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Research article

Synthesis and *in silico* investigation of thiazoles bearing pyrazoles derivatives as anti-inflammatory agents

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ABSTRACT

Searching novel, safe and effective anti-inflammatory agents has remained an evolving research enquiry in the mainstream of inflammatory disorders. In the present investigation series of thiazoles bearing pyrazole as a possible pharmacophore were synthesized and assessed for their anti inflammatory activity using *in vitro* and *in vivo* methods. In order to decipher the possible anti-inflammatory mechanism of action of the synthesized compounds, cyclooxygenase I and II (COX-I and COX-II) inhibition assays were also carried out. The results obtained clearly focus the significance of compounds **5d**, **5h** and **5i** as selective COX-II inhibitors. Moreover, compound **5h** was also identified as a lead molecule for inhibition of the carrageenin induced rat paw edema in animal model studies. Molecular docking results revealed significant interactions of the test compounds with the active site of COX-II, which perhaps can be explored for design and development of novel COX-II selective anti-inflammatory agents.

1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) include family of pharmacologically active compounds used in the therapeutic management of acute and chronic inflammation, pain, and fever. However, the long-term clinical usage of NSAIDs has been proved to be detrimental owing to the onset of gastrointestinal lesions, hemorrhaging, and nephrotoxicity (Bombardier et al., 2000; Silverstein et al., 2000; Kapupara et al., 2010). Despite the continuous efforts to improve the pharmacological index of NSAIDs, ulcerogenicity remains the most restraining obstructions in their clinical use (Bekhit et al., 2010). Cyclooxygenase (COX), the rate limiting enzyme of the prostanoid biosynthetic pathway, catalyzes the conversion of arachidonic acid to important anti-inflammatory mediators such as prostaglandins (PGs), prostacyclin (PGI) and thromboxane (TXA 2) (Bandgar et al., 2012; Mohamaadzaden and Sheibani, 2012; Celecoxib Drugs Future, 1997). These enzymes catalyze the formation of key mediators in recruiting inflammatory response. There are two isoforms of COXs (COX-I and COX-II) The COX-I is constitutively expressed in most tissues and organs and catalyzes the synthesis of PGs involved in the regulation of physiological, cellular activities, whereas COX-II is mainly stimulated by various stimuli such as cytokines, mitogens, and endotoxins in inflammatory sites (Kapupara et al., 2010; Song et al., 1999; Chandrasekharan et al., 2002). Classical NSAIDs, such as Indomethacin, inhibit both isoforms of COX (Ottana et al., 2005). Therefore, research on finding safe anti-inflammatory agents that selectively curtail the COX-II activity has recently gained a central importance in anti-inflammatory discourse.

In the previous state of the art literature, the heterocyles containing sulfur and nitrogen possess a broad range of pharmaceutical activities. For example, Penicillin: a classical antibiotic isolated from *Penicillium* fungi possess therapeutically active thiazole moiety (Vane and Botting, 1996). Thiazoles and their derivatives are already characterized to hold an array of interesting biological activities such as anti-tubercular (Kapupara et. al, 2010; Smith and Dewitt, 1996) and anti-HIV (Fu et al., 1990; Herschman, 1996). Conversely, pyrazole derivatives have been reported in the literature to demonstrate anti-inflammatory (Dubois et al., 1998), antihypertensive (Gupta et al., 1999), and antimicrobial activities (Ashtekar et al., 1987; Maass et al., 1993; Barreca et al., 2002).

Recently, multicomponent reactions (MCRs) have emerged as an imperative key model in combinatorial chemistry owing to its

Abbrevations: AA, arachidonic acid; COX, cyclooxygenase; EPA, eicosapentaenoic acid; NSAIDs, nonsteroidal anti-inflammatory drugs; PGs, prostaglandins; PGI, prostacyclin; TXA, thromboxane.

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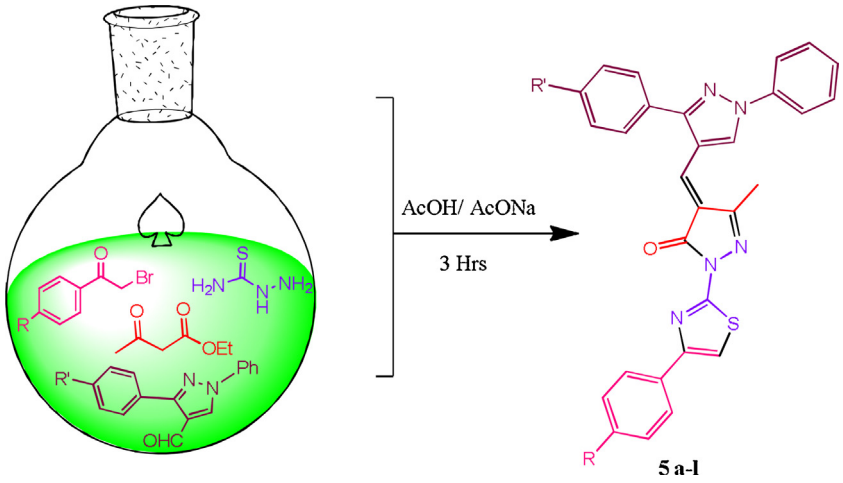

Fig. 1. Synthetic protocol of compounds (**5a–l**).

utility in synthesis of structurally diverse small drug-like molecules. Nevertheless, this methodology also allows the synthesis of compounds in a few steps and usually in a one pot operation. Another additional symbolic advantage from these reactions is the simplified purification, because all of the reagents are incorporated into the final product (Nasr and Said, 2003).

The circumstantial literate and the current perspective promoted us to design the synthesis and investigate the biological role of thiazole bearing pyrazole derivatives as possible anti-inflammatory agents and focus on development of an effective pharmacophore model of the class. Hence, here we report the synthesis of thiazole bearing pyrazole derivatives **5a–5l**. The effect of NO_2, Cl, Br, OCH_3 and CH_3 substituent of the pyrazol-4-yl moiety on the anti-inflammatory profile was studied (Ozdemir et al., 2007; Kaplancikli et al., 2007). These substituents were selected on the basis of structure activity relationship (SAR) studies using *in vitro* COX-I and COX-II inhibition assay and *in vivo* rat models. Molecular docking studies were further performed to better understand detail interactions of thiazol compounds with COX-II structural model.

2. Result and discussion

2.1. Chemistry

In continuation of our investigations on the synthesis of biologically active heterocyclic compounds (Kamble et al., 2015; Acharya et al., 2014; Chobe et al., 2013; Mandawad et al., 2013; Dawane et al., 2010) here we report the Hantzsch-thiazole synthesis and the concurrent formation of thiazolyl-pyrazolone along with condensation of heteroaldehyde moiety on an active methylene group of pyrazolone under Knoevenagel reaction conditions. This is a four-component reaction (Bailey et al., 1996; Venkata and Rao, 2011) (Fig. 1).

The synthesis was carried out as per method reported in the literature (Bailey et al., 1996; Venkata and Rao, 2011), which involves heating an equimolar mixture of phenacyl bromides, thiosemicarbazide, ethyl acetoacetate and heteroaldehyde in acetic acid using sodium acetate as a base. In the present reaction scheme, the thiocarboxamide part of thiosemicarbazide reacts with phenacyl bromide to furnish a Hantzsch-thiazole product (Bailey et al., 1996; Hantzsch and Weber, 1887; Polshettiwar and Varma, 2008). The hydrazine part of thiosemicarbazide reacts with ethyl acetoacetate to capitulate the pyrazolone (Bailey et al., 1996; Ryabukhin et al., 2007) moiety having an active methylene group. Furthermore, without isolation of the intermediate, addition of sodium acetate and heteroaldehyde to the reaction mixture gave the Knoevenagel product (Bailey et al., 1996; Ryabukhin et al., 2007; Madje et al., 2008) thiazol-2-yl-1H-pyrazol-4-yl-pyrazol-5 (4H)-one **5a–5l**.

The structures of compounds **5a–5l** were established on the basis of obtained spectral analysis (FTIR, ^{1}H NMR, ^{13}C NMR, Mass) and elemental analysis. The IR spectrum of compound **5a** showed —C=O stretching band at 1674 cm^{-1} of pyrazolone. ^{1}H NMR of compound **5a** exhibits typical singlet for CH_3 of tolyl at δ 1.37 and downfield singlets for CH_3 of pyrazolone at δ 2.91 ppm. The ^{13}C NMR of **5a** showed the peaks at δ 17.9 for CH_3 of pyrazolone, peak at δ 26.7 for tolyl CH_3 and δ 162.1 for C=O of pyrazolone. The obtained elemental analysis values are in good agreement with theoretical data. Mass spectra of all the compounds gave expected M^+ peak corresponding with proposed molecular mass. Physico-chemical properties of all the derivatives are shown in Table 1.

2.2. Biology

2.2.1. In vitro *COX inhibition assays*

The NSAIDs, Celecoxib, Indomethcine and Aspirin were selected as standard drugs due to their structural similarities with the synthesized compounds. Like celecoxib, the present panel of synthetic compounds contains pentacyclic ring with two adjacent nitrogens. It has been reported in earlier studies that celecoxib and several NSAIDs exert their anti-inflammatory action primarily by inhibiting COX-II enzyme (Celecoxib Drugs Future, 1997). Of the tested compounds, six derivatives demonstrated selective COX-II inhibitors. Compound **5h** exhibited significant COX-II inhibition (78.91 ± 0.80%), followed by **5i** (71.42 ± 0.82%), **5d** (66.99 ± 0.33%) and **5j** (61.84 ± 0.73%). Structure activity relationship (SAR)

Table 1
Substitution pattern and yields for compounds **5a–5l**.

Sr. no.	Product	R	R′	Yield in %	MP (°C)
1	**5a**	4-Cl	4-Me	76	272–274
2	**5b**	4-Cl	4-NO_2	70	205–207
3	**5c**	4-Cl	4-Cl	78	253–255
4	**5d**	4-Cl	—H	81	210–212
5	**5e**	—H	4-Cl	72	226–228
6	**5f**	—H	4-Me	79	198–200
7	**5g**	—H	4-NO_2	68	258–260
8	**5h**	—Cl	4-OMe	75	214–216
9	**5i**	—H	—H	79	189–192
10	**5j**	—H	4-OMe	78	243–245
11	**5k**	4-Br	4-OMe	74	263–265
12	**5l**	4-Br	4-Me	82	233–235