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Original article

Synthesis, antibacterial and antitubercular activities of benzimidazole bearing substituted 2-pyridone motifs

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ABSTRACT

A series of benzimidazole bearing 2-pyridones **5a**–**r** were synthesized and evaluated for their *in vitro* antibacterial and antitubercular activity. Further, all compounds were examined for their cytotoxic study on VERO cell line and characterized by well-known spectral techniques. It was observed that the compounds **5h**, **5i**, **5k**, **5q** and **5r** were found to possess significant broad spectrum antibacterial activity (12.5–100 μg/mL of MIC), while compounds **5g**-**5i**, **5k** and **5l** proved to be the most potent antitubercular activity in range of 2.76–20.4 μM of MIC at low level of cytotoxicity, indicating good selectivity. From SAR studies, lipophilic profile of compounds was remarkably vital for antibacterial activity, while MIC values of antitubercular activity could not be directly correlated with lipophilicity.

1. Introduction

The current first-line tuberculosis drug treatment is more than 40 years old and consists primarily of rifampicin and isoniazid. These antibiotics are drug-susceptible and require longer time and large number of doses, which are multi-drug resistant (MDR) and extensively drug resistant (XDR) to tuberculosis strains [1–3]. However, the rapid increase of multi-drug-resistant tuberculosis (MDR-TB) (resistant to at least isoniazid and rifampicin) and extensively drug-resistant tuberculosis (XDR-TB) (resistant to isoniazid, rifampicin in addition to fluoroquinolone, kanamycin, amikacin or capreomycin among second line anti-TB drugs) has led to an urgent need for the identification of new drug targets and the growth of novel anti-TB drugs. Furthermore, the most commonly encountered antibiotic-resistant bacteria, methicillin-resistant *Staphylococcus aureus* (MRSA), has a major impact on infections in both hospitals and community settings [4,5]. Unfortunately, as antibiotic resistant organisms have become more commonplace, the pipeline for the discovery of new antimicrobial agents has decreased [6]. Thus, there is a pressing need for new antimicrobial agents that are capable of treating resistant bacterial strains.

Moreover, dihydrofolate reductase (DHFR) catalyzes the NADPH-dependent reduction of dihydrofolate to tetrahydrofolate that is essential for DNA synthesis. Inhibition of its activity leads to arrest of DNA synthesis and hence cell death [7]. *Mycobacterium tuberculosis* DHFR is an attractive novel drug target for developing anti-tuberculosis drugs. To overcome this problem, we have synthesized a series of benzimidazole bearing 2-pyridones by replacing the pyrazole motif in our previously synthesized compounds **NCD$_{1-20}$** [8] and screened them for their antibacterial property. In this attempt, we got excellent antibacterial results. Antibacterial studies impelled us to inspect **5a**–**r** for their *in vitro* antitubercular activity as well. Structural relevance of title compounds **5a**–**r** with previously synthesized compounds is shown in Fig. 1.

Benzimidazole nucleus is the key building block for numerous compounds that play beneficial roles in the functioning of biologically important molecules [9] and are remarkably effective both with respect to their inhibitory activity and favorable selectivity ratio [10–12]. Benzimidazoles are considered a promising class of bioactive heterocyclic compounds encompassing a diverse range of biological activities such as antiulcer [13], antihelminthic [14], antihypertensive [15], anticoagulant [16], anti-inflammatory [17], antimicrobial [18–20] and antiparasitic [21]. The azole group of heterocyclic compounds possesses significant pharmacokinetic profile and lipophilicity that influence the ability of drug to reach the target by transmembrane diffusion and along with promising

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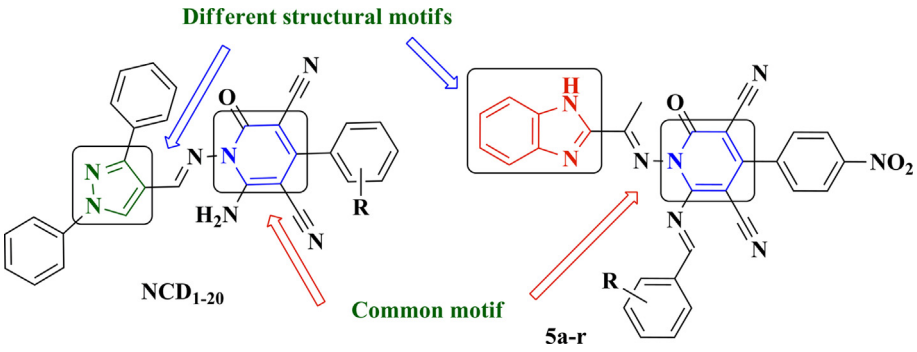

Fig. 1. Structural relevance of title compounds **5a**–**r** with previously synthesized compounds **NCD$_{1-20}$**.

activity against resistant TB by inhibiting the biosynthesis of lipids [22,23]. Further, 2-pyridones represent a unique class of pharmacophores, which are observed in various therapeutic agents [24]. In recent years, 2-pyridones have assimilated much importance as they exhibit several biological activities such as antitumoral [25], antimalarial [26], analgesic [27] and anti-HIV [28] properties. Moreover, 2-pyridones are a class of recently discovered potent antibacterial agents that are of particular interest due to their *in vitro* and *in vivo* antibacterial potencies against the bacterial type II DNA topoisomerases, which include two highly homologous enzymes-DNA gyrase and topoisomerase IV [29,30]. Moreover, among the pharmacokinetic properties, a low and highly variable bioavailability is indeed the main reason for stopping further development of the drug [31].

Motivated by the above findings and from our previous work [32,33], the main aim of the work is to obtain more active antibacterial and antitubercular agents with plausible novel mechanisms of action. It was thought worthwhile to synthesize some new benzimidazole bearing 2-pyridone derivatives comprising of the above aforementioned moieties in single molecular framework in order to investigate their *in vitro* antibacterial and antitubercular activity. In continuation to this, in our present communication, we have synthesized benzimidazole bearing 2-pyridones **5a**–**r** and evaluated them for their *in vitro* antibacterial and antitubercular activity. In addition, cytotoxicity studies were also conducted in VERO cell lines to evaluate the ability of these compounds to inhibit the cell growth. Most active compounds **5h**, **5q** and **5r** were also screened against MRSA strain.

2. Results and discussion

2.1. Chemistry

We have synthesized new analogues in which 2-pyridone motif is connected to the benzimidazole system. The synthetic strategies adopted for the synthesis of target benzimidazole bearing 2-pyridone derivatives **5a**–**r** are depicted in Schemes 1 and 2. In Scheme 1, condensation of 1-(1*H*-benzo[*d*]imidazol-2-yl)ethanone **1** with equimolar quantity of cyanoacetic acid hydrazide in refluxing 1,4-dioxane afforded a single product, that was identified as *N*′-(1-(1*H*-benzo[*d*]imidazol-2-yl)ethylidene)-2-cyanoacetohydrazide **2**. The elemental analysis and spectral data were in accordance with the proposed *N*′-(1-(1*H*-benzo[*d*]imidazol-2-yl)ethylidene)-2-cyanoacetohydrazide **2** structure. The IR spectrum of **2** showed strong absorption bands at 2248 and 1681 cm^{-1} due to cyanide and carbonyl group, respectively. Its ^{1}H NMR spectrum apart from the expected aromatic signals, showed two new singlets at δ 3.38 and 8.92 ppm due to the presence of reactive methylene protons and proton of secondary amine attached with carbonyl group respectively. The ^{13}C NMR spectrum displayed nine carbon signals, the most important signals appeared at δ 13.4, 27.3, 125.8, 171.3 ppm characteristic of methyl, methylene, cyanide and carbonyl carbons, respectively. The mass spectrum revealed a molecular ion peak at m/z = 241.11 (M^{+1}), in agreement with its proposed structure. The presence of the reactive methylene group in hydrazide **2** makes it a versatile precursor for the Michael type condensation with Knoevenagel product *p*-nitrobenzaldehyde and malononitrile compound **3** in presence of catalytic amount of piperidine. Utilizing Ethanol (95%) was used as a solvent to furnish 2-pyridone derivative identified as 1-((1-(1*H*-benzo[*d*]imidazol-2-yl)ethylidene)amino)-6-amino-4-(4-nitrophenyl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile **4**. Structure of the latter product was confirmed by IR spectra which showed characteristic absorption bands at 1688 and 3446 cm^{-1} for conjugated > C=O and primary amine group respectively. Their ^{1}H NMR spectra displayed a broad singlet at δ 8.78 ppm for primary amine protons, besides the disappearance of reactive methylene group and secondary amine singlets due to its involvement in cyclization. Condensation of 2-pyridone derivative **4** with appropriate aromatic aldehydes in boiling ethanol afforded the respective targeted benzimidazole bearing 2-pyridones acknowledged as 1-((1-(1*H*-benzo[*d*]imidazol-2-yl)ethylidene)amino)-6-((arylbenzyli-dene)amino)-4-(4-nitrophenyl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitriles **5a**–**r**. The structures of the final analogues **5a**–**r** were established by IR spectra which showed disappearance of $-NH_2$ band in compound **4**. A strong absorption band between 1679 and 1688 cm^{-1} was assigned to conjugated > C=O group. Their ^{1}H NMR spectra revealed a singlet of methine proton at δ 9.40–9.57 ppm involved in azomethine formation, along with the vanishing of primary amine singlet. The ^{13}C NMR spectrum of compound **5l** displayed, besides the expected methyl and aromatic signals, three characteristic signals at δ 115.9, 160.1 and 163.8 ppm due to the carbons of CN, C=O and CH=N respectively. The mass spectrum of **5l** showed molecular ion peak at m/z = 540.17 (M^{+1}), in agreement with its proposed structure. Similarly, the spectral values for all the compounds and C, H, N analysis are presented in the experimental part.

A plausible mechanistic pathway for the formation of compounds **5a**–**r** is suggested in Scheme 2. Firstly, hydrazone (***A***) underwent Michael addition with Knoevenagel product (***B***) and furnished the intermediate (***C***), which further experienced intramolecular nucleophilic attack on cyanide carbon followed by annulation to yield intermediate (***D***). The intermediate (***D***) transformed to compound (***E***) by intramolecular electron transfer to nitrogen atom. In the last step, intermediate (***E***) was transformed to targeted compounds by intermolecular nucleophilic attack on carbonyl carbon of different aromatic aldehydes.