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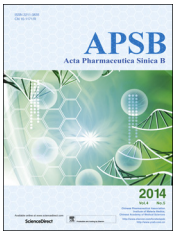

ORIGINAL ARTICLE

Novel coumarin–benzimidazole derivatives as antioxidants and safer anti-inflammatory agents

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Abstract Inspired from occurrence of anti-inflammatory activity of 3-substituted coumarins and antiulcer activity of various 2-substituted benzimidazoles, novel compounds have been designed by coupling coumarin derivatives at 3-position directly or through amide linkage with benzimidazole nucleus at 2-position. The resultant compounds are expected to exhibit both anti-inflammatory and antioxidant activities along with less gastric toxicity profile. Two series of coumarin–benzimidazole derivatives (**4a–e** and **5a–e**) were synthesized and evaluated for anti-inflammatory activity and antioxidant activity. Compounds **4c**, **4d** and **5a** displayed good anti-inflammatory (45.45%, 46.75% and 42.85% inhibition, respectively, *versus* 54.54% inhibition by indomethacin) and antioxidant (IC_{50} of 19.7, 13.9 and 1.2 μmol/L, respectively, *versus* 23.4 μmol/L for butylatedhydroxytoluene) activities. Evaluation of ulcer index and *in vivo* biochemical estimations for oxidative stress revealed that compounds **4d** and **5a** remain safe on gastric mucosa and did not induce oxidative stress in tissues. Calculation of various molecular properties suggests the compounds to be sufficiently bioavailable.

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1. Introduction

Inflammation is an important indication in many pathological conditions such as rheumatoid arthritis, osteoarthritis, gout, Alzheimer's disease and obesity related diseases[1]. Chronic inflammatory states lead to a vicious cycle of inflammation and the accompanying pathological states, like obesity can lead to inflammation and the chronic inflammation can promote obesity associated diabetes by inducing insulin resistance[2]. Therefore, control of inflammation becomes more important in all pathological conditions. The well-known non-steroidal anti-inflammatory drugs (NSAIDs) *viz.* indomethacin, ibuprofen and naproxen are commonly employed drugs in first line treatment of various chronic inflammatory disease states[3].

The major limitation of use of NSAIDs is gastric intolerance, which is manifested by dyspepsia, bleeding and ulcers. It occurs due to prostaglandin synthesis blockade as a result of cyclooxygenase (COX) inhibition by NSAIDs as well as due to acidic character of the NSAIDs themselves[4]. Users of NSAIDs are found to be at 3 times greater risk of developing serious gastro-intestinal (GIT) adverse effects than the non-users[5]. Another statistical analysis displays that 23%–31% of patients develop gastric lesions when prescribed NSAIDs for arthritis[6]. All these facts have arose the need to improve the safety profile of existing NSAIDs or to discover better alternatives. Various COX-2 selective inhibitors have exhibited marked anti-inflammatory effect with reduced GIT toxicity. MK-0966, rofecoxib and celecoxib are selective COX-2 inhibitors with significant anti-inflammatory activity but induce less GIT side-effects in comparison to those of aspirin and ibuprofen[7,8]. Coupling of NSAIDs with an antioxidant cysteamine has produced compounds having good activity with less GIT intolerance. Coupling of nitric oxide, a cellular antioxidant with NSAIDs has also been explored successfully to design anti-inflammatory agents with markedly reduced ulcerogenic potential[4,9].

Coumarins form an elite class of compounds, which exhibit a variety of therapeutic activities including antioxidant, anti-inflammatory, antitumor, antiviral, antituberculosis and antimicrobial[10–13]. Anti-inflammatory activity of coumarin derived compounds has been reviewed extensively and a structure activity relationship (SAR) has been established wherein it is found that an aromatic group when directly fused or linked through amide linkage at 3-position of coumarin nucleus incurs anti-inflammatory activity (Fig. 1)[14–16]. Many such derivatives also possess antioxidant activity through scavenging mechanisms[17,18].

Benzimidazole is another multifacet nucleus possessing a wide range of biological activities[19]. This nucleus bearing at its 2-position a heterocycle through linker has been found in many clinically available antiulcer drugs. It reveals that benzimidazole substituted with an appropriate group at 2-position is an important structural feature for gastric safety of the molecule[20]. Therefore, the present study is undertaken to design novel molecules through coupling of 2-position of benzimidazole nucleus with 3-position of 6-substituted coumarin nucleus (Fig. 1), which can be exploited as viable alternatives to the existing NSAIDs. The resultant molecules are expected to exhibit both anti-inflammatory and antioxidant activities but still being less gastro-toxic.

2. Results and discussion

2.1. Chemistry

The target compounds were synthesized as shown in Scheme 1. Initially, Meldrum's acid (**1**) was prepared by treating malonic acid with acetone in the presence of catalytic amounts of sulphuric acid and acetic anhydride. Different salicylaldehydes (**2a–e**) were reacted with **1** in the presence of piperidinium acetate to obtain 6-substituted coumarin-3-carboxylic acids (**3a–e**). These intermediates were then used to synthesize target compounds **4a–e** by refluxing them with *o*-phenylenediamine under inert environment (nitrogen) in the presence of catalytic amounts of polyphosphoric acid (PPA). The target compounds **5a–e** were synthesized by coupling these intermediates with 2-aminobenzimidazole in the presence of dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP). Many literature reports reveal the use of both orthophosphoric acid (OPA) and PPA for the formation of benzimidazole nucleus[21,22]. In the present study, PPA was used as catalyst as OPA produced more by products and took longer reaction times. All target compounds were obtained in good yields and were found to be pure as assured by single spots in thin layer chromatographic plates (TLC). Structures of the synthesized compounds were confirmed by IR, NMR and high resolution mass (HRMS) spectral techniques. Formation of benzimidazole nucleus in compounds **4** and **5** was ascertained by the disappearance of –COOH band, due to –COOH group of compounds **2**, in their IR spectra. In addition, IR spectra of **5** showed an amide band in the range of 1640–1690 cm^{-1}. Presence of benzimidazole nucleus was confirmed by appearance of signals due to –NH and four aromatic protons of the nucleus in their ^{1}H NMR spectra. The labile –NH protons were detected in the range of δ 8.9–9.2, which was confirmed by deuterium exchange experiments. The ^{13}C NMR spectra showed distinct resonances in agreement with the proposed structure. The methoxy derivatives **4b** and **5b** showed distinct peaks due to methoxyl carbon at around δ 55. The benzimidazole carbons were detected at δ 116.21–125.51, and the coumarinyl carbonyl carbon was found at δ 155.62–164.05. Carbon atoms of the benzene ring of coumarin nucleus showed downfield or upfield shifts in consonant with the type of substituent present on the ring. Finally, the HRMS data, recorded with electrospray ionization in positive polarity (+ESI), of each compound showed that the mass of $[MH^{+}]$ ion was in close agreement with its accurate theoretical mass.

2.2. Anti-inflammatory activity

It was evaluated in terms of percent (%) inhibition of formalin induced oedema in rat paw. The activity was monitored at 0.5, 1, 2, 4 and 6 h after administration. It was found that the activity continuously increased with time. All test compounds exhibited good to moderate anti-inflammatory activity, which was comparable to indomethacin at each time period (Fig. 2). Further, the inhibition profile of each compound was similar to that of indomethacin at each time slab, which suggested that the mechanism of action of the compounds might be similar to that of indomethacin. Compounds **4a–e** showed anti-inflammatory effects better than those of compounds **5a–e**. Compounds **4d** and **4c** were maximally potent with 46.75% and 45.45% inhibition of paw oedema, respectively. From the other series, compound **5a** was the most potent with 42.85% inhibition. These results suggest that an electron withdrawing group (–Cl or –Br) increases the anti-inflammatory potency whereas electron releasing group ($-OCH_3$) decreases the potency. Further, it was found that an amide linkage in the molecule decreased the activity.

2.3. In vitro antioxidant activity

The antioxidant potential was evaluated as radical scavenging capacity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. DPPH being