed that compound **2** exhibited an equal COX-2 inhibitory activity ($IC_{50} = 0.46\ \mu M$) compared with Celecoxib ($IC_{50} = 0.38\ \mu M$). Inspired by the interesting result, we expected that incorporation of the ferrocene fragment into the diarylpyrazole sulfonamides scaffold might have an attracting structural result for the development of novel COX-2 inhibitors.

Considering the side-effects of tNSAIDs, NO donors were introduced into the ferrocene-pyrazole sulfonamide scaffold for purpose of minimizing the side-effects and enhancing the anticancer activities. Herein, we designed and synthesized a series of ferrocene-pyrazole sulfonamide hybridizing NO donors with different length to explore their COX-2 inhibition and anticancer activities.

2. Results and discussion

2.1. Chemistry

In this study, a series of novel ferrocene-pyrazolo sulfonamide derivatives containing nitric oxide donors were synthesized and their structures were outlined in Tables 1 and 2. As depicted in Scheme 1, the target compound **2** was synthesized from 5-(1,2,3-trifluoromethyl-2,4-dione) ferrocene (**1**) and 4-