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Rapid 'one-pot' synthesis of a novel benzimidazole-5-carboxylate and its hydrazone derivatives as potential anti-inflammatory and antimicrobial agents

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ABSTRACT

A novel series of *N*-arylidene-2-(2,4-dichloro phenyl)-1-propyl-1*H*-benzo[*d*] imidazole-5-carbohydrazides having different substitution on the arylidene part were synthesized in good yield. The core nucleus benzimidazole-5-carboxylate (**5**) was efficiently synthesized by 'one-pot' nitro reductive cyclization reaction between ethyl-3-nitro-4-(propylamino)benzoate and 2,4-dichlorobenzaldehyde using sodium dithionite in dimethylsulfoxide. This 'one-pot' reaction was proceeded very smoothly, in short reaction time with an excellent yield. All the compounds (**7a**–**r**) were screened for their in vivo anti-inflammatory and in vitro antimicrobial activity. Most of the compounds exhibited remarkable paw-edema inhibition in the initial one hour of administration indicating the higher potentiality of these molecules. In particular, compounds **7a**, **7d**, **7f** and **7g** displayed a high level of carrageenan-induced paw edema inhibition compared to that of indomethacin. Compound **7p** exhibited very good antibacterial activity and antifungal activity with a MIC of 3.12 μg/mL against most of the tested organisms. Furthermore, compounds **7d**, **7f**, **7h** and **7p** found to be good inhibitors of *Aspergillus niger* with MIC of 3.12 μg/mL. Cytotoxicity of the potent compounds **7d**, **7f** and **7p** was checked using MDA MB-231 breast cancer cell line and are found to be non toxic at the highest concentration used (i.e., 10 μg/mL).

Inflammation is a multiple and complex process enacted by activated immune-associated cells.[1] Nonsteroidal anti-inflammatory drugs are mainly used for managing inflammatory disorders such as osteoarthritis (OA) and rheumatoid arthritis (RA). The entire known NSAIDs act by inhibiting the COX enzymes, which are mainly responsible for the conversion of arachidonic acid to prostaglandins.[2] In 1991, the isoform of cyclooxygenase involved in the conversion of arachidonic acid to prostaglandin pathway was discovered[3,4] and the enzyme involved in this pathway was named as cyclooxygenase II (COX-II) or prostaglandin G/H synthase II. COX-II is mainly responsible for the inflammation process, whereas COX-I is mainly responsible for the production of homeostatic prostaglandins which are associated with gastrointestinal ulceration, perforation and hemorrhage. This leads to the search of novel selective COX-II inhibitors, which are more effective in inhibiting the production of prostaglandins with diminished side effects.[5,6] On the other hand, the increase in the infectious diseases and their treatment remains a challenging problem worldwide.[7] The mortality rate is drastically increasing in the recent years due to infections caused by the pathogens. Even though there are several drugs are available in the market to treat infectious diseases, these drugs are being used enormously without precautions, due to which microbial pathogens have developed resistivity and meanwhile a number of new resistant pathogens are emerging. Some of these newer pathogens are resistant to the entire known library of antibiotics. Initially, the drug resistivity problem was limited to hospitalized patients, but now an even healthy population suffers from it. So, successful treatment of infectious disease has a downside and to overcome this, substantial effort has been devoted towards finding newer drug candidates.

Benzimidazoles are the privileged class of heterocyclic molecules in medicinal chemistry having a broad range of biological activity. Benzimidazoles and its derivatives exhibit antimicrobial,[8] antimicrobial/antioxidant,[9] anti-inflammatory,[10] anticancer,[11]

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antituberculosis[12] and antihypertensive[13] activities. Moreover, recently benzimidazole-5-carboxylic acid and its derivatives were emerged as promising agents for hepatitis C virus infections.[14] Extensive research has been devoted for these molecules in order to optimize a novel compound for HCV infections.[15,16] Moreover, benzimidazole-5-carboxylic acid derivatives exhibit glycoprotein IIb/IIIa inhibitor,[17] glutaminyl cyclase inhibitor,[18] antitumour[19] and checkpoint kinase inhibitor[20] activities. Acylhydrazones are well renowned class of compounds which possess a wide range of biological activities. These hydrazide–hydrazone derivatives have exerted different pharmacological activities such as antimicrobial,[21,22] analgesic/anti-inflammatory,[23,24] anticonvulsant,[25] anticancer,[26,27] antimalarial,[28] antileishmanial[29] and antiamoebic.[30] It was well documented that the –CH=N–NH– functionality present in it is responsible for the pronounced activity[31] and plays a key role by acting as a binding site for the receptors.[32]

'One-pot' method of synthesis of benzimidazole has gained much attention in the recent years.[33,34] It has been an efficient tool in the organic synthesis[35] and has several advantages over the stepwise method in terms of yield, purity and reaction time. Hence, in our present study, we utilized the 'one-pot' nitro reductive cyclization method for the synthesis of a novel benzimidazole-5-carboxylate. In view of the above biological profiles of benzimidazole and hydrazone derivatives, we carefully optimized structure for the new target compounds. A recent study pronounced that the propyl group at the 1st position would be much favorable wherein the benzimidazole derivatives with this propyl chain exhibited remarkable anti-inflammatory activity[36] and selective mGlu5 receptor antagonist activity.[37] Further, the presence of 2,4-dichloro phenyl moiety at 2nd position has shown remarkable topoisomerase I inhibitor[38], glycogen synthase kinase 3β inhibitor[39] and anticancer[40] activities with few benzimidazole derivatives. Moreover, benzimidazoles moiety endowed with halogenated aryl unit are known for their excellent anti-microbial activity.[41,42] Especially, several marketed antifungal agents like omoconazole, ketoconazole, oxiconazole, etc., contains this functionality.

In view of all these observations and in continual part of our work on 'one-pot' nitro reductive cyclization methodology,[43] herein we report the synthesis of a novel benzimidazole-5-carboxylate and its acyol hydrazone derivatives with the propyl group at the 1st position, 2,4-dichlorophenyl group at 2nd position (Fig. 1) and evaluated them for their in vivo anti-inflammatory and in vitro antimicrobial activity.

Synthetic route for the target compounds **7a**–**r** is depicted in Scheme 1. The core moiety ethyl 2-(2,4-dichlorophenyl)-1-propyl-1*H*-benzo[*d*]imidazole-5-carboxylate **5** was prepared by 'one-pot' nitro reductive cyclization of **3** with 2,4-dichlorobenzaldehyde **4** using sodium dithionite reagent under dimethyl sulfoxide medium at 90 °C. The 'one-pot' reaction produced the compound **5** within 3 h in an excellent yield (94%) and purity. Further, this method was found to be superior over the stepwise method of cyclization between 1,2-diamine and aldehydes/acid in terms of yield and reaction time. The stepwise method involves the preparation of amine intermediate and its isolation.[44] The reduction of nitro group to amine can be achieved by H_2/Pd, H_2/Pt mediated hydrogenation reaction or Sn/HCl, or Zn/HCl or Fe/HCl reduction reactions. The hydrogenation reactions are expensive and Sn, Zn or Fe in HCl mediated reduction involves tedious work up procedure and also results in low yield. The next cyclization step involves the use of either strong acidic condition[45] or oxidizing conditions.[12] But these methods suffer from several drawbacks such as low yield, tedious work up procedures and long reaction time. Hence, when compared to stepwise method this one-pot method was found to be more superior. The intermediate compound ethyl 4-chloro-3-nitro benzoate **2** was prepared according to the literature procedures.[46] The compound ethyl 3-nitro-4-(propylamino)-benzoate **3** was obtained by the nucleophilic displacement reaction of **3** with *n*-propylamine in the presence of triethylamine base in THF medium. The ester group was then refluxed with hydrazine hydrate in ethanol medium for 6 h to obtain hydrazide **6**. Finally, the target hydrazones **7a**–**r** were obtained by condensing **6** with various substituted aldehydes in ethanol with catalytic amount of glacial acetic acid.

The newly synthesized compounds were characterized by FTIR, ^{1}H NMR, ^{13}C NMR, LCMS and elemental analysis. 'One-pot' reductive cyclization reaction yielding the core moiety ethyl 2-(2,4-dichlorophenyl)-1-propyl-1*H*-benzo[*d*]imidazole-5-carboxylate **5** was confirmed by the absence of NH stretching at 3365 cm^{-1} of the previous amine compound **3**. Further, its ^{1}H NMR spectra showed a doublet at 7.67 ppm, a multiplet in the region of 7.94–7.98 ppm corresponding to the three protons of 2,4-dichlorophenyl moiety clearly confirms the cyclization. In its ^{13}C NMR spectrum it showed a signal at 166.59 ppm corresponding to the C=O of ester and two distinct signals for –CH_2 and –CH_3 carbons at 61.06 ppm and 14.72 ppm, respectively. The shifting of carbonyl stretching frequency to a lower region of 1649 cm^{-1} from 1713 cm^{-1} clearly indicates the formation of hydrazide **6** from the ester **5** and presence NH/NH_2 stretching frequencies at 3363 cm^{-1}, 3319 cm^{-1} and 3292 cm^{-1} will give the evidence for the formation of hydrazide. In the ^{1}H NMR spectrum of **6**, two singlet signals displayed at 9.79 ppm and 4.51 ppm corresponding to the NH and NH_2 protons, respectively. Moreover, absence of two signals at 61.06 ppm and 14.72 ppm of –CH_2 and –CH_3, respectively, in its ^{13}C NMR spectrum confirms the formation of hydrazide **6** from ester **5**. The final compounds **7a**–**r** were confirmed by their IR, ^{1}H NMR, ^{13}C NMR, LCMS and elemental analysis. ^{1}H NMR spectrum of **7i** showed a singlet for –NH proton at 10.26 ppm and disappearance

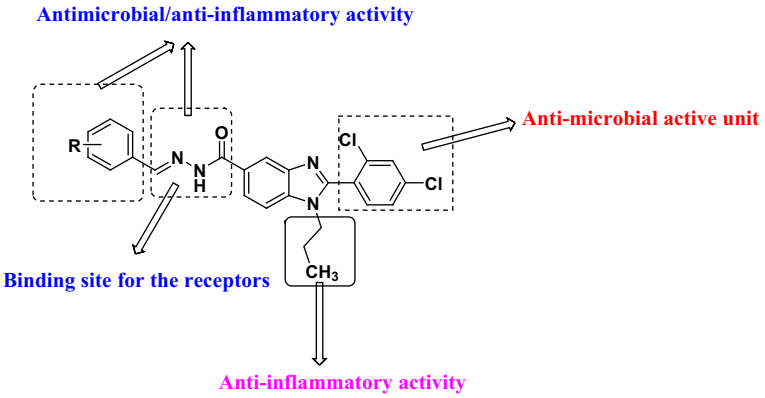

Figure 1. Synthetic strategy for the target compounds **7a**–**r**.