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Design, synthesis, analgesic, anti-inflammatory activity of novel pyrazolones possessing aminosulfonyl pharmacophore as inhibitors of COX-2/5-LOX enzymes: Histopathological and docking studies

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ABSTRACT

A series of newly synthesized 4-aryl-hydrazonopyrazolones were designed and their structures were confirmed by spectral and elemental analyses. All synthesized compounds were evaluated for their *in vitro* COXs, 5-LOX inhibition, *in vivo* analgesic and anti-inflammatory activities. Compounds **5d**, **5f** and **5i** were found to be the most potent COX-2/5-LOX inhibitors with superior COX-2 selectivity index values (SI = 5.29–5.69) to reference standard celecoxib (SI = 3.52). Four compounds; **5b**, **5c**, **5d** and **5f** showed excellent anti-inflammatory activity (% edema inhibition = 72.72–54.54%) and perfect ED_{50} values (ED_{50} = 0.044–0.104 mmol/kg) relative to celecoxib (ED_{50} = 0.032 mmol/kg). To explore the most active compounds, ulcerogenic effect on stomach in comparison with indomethacin and celecoxib in addition to histopathological investigations were performed. Compound **5f** showed better gastric profile (UI = 2.33) than celecoxib (UI = 3.00). Also, **5f** caused 50% increase in thermal pain threshold close to reference drug indomethacin (53.13%). Docking study of all the target compounds into COX-2 and 5-LOX active sites was performed to rational their anti-inflammatory activities.

1. Introduction

The inflammatory mediators, prostaglandins (PGs), leukotrienes (LTs) and thromboxanes (TXs) are responsible for inflammation, other pathological and physiological processes [1]. They are generated from arachidonic acid (AA), a poly saturated fatty acid released from membrane phospholipids metabolism by the action of cyclooxygenase (COX-1, -2, -3) and lipoxygenase (5-LOX, 8-, 12-, 15-) enzymes [2–4].

COX enzymes are responsible for the production of PGs and TXs. COX-1 is a constitutive enzyme found in the stomach, platelets and kidneys as a "house-keeper" enzyme and involved in gastric protection, platelet aggregation and normal kidney functions. COX-2 is an inducible enzyme found in macrophages, fibroblasts and leukocytes and stimulated in response to pro-inflammatory mediators. COX-3, the third cyclooxygenase is latterly discovered and present in central nervous system [5–8].

Classical non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin and indomethacin exert their therapeutic action *via* suppressing PGs bio-synthesis through non-selective inhibition of COX-1 and COX-2 enzymes resulting in serious adverse effects like gastric pain, bleeding, ulcer and kidney complications [9,10].

COX-2 selective NSAIDs (Coxibs) illustrated by celecoxib (Celebrex®), valdecoxib (Bextra®) and rofecoxib (Vioxx®) have no effect on gastric mucosal prostaglandin. But recent studies have shown the risk of some highly selective COX-2 inhibitors to increase the incidence of myocardial infarction leading to cardiac arrest due to alteration in the COX-1/COX-2 biochemical pathway [3,11,12].

5-LOX is a human non-heme enzyme responsible for the production of LTs involved in the inflammatory process. Zileuton, licofelone and meclofenamate sodium (Meclomen®) are examples of orally active 5-LOX inhibitors [13,14].

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The incident of NSAIDs side effects is thought to be due to inhibition of one enzyme pathway (COX) over the other (LOX) pathway leading to shift in AA metabolism [15], hence the development of new anti-inflammatory (AI) agents targeting both metabolic pathways of AA (COX-2 and 5-LOX) inhibition is a worthy rational approach to obtain effective and safe NSAIDs [14,16,17].

Pyrazolone ring system is a core structure in numerous drugs displaying analgesic and AI activities such as aminoantipytine, propyphenazone and famorofazone [18–20].

Several research studies reported the enhanced biological activities of heterocyclic compounds incorporating hydrazono pharmacophore as analgesic and AI agents with improved gastric profile due to its dual COX/5-LOX inhibitory activities [18,21–23].

Furthermore, structure activity relationship studies of selective COX-2 inhibitors demonstrated the importance of aminosulfonyl (SO_2NH_2) pharmacophore for COX-2 selectivity [24–26].

On the basis of these findings and in continuation of our previous work [27–32] to develop effective AI agents devoid from adverse effects, we describe the design, synthesis, analgesic and AI activities of novel 4-aryl-hydrazonopyrazolone derivatives incorporating both sulfonamoyl and hydrazone pharmacophores as COX-2 and 5-LOX inhibitors (Fig. 1). Ulcerogenic liability and histopathological screening were performed in order to identify the non-ulcerogenic AI active compounds. Docking studies were also performed to understand the possible binding modes of the synthesized compounds into both COX-2 and 5-LOX active sites in order to explain their AI activities.

2. Results and discussion

2.1. Chemical synthesis

The synthetic pathways adopted for starting materials **2a–e** and target compounds **3**, **4**, **5a–i** are illustrated in Scheme 1, 2.

Hydrazones **2a–d** were prepared *via* coupling the diazonium salt of different primary aromatic amines with the active methylene group of ethyl acetoacetate [33].

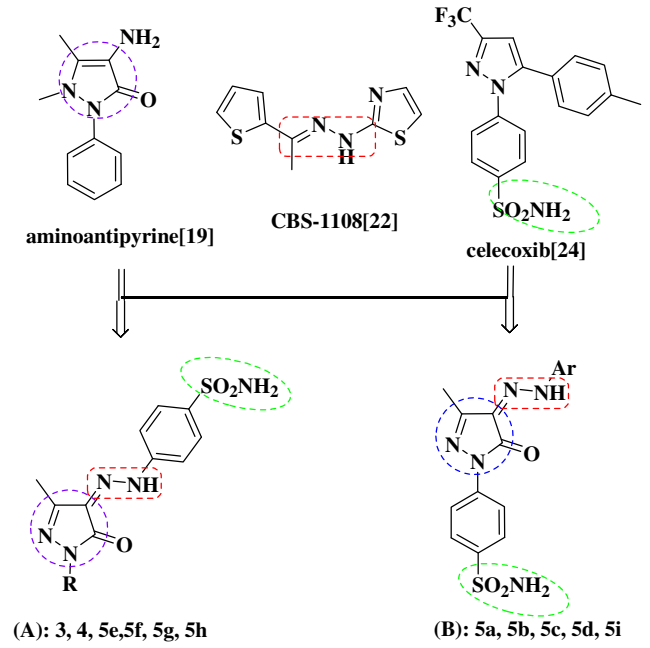

Fig. 1. Structure of aminoantipyrine, CBS 1108, celecoxib, and general structure of targeted pyrazolones (A) and (B).

3-Oxo-2-[(4-sulfamoylphenyl)-hydrazono]-butyric acid ethyl ester (**2e**) was prepared through diazotization of sulfanilamide **1e** and coupling the formed diazonium salt with ethyl acetoacetate in presence of sodium acetate. IR spectrum of compound **2e** demonstrated two absorption bands at 1690 and 1676 cm^{-1} corresponding to two C=O groups while ^1H NMR spectrum showed a D_2O exchangeable peak at δ 11.60 ppm indicating NH proton. Also, the presence of a singlet CH_3, a triplet CH_3 and a quartet CH_2 peaks at δ 2.47, 1.28 and 4.29 ppm corresponding to acetyl ($COCH_3$) and ethoxy (OCH_2CH_3) protons respectively confirmed the structure**.** These spectral data demonstrated the existence of compounds **2a–f** in hydrazone form (*i*) rather than azo form (*ii*) (Scheme 1).

Cyclo-condensation ethanolic solution of **2e** with an equimolar amount of hydrazine hydrate afforded pyrazolone **3** in 74% yield. ^1H NMR spectrum of **3** revealed the disappearance of the signals due to ethoxy protons of the parent ester **2e**. The presence of additional D_2O exchangeable singlet signal at δ 13.22 ppm corresponding to pyrazolone NH proton, confirmed the reaction. In addition, the mass spectra of **3** displayed molecular ion peak at m/z 281 (92.94%).

Heating pyrazolone **3** with acetyl chloride afforded the *N*-acetylpyrazolone **4** in a good yield of 63%. IR spectrum of **4** displayed an additional absorption band at 1749 cm^{-1} due to the carbonyl group of *N*-acetyl moiety. The absence of pyrazolone NH peak of the precursor **3** and presence of a peak of an additional signal of three protons integration due to acetyl moiety at δ 2.45 ppm in ^1H NMR spectrum of **4** confirmed the reaction. Also, ^{13}C NMR spectrum of compound **4** revealed the presence of two peaks at δ 24.20 and δ 171.61 ppm corresponding to $COCH_3$ and $COCH_3$, respectively.

Different substituted phenylhydrazine hydrochlorides were heated with hydrazones **2a–e** under reflux in absolute ethanol to give the target compounds **5a–i** in excellent yield (68–85%). The reaction proceeds *via* addition of the more nucleophilic NH_2 hydrazine group to the reactive acetyl carbonyl group ($COCH_3$) followed by intra-molecular cyclization through nucleophilic substitution of the good leaving ethoxy group and loss of an ethanol molecule (Scheme 2).

The structure of diarylpyrazolones **5a–i** was investigated by elemental and spectral analyses. The IR spectra of compounds **5a–i** indicated the presence of two absorption bands at 3460–3313 cm^{-1} and 3279–3247 cm^{-1} corresponding to NH_2 and NH groups in addition to an absorption band at 1672–1650 cm^{-1} indicating C=O group at C-5 of pyrazolone ring. Carboxy compounds, **5b, 5c, 5d, 5f, 5g, 5h** displayed two additional absorption bands at 3440–3416 cm^{-1} and 1701–1680 cm^{-1} due to carboxylic OH and C=O groups, respectively. ^1H NMR spectra of **5a–i** showed the presence of a singlet signal at δ 2.29–2.34 ppm corresponding to methyl protons at C-3 of pyrazolone ring, in addition to two exchangeable singlet signals at δ 7.22–7.42 corresponding to NH_2 protons of aminosulfonyl moiety, and at δ 13.03–13.66 for NH protons. Carboxy compounds **5b, 5c, 5d, 5f, 5g** and **5h** displayed carboxyl protons at δ 13.03–15.05 ppm. Also, ^{13}C NMR spectra of **5a–i** exhibited the methyl carbon at δ 12.14–12.30 ppm and carbonyl carbons at δ 154.71–156.99 ppm. In Addition, ^{13}C NMR spectra of carboxy compounds **5b, 5c, 5d, 5g, 5h** displayed carboxy carbons at δ 167.17–168.67 ppm which confirmed the structure.

2.2. Biological activity

2.2.1. Analgesic activity (hot plate latency test)

Analgesic activity of compounds **3**, **4** and diarylpyrazolones **5a-i** was evaluated applying hot plate latency test [34]. Oral administration of tested compounds produced a significant delay in the latency time relative to basal values except for compounds **3**, **4**,