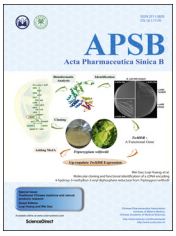
Acta Pharmaceutica Sinica B 2017;7(2):230–240

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com



ORIGINAL ARTICLE

Synthesis, characterization and pharmacological evaluation of pyrazolyl urea derivatives as potential anti-inflammatory agents

Kanagasabai Somakala, Mohammad Amir*

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Hamdard University, New Delhi 110062, India

Received 8 June 2016; revised 5 July 2016; accepted 17 July 2016

KEY WORDS

Pyrazolyl urea;
p38;
MAPK;
DPPH;
Anti-inflammatory;
Gastric toxicity;
Lipid peroxidation

Abstract p38α mitogen activated protein kinase (MAPK) inhibitors provide a novel approach for the treatment of inflammatory disorders. A series of fifteen pyrazolyl urea derivatives (**3a–o**) were synthesized and evaluated for their p38α MAPK inhibition and antioxidant potential. Compounds **3a–e, 3g** and **3h** showed low micromolar range potency (IC_{50} values ranging from 0.037 $\pm$ 1.56 to 0.069 $\pm$ 0.07 μmol/L) compared to the standard inhibitor SB 203580 (IC_{50} = 0.043 $\pm$ 3.62 μmol/L) when evaluated for p38α MAPK inhibition by an immunosorbent-based assay. Antioxidant activity was measured by a 2,2′-diphenyl-1-picryl hydrazyl radical (DPPH) free radical scavenging method and one of the compounds, **3c**, showed better percentage antioxidant activity (75.06%) compared to butylated hydroxy anisole (71.53%) at 1 mmol/L concentration. Compounds **3a–e, 3g** and **3h** showed promising *in vivo* anti-inflammatory activity (ranging from 62.25% to 80.93%) in comparison to diclofenac sodium (81.62%). The ulcerogenic liability and lipid peroxidation activity of these compounds were observed to be less in comparison to diclofenac sodium. These compounds also potently inhibited the lipopolysaccharide (LPS)-induced TNF-α release in mice (ID_{50} of **3a–c** = 19.98, 11.32 and 9.67 mg/kg, respectively). Among the screened compounds, derivative **3c** was found to be the most potent and its binding mode within the p38α MAPK is also reported.

*Corresponding author. Tel.:+91 9013476217.
E-mail address: mamir_s2003@yahoo.co.in (Mohammad Amir).

Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.

http://dx.doi.org/10.1016/j.apsb.2016.08.006

1. Introduction

Inflammation is a multifactorial, protective attempt of the non-specific immune system. In response to infection stimulus, monocytes/macrophages lineage cells are activated, thereby generating an inflammatory environment by secreting proinflammatory cytokines. It is an important aspect in rheumatoid arthritis, osteoarthritis, Alzheimer's disease and obesity related diseases[1]. Non-steroidal anti-inflammatory drugs (NSAIDs) are amongst the most widely prescribed agents for the management of various inflammatory diseases[2,3]. NSAIDs act by counteracting the cyclooxygenase (COX) that converts arachidonic acid into prostaglandins in inflammatory processes. Commonly used NSAIDs, such as aspirin, indomethacin and diclofenac, are non-selective inhibitors and are responsible for adverse side effects, such as gastric ulceration, bleeding and renal function suppression[4]. There are at least two mammalian COX isoforms[5,6]. COX-1 is constitutive and provides cytoprotection in the gastrointestinal (GI) tract, while COX-2 is induced and responsible for pro-inflammatory conditions[4]. Various selective COX-2 inhibitors, such as celecoxib, rofecoxib and valdecoxib, showed anti-inflammatory activity with minimum gastric side effects[7]. Unfortunately, selective COX-2 inhibitors were found to cause cardiovascular side effects[8]. Therefore, in view of the GI toxicity of conventional NSAIDs and the adverse cardiovascular side effects of selective COX-2 inhibitors, there is a need to develop anti-inflammatory agents with an improved safety profile.

p38α mitogen activated protein kinase (MAPK) has attracted considerable attention as a major target in developing anti-inflammatory drugs. Activated p38α phosphorylates a range of intracellular protein substrates that transcriptionally regulate the biosynthesis of inflammatory cytokines like tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β)[9]. The identification of p38 MAPK as the target of some pyridinyl-imidazole compounds, *e.g.* SB203580 (Fig. 1A), confirmed the role of this intracellular enzyme in the regulation of many physiological and pathological states and reinforced the importance of its modulation in the therapy of many inflammatory diseases[10]. p38α MAPK belongs to the serine/threonine family of kinases and is a key enzyme of a cascade leading to the production of pro-inflammatory cytokines, such as IL-1β and TNF-α[11]. Excessive levels of these cytokines are known to be involved in the progression of many inflammatory disorders, such as rheumatoid arthritis, inflammatory bowel disease, and psoriasis[12–14]. TNF-α is a major pleiotropic pro-inflammatory cytokine and is known to activate platelets and also participates in the genesis of fever and anemia[15]. Increased production of TNF-α also modulates processes, such as immune cell activation, proliferation, apoptosis and leukocyte migration and is thereby associated with many inflammatory diseases, like Crohn's disease, psoriasis, multiple sclerosis and rheumatoid arthritis[16]. p38α MAPK inhibition is therefore a promising therapeutic strategy to block the biosynthesis of TNF-α.

Pyrazole derivatives are an important class of heterocycles because of their diverse pharmacological properties, such as antioxidant, anti-inflammatory, antimicrobial and anti-viral/anti-tumor effects. Rofecoxib and celecoxib (selective COX-2 inhibitors) having a pyrazole moiety have exhibited significant anti-inflammatory activity with reduced GI toxicity. Furthermore, pyrazoles[17] and pyrazolyl urea derivatives have also been reported to be potential anti-inflammatory agents[18–20]. Compound BIRB-796[20] (Fig. 1B) having pyrazolyl urea moiety has shown significant anti-inflammatory activity through p38α MAPK and TNF-α inhibition and had advanced into clinical trials.

Encouraged by these observations and in the course of our research program on the synthesis of five membered heterocyclic compounds as anti-inflammatory agents[10,21–24], we report herein the synthesis and evaluation of some new pyrazolyl urea derivatives as potential anti-inflammatory agents.

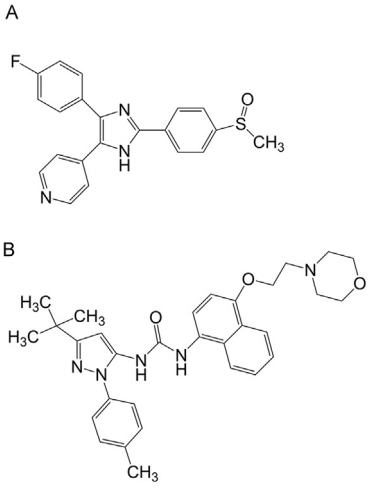


Figure 1 Compound structures of (A) SB 203580 and (B) BIRB 796 (Doramapimod).

2. Results and discussion

2.1. Chemistry

The titled compounds **3a–o** were synthesized as illustrated in Scheme 1. The pyrazoles **1a–o** were synthesized as reported earlier[25]. Compounds **1a–o** were then treated with 4-nitrophenylchloroformate in acetonitrile in the presence of pyridine to afford phenylcarbamate derivatives **2a–o**. The pyrazolyl urea derivatives **3a–o** were synthesized by treating compounds **2a–o** with ammonium acetate in THF in the presence of triethylamine. Many literature revealed the use of benzylisocyanate for converting amino groups into ureas. In the present study, benzylisocyanate was used for the preparation of urea derivatives but the reaction failed to yield the desired products. Further literature surveys revealed that amines when treated with 4-nitrophenylchloroformate, followed by the treatment with ammonium acetate, provided the corresponding ureas in high yield and purity even in aqueous environment. Following this method the titled compounds were obtained in good yields and were found to be pure.

The NH_2 protons of the pyrazoles **1a–o** were observed at δ 5.17–5.24, which disappeared in the phenyl carbamate derivatives **2a–o**. Appearance of a singlet for CONH protons from δ 10.21–10.47 confirmed the formation of phenyl carbamate derivatives. The formation of **3a–o** was confirmed by the appearance of a singlet for NH_2 protons from δ 6.12–6.23 showing the presence of a urea group in the compounds. Compounds **3a–o** also showed a singlet for the CONH protons from δ 10.13 to 10.55. The ^{13}C NMR spectral data of **3a–o** showed characteristic peaks for C=O carbons from δ 170.11 to 171.96. The pyrazole carbons were detected at δ 159.21 to 161.48 (pyrazole C_3), δ 102.48 to 105.68 (pyrazole C_4) and δ 150.97 to 159.66 (pyrazole C_5). The OCH_2 carbon showed distinct peaks at δ 70.19 to 72.54. Mass spectra of compounds **3a–o** showed molecular ion peaks M^+ at an *m/z* corresponding to their molecular formula.