Journal of Photochemistry & Photobiology, B: Biology 166 (2017) 285–300

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

Anti-inflammatory, analgesic and COX-2 inhibitory activity of novel thiadiazoles in irradiated rats

Fatma A. Ragab [a], Helmi I. Heiba [b], Marwa G. El-Gazzar [b,*], Sahar M. Abou-Seri [a], Walaa A. El-Sabbagh [b], Reham M. El-Hazek [b]

[a] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Cairo University, Egypt
[b] Department of Drug Radiation Research, National Centre for Radiation Research and Technology (NCRRT), Egyptian Atomic Energy Authority (EAEA), PO box 29, Nasr City, Cairo, Egypt

ARTICLE INFO

Article history:
Received 26 August 2016
Accepted 10 December 2016
Available online 16 December 2016

Keywords:
Thiadiazoles
COX-2 inhibitors
Anti-inflammatory
Analgesic
Toxicological studies
Irradiated rats
Docking

ABSTRACT

In this work, novel series of pyran, thiophene and thienopyrimidine derivatives based on 2-acetamide-thiadiazole scaffold were designed and synthesized for evaluation as selective COX-2 inhibitors *in-vitro* and investigated *in-vivo* as anti-inflammatory and analgesic agents against carrageenan-induced rat paw oedema model in irradiated rats, since its well-known that ionizing radiation plays an important role in exaggerating the inflammatory responses and in enhancing the release of inflammatory mediators in experimental animals. Toxicological studies were carried out to evaluate the ulcerogenic activity, acute toxicity and kidney and liver functions for the most potent compounds. In order to understand the binding mode of the synthesized compounds into the active site of COX-2, docking study was performed. Most of the tested compounds showed high inhibitory ability to COX-2. Among them, thiadiazole derivatives bearing thiophene and thienopyrimidine moieties were the most active derivatives, compound **26** showed extremely high selectivity index (SI) of >555.5 μM which is nearly two folds better than celecoxib (>277.7 μM), in addition to compounds **3**, **16**, **17**, **21** and **26** with SI in the range of >308.6- >384.6 μM. The 4-chlorothieno[2.3-*d*]pyrimidine derivative of thiadiazole **21** showed the highest anti-inflammatory activity in this study having 24.49% of oedema compared to celecoxib (18.61%) in addition to compounds **17** and **26** with 24.70 and 25.40% of oedema, respectively, while the thiadiazol-2-acetamide derivative **2** was the most potent analgesic compound with the highest nociceptive threshold (85.72 g) very close to that of celecoxib (90.23 g). These compounds showed high safety margin on gastric mucosa with no ulceration effect. Also the most active *in-vivo* anti-inflammatory compounds **17**, **21** and **26** were found to be non-toxic in experimental rats with normal kidney and liver functions. Docking study of the synthesized compounds showed similar orientation as celecoxib within the active site of COX-2 enzyme and similar ability to emerge deeply in the additional pocket and binding with Arg513 and His90 the key amino acids responsible for selectivity.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) continue to be widely used group of therapeutic agents, which inhibit both COX-1 and COX-2 with an extremely varying level of selectivity [1,2]. Their clinical use as analgesics and anti-inflammatory agents is always accompanied with adverse gastrointestinal disorders and the design of novel NSAIDs with an advanced safety profile on GIT is a challenge in pharmaceutical industry. Since the discovery celecoxib, researchers have focused on the synthesis of novel derivatives of this class which reduce inflammation with fewer side effects [3,4].

Inflammation is a complex biological response to harmful stimulus which may vary from a localized response to a generalized one and is mediated by prostaglandins (PGs) [5]. The biosynthesis of (PGs) is carried out by the bifunctional enzyme prostaglandin H2 synthase (PGHS or cyclooxygenase, COX), which exhibits both cyclooxygenase and peroxidase activities. There are three distinct COX isoforms: COX-1, the constitutive which is involved in the regulation of physiological functions and production of cycloprotective prostaglandin in GIT and maintaining platelet aggregation by production of proaggregatory thromboxane. COX-2, the inducible form which is released in inflammatory cells in response to cytokines such as tumor necrosis factor-α (TNF-α), interleukines, growth factors, and other inflammatory mediators. COX-3, a third full active isoform, and two partial isoforms, pCOX1a and b recently reported to be detected in the cerebral cortex and in human heart [6–8].

Most studies on new anti-inflammatories have been focused on healthy population. However, inflammatory processes have particular relevance in the context of cancer, as inflammation is increasingly

* Corresponding author.
E-mail address: marwa.galal@eaea.org.eg (M.G. El-Gazzar).

http://dx.doi.org/10.1016/j.jphotobiol.2016.12.007
1011-1344/

recognized as a contributor to cancer development and progression especially in breast and prostate cancer [9]. Moreover, radiation therapy activates pro-inflammatory cytokine production as part of a coordinated response designed to control damage and promote tissue repair [10]. Pain management and controlling of inflammatory responses should be concluded in protocols before starting of radiation treatment [11].

Carrageenan-induced paw oedema is an acute inflammatory model commonly used in experimental rats [12]. Ionizing radiation has been shown to exaggerate the inflammatory responses induced by this model due to enhancement of release of inflammatory mediators through the cyclooxygenase and lipoxygenase pathways and also through the release of reactive oxygen metabolites resulting from the interaction between radiation and water from the cellular environment [13–15].

The common structure of COX-2 inhibitors includes two classes: tricyclic and non-tricyclic compounds [16,17], the tricyclic group consists of two aryl rings linked to a central homocyclic, heterocyclic or fused ring moieties such as, thiophene, pyrazole, furanone, isoxazole, cyclopentene and fused heterocyclics. One of the two aryl rings carries a sulfonamide moiety which is deeply immerged into the additional hydrophilic pocket of COX-2 enzyme and is capable of binding to the key amino acids His90 and Arg513 responsible for selectivity [16,17]. In the non-tricyclics, the cyclic core is replaced by acyclic centre such as olefinic, iminic, azo, urea, and a,b-unsaturated structures [18–20]. This common pharmacophore presents a wide framework which allows medicinal chemists to design novel selective COX-2 inhibitors with varying structures.

Pyran derivatives have recently attracted considerable attention due to their wide spectrum of biological activity [21–24], Hyup et al. [25] introduced 2.3-diaryl benzopyrans as a part of the vicinal diaryl heterocyclic family as a promising lead structure for selective COX-2 inhibition. Caturla et al. [26] reported a new class of 2-phenylpyran-4-ones as selective COX-2 inhibitors. Moreover, the anti-inflammatory activity of thiophene and thienopyrimidine derivatives is well-documented in addition to their array of pharmacological activities [27–30]. In 1995, Gierse et al. [31] introduced a new generation of selective COX-2 inhibitors that include 5-bromo-2-(4-fluorophenyl)-3-(methylsulfonyl) thiophene (DuP-697). This new class binds tightly to the COX-2 active cite and dissociate slowly showing a long lasting action. Hence, different series of thiophene and thienopyrimidines have been synthesized with optimal COX-2 inhibition [32].

The 1,3,4-thiadiazole ring is endowed with relatively high aromaticity and weak basicity due to its sulfur inducible effect, the electron withdrawing effect of its nitrogen atoms are responsible for its electron deficiency and susceptibility to nucleophilic attack, it is relatively stable in aqueous acid solutions but can undergo ring cleavage with aqueous base. Thus, with these structural properties, 1,3,4-thiadiazole derivatives are widely applied in medicinal chemistry and displayed significant biological activities [33,34].

Based on these facts, we decided to synthesize novel series of non-tricyclic COX-2 inhibitors possessing a central azo acyclic core, the two aromatic rings are replaced by 1,3,4-thiadiazole, and by either pyran, thiophene or thienopyrimidine. By using the structural features of these biologically active moieties and by diverting the substituents, novel inhibitors were synthesized. The aim is to study the SAR and the effect of thiadiazole ring on the orientation and binding mode of pyran, thiophene and thienopyrimidine within COX-2 active site in order to reach the best inhibitory activity with higher selectivity index which could lead to the discovery of new class of COX-2 inhibitors (Fig. 1). These new derivatives were screened using *in-vitro* COX inhibitory assay, the most potent candidates were subjected to anti-inflammatory and analgesic testing. Toxicological studies were performed by evaluation of their ulcerogenic activity, acute toxicity, and kidney and liver functions in experimental rats. Molecular docking was carried out for all the synthesized compounds into the active site of COX-2 to explore their binding interactions and their accessibility to the additional pocket responsible for selectivity.

2. Material and Methods

2.1. Instruments

The melting points were taken in an open capillary tube on a Stuart melting point apparatus (Stuart Scientific, Redhill, UK) and are uncorrected. The IR spectra of the compounds were recorded on FT-IR Shimadzu spectrometer (Shimadzu, Tokyo, Japan). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury Plus Oxford 400 MHz spectrometer (Varian Inc., Palo Alto, CA) using TMS as an internal Standard and DMSO-d_6 as solvent. Mass spectra were run on HP Model MS-5988 (Hewlett Packard, Palo, Alto, California, USA). Microanalyses were obtained on a Carlo Erba 1108 Elemental Analyzer (Heraeus, Hanau, Germany), all values were within ± 0.4% of the theoretical values. The purity of the compounds was checked by TLC on pre-

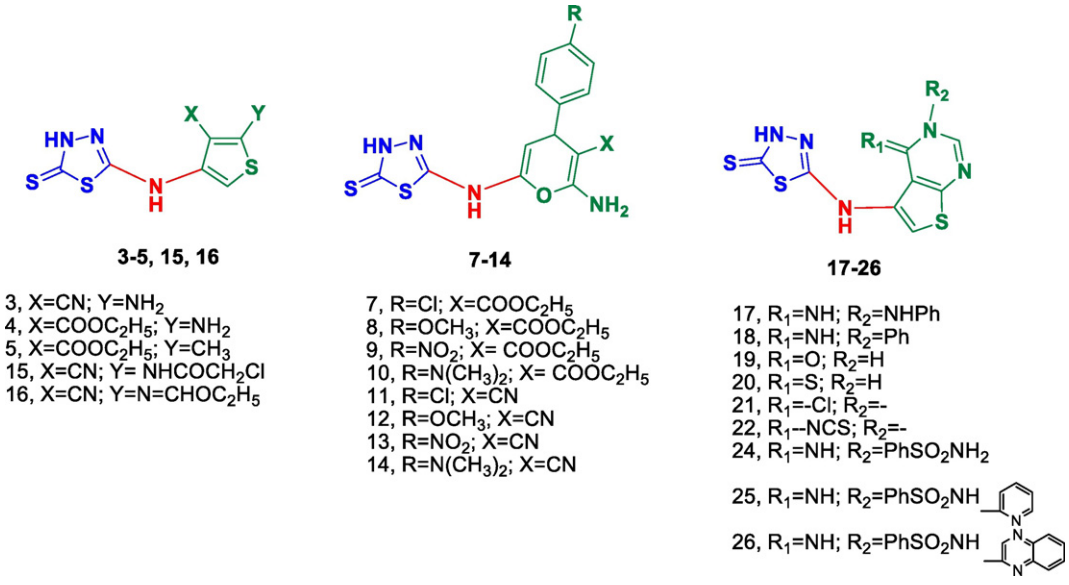

Fig. 1. The designed synthesized compounds.