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Original article

Facile synthesis of benzimidazole bearing 2-pyridone derivatives as potential antimicrobial agents

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ABSTRACT

A series of benzimidazole bearing 2-pyridones **5a**–**k** were synthesized and assessed *in vitro* for their activity as antimicrobial agents using the conventional broth dilution method. The results of the antimicrobial study revealed that compounds **5b**, **5c**, **5j** and **5k** exhibited substantial antibacterial activity while compound **5d** emerged as amore potent antifungal agent compared to the standard drugs chloramphenicol and ketoconazole, respectively. It was observed that the presence of inductively electron withdrawing groups remarkably enhance the antibacterial activity of the newly synthesized compounds. Cytotoxicity studies suggested that none of the tested compounds exhibited any significant cytotoxic effects.

1. Introduction

The alarming rate of emerging and reemerging microbial threat of bacterial resistance has heightened the urgency to discover and develop effective agents with novel mechanisms of action and enhanced activity. Despite the development of several new antibacterial agents, their clinical value is limited in treating an increasing array of life threatening systemic infections. Thus, the development of potent and effective antimicrobial agents is most important to overcome the emerging multi-drug resistance strains of bacteria and fungi.

2-Pyridones represent a unique class of pharmacophores, which are observed in various antibiotics and therapeutic agents [1]. In recent years, 2-pyridones have been found to exhibit several biological activities, such as analgesic [2], anti-HIV [3] and antitumoral [4] 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 potency against the bacterial type II DNA topoisomerases, which include two highly homologous enzymes-DNA gyrase and topoisomerase IV [5]. In addition, benzimidazoles are considered as a promising class of bioactive heterocyclic compounds surrounding a diverse range of biological activities such as antihypertensive [6], anticoagulant [7], anti-inflammatory [8] and antimicrobial [9]. The azole group of heterocyclic compounds possessed favorable pharmacokinetic properties and lipophilicity that influence the ability of drug to reach the target by transmembrane diffusion and showed promising activity against resistant TB by inhibiting the biosynthesis of lipids [10].

Molecular hybridization is an important tool for discovery of new chemical entities. In the past several decades much attention has been given to the design and synthesis of new types of pharmacologically diverse structural hybrid molecules [11]. One shining example is vilazodone, which combined serotonin reuptake inhibitor (SSRI) and 5-HT_{1A} receptor partial agonist and was marketed under the tread name Viibryd [12]. Motivated by the above findings and our previous work [13,14], it was thought worthwhile to synthesize new benzimidazole bearing 2-pyridone derivatives **5a**–**k** that contain the aforementioned moieties in a single molecular framework in order to investigate their *in vitro* antibacterial and antifungal activity.

2. Experimental

Elemental analysis (% C, H, N) was carried out by a Perkin-Elmer 2400 CHN analyzer. IR spectra of all compounds were recorded on a Perkin-Elmer FT-IR spectrophotometer in KBr. ^{1}H NMR spectra were recorded on a Varian Gemini 300 MHz and ^{13}C NMR spectra on a Varian Mercury-400, 100 MHz in DMSO-d_6 as a solvent and tetramethylsilane (TMS) as an internal standard. Mass spectra were scanned on a Shimadzu LC–MS 2010 spectrometer.

General procedure for the synthesis of title compounds **5a**–**k**: Compound **4** (0.01 mol), different substituted aromatic aldehydes (0.01 mol) and ethanol (50 mL) were taken in a round bottom flask and refluxed for 5 h. After 5 h, the reaction mass was poured onto

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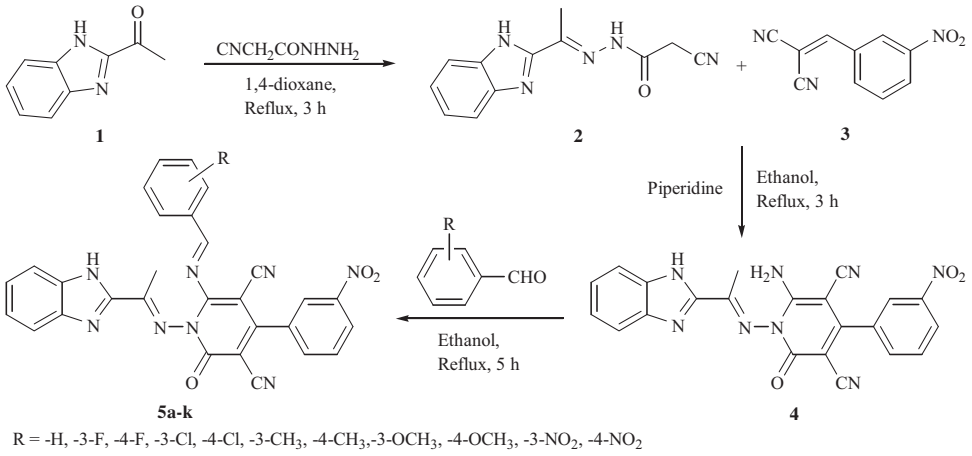

Scheme 1. Synthetic route for the preparation of title compounds **5a–k**.

crushed ice and separated solid was filtered, dried and recrystallized from DMSO. The detail characterization data of intermediate compounds **2**, **4** and **5a–k** were given in Supporting information.

3. Results and discussion

The synthetic strategies adopted for the synthesis of target benzimidazole bearing 2-oxopyridine derivatives **5a–k** are depicted in Scheme 1. In Scheme 1, the synthesis 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 intermediate **2**. The presence of the reactive methylene group in the hydrazide **2** makes it a versatile precursor for the Michael type condensation with Knoevenagel product **3** in the presence of a catalytic amount of piperidine in ethanol (95%) as a solvent giving 2-oxopyridine compound **4**. Condensation of 2-oxopyridine derivative **4** with appropriate aromatic aldehydes in boiling ethanol produced the targeted benzimidazole bearing 2-pyridones **5a–k**. The structures of the final compounds **5a–k** were established by their spectral analysis. Using compound **5i** as a representative example, its IR spectrum showed the disappearance of -NH_2 band of derivative **4**. A strong absorption band appeared at 1682 cm^{-1} and was assigned to ⟩C=O group. Its ^1H NMR spectrum revealed a singlet of emine proton at 9.48, alongside the vanishing of the primary amine singlet. The ^{13}C NMR spectrum of compound **5e** displayed, besides the expected methyl and aromatic signals, three characteristic signals at 115.9, 160.1 and 163.8 due to the carbons of CN, C=O and CH=N, respectively. The mass spectrum of **5e** showed a molecular ion peak at *m/z* 560.12 (M + 1), which is in agreement with its proposed structure [15].

All the newly synthesized compounds were evaluated for their *in vitro* antimicrobial activity against different bacterial and fungal strains by the conventional broth-dilution method [16] using standard drugs. The results of antimicrobial studies are presented in Table 1. Intermediates **2** and **4** showed poor antimicrobial activity against all tested bacterial and fungal strains as compared to final derivatives **5a–k**. In general, compounds **5a–k** showed improved antibacterial activity compared to antifungal activity. Compounds **5b**, **5c**, **5j** and **5k** were found to be highly active against all the bacterial strains, showing

Table 1
Antimicrobial screening results of compounds **2**, **4** and **5a–k**.

Entry	R	Minimum inhibitory concentration (MIC) μg/mL						
		Gram positive bacteria		Gram negative bacteria		Fungi		
		S. aureus	*S. pyogenes*	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. niger*	*A. clavatus*
2	–	1000	1000	>1000	>1000	>1000	>1000	>1000
4	–	250±2.65[c]	500±3.05[c]	1000	500±1.05[a]	1000	500±2.65[c]	1000
5a	-H	250±1.41[a]	250±1.72[b]	500±2.15b	500±3.04	250±1.65[b]	500±2.24[b]	250±1.54[a]
5b	-3-F	12.5±1.05[a]	12.5±1.21[a]	25±1.35a	25±2.80[b]	500±1.57[b]	100±1.24[a]	250±2.78[c]
5c	-4-F	50±1.54[a]	50±1.31[a]	100±2.65c	100±1.61[c]	500±2.15[c]	250±2.21[c]	250±1.24[a]
5d	-3-Cl	62.5±1.35[a]	100±1.65[b]	125±1.42a	125±1.71[c]	25±1.41[a]	50±1.14[a]	62.5±1.35[a]
5e	-4-Cl	100±1.85[b]	100±2.25[b]	250±2.21b	250±1.25[a]	100±1.72[b]	250±1.65[b]	100±1.81[b]
5f	-3-CH_3	250±2.35[c]	250±2.12[b]	500±1.81a	500±2.91[c]	250±2.14[b]	100±1.28[a]	500±2.15[c]
5g	-4-CH_3	500±3.53	500±2.85[c]	1000	500±3.41	500±2.45[c]	250±3.05	500±2.34[c]
5h	-3-OCH_3	250±1.41[a]	500±3.45	500±3.15	500±2.51[b]	500±1.82[b]	250±1.32[a]	100±1.64[b]
5i	-4-OCH_3	250±3.62	500±2.45[b]	1000	>1000	250±2.25[c]	500±2.38[c]	1000
5j	-3-NO_2	12.5±1.48[b]	25±2.15[b]	25±1.35a	25±1.15[a]	100±1.64[b]	500±1.85[b]	250±1.32[a]
5k	-4-NO_2	25±1.21[a]	50±1.81[a]	25±1.54b	50±1.51[a]	250±1.32[a]	>1000	500±2.32[b]
Chloramphenicol	–	50±1.24	50±2.04	50±1.00	50±2.06	–	–	–
Ketoconazole	–	–	–	–	–	50±0.50	50±1.20	50±1.10

All values are presented as mean of 6 experiments ($n = 6$). All significant differences are considered from control value (0.00).
[a] $P < 0.001$ extremely significant.
[b] $P < 0.01$ moderately significant.
[c] $P < 0.05$ significant.

inhibition in the range of 12.5–100 μg/mL. Among them, compounds **5b** and **5j** emerged as the most effective antibacterial agents with a 2 to 4-fold higher MIC (12.5–25 μg/mL) than the reference drug chloramphenicol. Compounds **5c** and **5k** exhibited comparable antibacterial activity with MIC values of 25–100 μg/mL. From these results, it can be observed that the antibacterial activity was considerably affected by the substitution pattern on the phenyl ring and the most active compounds contain an inductively electron withdrawing substituent at the *meta* or *para* position of the phenyl ring ($m > p$). In contrast, the presence of electron donating groups on the phenyl ring resulted in a substantial decrease in antimicrobial activity for compounds **5f**–**5i**. Compounds **5b** and **5j**, substituted with inductively electron withdrawing fluoro and nitro groups, respectively, at the *meta* position showed the highest antibacterial activity ($F > NO_2$). The presence of lipophilic substituent at the *meta* position of phenyl ring provided a positive influence on the antibacterial activity. Further, the results of the antifungal activity indicated that compound **5d** endowed with chlorine emerged as the most effective antifungal agent and showed an MIC in range of 25–62.5 μg/mL against three fungal strains using ketoconazole as a positive control.

The cytotoxic potential of compounds **5b**, **5c**, **5j**, **5k** and **5d** was also determined in human cancer cell lines such as A549, HL-60, and HepG2 according protocols [17]. None of the tested compounds exhibited significant cytotoxic activity ($IC_{50} > 100$ μg/mL) at the highest does used, indicating good selectivity. They possessed potent antibacterial and antifungal activity without the cytotoxicity in mammalian cells [18].

4. Conclusion

In summary, we have accomplished the synthesis of new derivatives of benzimidazole bearing 2-pyridones **5a**–**k** having two cyano groups with the hope of generating new bioactive molecules that could be useful as potent antimicrobial agents. Among the eleven newly synthesized compounds, analogs **5b**, **5c**, **5j** and **5k** possessing electron withdrawing atom/group such as fluoro and nitro at the *meta* or *para* position were identified as the most potent antibacterial agents and compound **5d** was found to be the most effective antifungal agent with relatively low cytotoxicity. The results described here merit further investigations in our laboratory using a forward chemical genetic approach in finding lead molecules as antimicrobial agents.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cclet.2013.11.026.

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