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RESEARCH ARTICLE

Synthesis, antimicrobial and antimycobacterial evaluation of [2-(substituted phenyl)-imidazol-1-yl]-pyridin-3-yl-methanones

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Abstract

A series of [2-(substituted phenyl)-imidazol-1-yl]-pyridin-3-yl-methanones (**1–11**) were synthesized and screened for their antimicrobial and antimycobacterial activities. Further, a series of [2-(substituted phenyl)-benzimidazol-1-yl]-pyridin-3-yl-methanones (**12–20**) reported in our earlier study was also screened for their antimycobacterial activity. The antimycobacterial activity results indicated that [2-(4-Nitro-phenyl)-imidazol-1-yl]-pyridin-3-yl-methanone (**8**, minimum inhibitory concentration [MIC] = 3.13 μg) was equipotent as standard drug ciprofloxacin and [2-(4-Nitro-phenyl)-benzimidazol-1-yl]-pyridin-3-yl-methanone (**16**, MIC = 1.56 μg) was equipotent as standard drug ethambutol. The results of antimicrobial screening demonstrated that 2-[1-(Pyridine-3-carbonyl)-1H-imidazol-2-yl]-benzoic acid (compound **11**, MIC = 0.002 μg) was two times more effective than standard drug ciprofloxacin (MIC = 0.004 μg) against tested bacterial strains and [2-(2,5-Dimethyl-phenyl)-imidazol-1-yl]-pyridin-3-yl-methanone (compound **3**, MIC = 0.005 μg) was equipotent to the reference compound, fluconazole against tested fungal strains.

Keywords: Imidazole/benzimidazole derivatives; antimycobacterial; antimicrobial

Introduction

Disease-causing microbes that have become resistant to drug therapy are an increasing public health problem nowadays[1]. There is a real need for discovery of new chemical entities endowed with antimicrobial activity, possibly acting through mechanisms which are distinct from the well-known classes of antimicrobial agents, to which many clinically relevant pathogens have become resistant[2].

Tuberculosis (TB), which is caused by single infectious agent *Mycobacterium tuberculosis*, is one of the most important infectious disease[3]. Recently, world health organization (WHO) reported that the global figure of total deaths by infectious diseases is 17 million, of which TB accounts for ~20 % of mortality. The gravity of problem increases even more by the fact that one new case of TB is reported every 4 sec and one patient dies every 10 sec[4]. The factors responsible for this are (i) patient non-compliance to existing drug regimens which has resulted in the emergence of single drug-resistant strains to all major anti-TB drugs; (ii) emergence of multidrug resistant TB (MDR-TB), which is defined as the disease caused by the strains of *M. tuberculosis* resistant to two mainstay first line anti-TB drugs, isoniazid and rifampicin, and (iii) association of human immunodeficiency virus (HIV) with TB, in which TB is the leading cause of death among patients who are HIV-positive[5]. The development of potent new anti-TB agents with low toxicity profiles effective against both drug-susceptible and drug-resistant strains of *M. tuberculosis* and capable of shortening the current duration of therapy are the need of the hour[6]. There are two basic

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approaches to develop a new drug for TB: (i) synthesis of analogues, modification, or derivatives of existing compounds for shortening and improving TB treatment and (ii) searching for novel structures, that the TB organism has never been presented with before, for the treatment of MDR-TB[7].

The prevalence of imidazoles in natural products and pharmacologically active compounds has instituted a diverse array of synthetic approaches to these heterocycles. The imidazole nucleus reported to have a wide range of pharmacological activities *viz.* anticancer, antimycobacterial, antiviral, antibacterial, antifungal, antidiabetic, anti-inflammatory, analgesic, and antiobesity activities[8–15]. In pursuit of this goal, our research efforts are directed toward discovery of new chemical entities that are effective as anti-TB agents *viz.* synthesis and antitubercular evaluation of [2-(substituted phenyl)-imidazol-1-yl]-pyridin-3-yl-methanones. Further, we have also screened the nicotinic acid-based benzimidazole derivatives reported in our previous study[16] for their antimycobacterial activity.

Experimental

Melting points were determined in open capillary tubes on a Sonar melting point apparatus and are uncorrected. Reaction progress was monitored by thin layer chromatography (TLC) on silica gel sheets (Merck silica gel-G) and the purity of the compounds is ascertained by single spot on TLC sheet. ^{1}H nuclear magnetic resonance (^{1}H NMR) and ^{13}C-NMR spectra were recorded in Bruker Avance II 400 NMR spectrometer using appropriate deuterated solvents and are expressed in parts per million (δ, ppm) downfield from tetramethylsilane (internal standard). Infrared (IR) spectra were recorded on a Shimadzu FTIR spectrometer.

General procedure for the synthesis of [2-(o-Chlorophenyl)-imidazol-1-yl]-pyridin-3-yl-methanone[4]

o-Chloroaniline (0.13 mol) in hydrochloric acid/water mixture (1:1) was diazotized using solution of sodium nitrite at 0–10°C. To the diazotized mixture, imidazole (0.004 mol) was added with vigorous shaking. A solution of sodium acetate (40 g in 100 ml) was added drop wise to the above mixture by maintaining temperature at 5–10°C. The above solution was stirred initially for 3 h at cold condition followed by continuation of stirring at room temperature for 48 h. The product, 2-(*o*-Chlorophenyl)-1-imidazole, obtained was filtered, dried, and recrystallized from alcohol.

A solution of 2-(*o*-Chlorophenyl)-1-H-imidazole (0.002 mol) in diethyl ether (50 ml) was added with a solution of nicotinyl chloride (0.002 mol) in diethyl ether (50 ml). The above mixture was stirred for 24 h at room temperature. The resultant product, [2-(*o*-Chlorophenyl)-imidazol-1-yl]-pyridin-3-yl-methanone, was isolated by evaporation of ether and purified by recrystallization from methanol.

The compounds **1–3** and **5–11** were synthesized by the similar procedure followed for **4** using corresponding substituted anilines.

Compound 1. mp (°C) 79–81; Yield– 50.76%; ^{1}H-NMR: δ 7.0 (d, 2H, CH of imidazole), δ 7.20–7.31 (m, 3H, CH of C_3-C_5 of benzene), δ 7.46–7.48 (d, 1H, CH of C_2 of benzene), δ 8.2 (d, 1H, CH of C_6 of benzene), δ 7.51–7.57 (m, CH of C_4-C_5 of pyridine), δ 8.81 (d, 1H, CH of C_6 of pyridine), δ 9.05 (s, 1H, CH of C_2 of pyridine); ^{13}C-NMR: 136, 122, 136.5, 126.8, 134.8, 128.5, 128.8, 127.2, 193, 131.9, 152.2, 155.2, 124.6, 134.9; IR (KBr pellets): cm^{-1} 1724.2 (C=O str.), 3080.0 (CH str., aromatic), 1027.8 (Ring bending mode, imidazole), 1597.9 and 1680.0 (C=C and C=N str. of pyridine ring); Anal. Calculated for $C_{15}H_{11}N_3O$: C, 72.28; H, 4.45; N, 16.86; O, 6.42. Found: C, 72.19; H, 4.41; N, 16.83; O, 6.41.

Compound 4. mp (°C) 94–96; Yield– 68.75%; ^{1}H-NMR: δ 7.29–7.34 (d, 2H, CH of C_4 and C_5 of imidazole), δ 7.83–7.86 (m, 2H, CH of C_5 and C_6 of ArCl), δ 7.99–8.02 (d, 1H, CH of C_6 of ArCl), δ 8.65–8.67 (d, 1H, CH of C_3 of ArCl), δ 8.81–8.83 (t, 1H, CH of C_5 of nicotinic acid), δ 8.98–8.99 (d, 1H, CH of C_6 of nicotinic acid), δ 9.21 (s, 1H, CH of C_2 of nicotinic acid), δ 8.94–8.95 (d, 1H, CH of C_4 of nicotinic acid); ^{13}C-NMR: 136.4, 121.9, 135.7, 128.3, 127.2, 128.7, 129.9, 131.8, 191, 131.7, 152.5, 155.4, 124.6, 135.7; IR (KBr pellets): cm^{-1} 1731.96 (C=O str.), 3084.93 (CH str., aromatic), 1025.1 (Ring bending mode, imidazole), 735.79 (C-Cl str., aromatic), 1601.77 and 1699.0 (C=C and C=N str. of pyridine ring); Anal. Calculated for $C_{15}H_{10}ClN_3O$: C, 63.50; H, 3.55; N, 14.81; O, 5.64; Cl, 12.5. Found: C, 63.48; H, 3.54; N, 14.78; O, 5.60; Cl, 12.3.

Compound 6. mp (°C) 99–101; Yield– 36.80%; ^{1}H-NMR:7.13 (d, 1H, CH of C5 of imidazole), δ 7.16 (d, 1H, CH of C4 of imidazole), δ 7.26–7.56 (m, 4H, ArH of ArCl), δ 7.87 (d, 1H, CH of C_4 of pyridine), δ 7.88 (d, 1H, CH of C_5 of pyridine), δ 7.97 (d, 1H, CH of C_6 of pyridine), δ 9.04 (s, 1H, CH of C_2 of pyridine); ^{13}C-NMR: 135.6, 122.6, 137.2, 125.9, 131, 128.4, 134.2, 128, 190, 131.9, 152.6, 155.4, 124.3, 135.9; IR (KBr pellets): cm^{-1} 1719.1 (C=O str.), 3070.0 (CH str., aromatic), 1019.8 (Ring bending mode, imidazole), 710.2 (C-Cl str., aromatic), 1594.7 and 1690.0 (C=C and C=N str. of pyridine ring); Anal. Calculated for $C_{15}H_{10}ClN_3O$: C, 63.50; H, 3.55; N, 14.81; O, 5.64; Cl, 12.5. Found: C, 63.48; H, 3.54; N, 14.78; O, 5.60; Cl, 12.3.

Compound 11. mp (°C) 145–147; Yield– 56.30%; ^{1}H-NMR:δ 6.95–7.0 (d, 2H, CH of imidazole), δ 10.50 (s, 1H, COOH), δ 7.31 (t, 1H, CH of C_4 of benzoic acid), δ 7.94 (d, 1H, CH of C_6 of benzoic acid), δ 7.32–7.35 (t, 1H, CH of C_6 of benzoic acid), δ 7.54 (d, 1H, CH of C_4 of pyridine), δ 7.48–7.49 (t, 1H, CH of C_5 of pyridine), δ 8.54 (d, 1H, CH of of C_6 of pyridine), δ 9.2 (s, 1H, CH of C_2 of pyridine); ^{13}C-NMR: 136.8, 122, 138.2, 127.4, 134.8, 128.9, 131, 129.8, 190, 131.5, 155.7, 152.6, 124.9, 136.1, 172.8; IR (KBr pellets): cm^{-1} 1720.0 (C=O str.), 3010.0 (CH str., aromatic), 1030.0 (Ring bending mode, imidazole), 1598.0 and 1653.4 (C=C and C=N str. of pyridine ring); Anal. Calculated for $C_{16}H_{11}N_3O_3$: C, 65.53; H, 3.78; N, 14.33; O, 16.37. Found: C, 65.50; H, 3.74; N, 14.30; O, 16.35.

Evaluation of antimycobacterial activity

All compounds were screened for their *in vitro* antimycobacterial activity against MTB, in Middlebrook 7H11agar medium supplemented with OADC by agar dilution method similar to that recommended by the National Committee for Clinical Laboratory Standards for the determination of minimum inhibitory concentration (MIC) in triplicate. The MIC is defined as the minimum concentration of compound required to give complete inhibition of bacterial growth.

Evaluation of antimicrobial activity

Determination of MIC

The antimicrobial activity was performed against Gram-positive bacteria: *S. aureus*, *B. sublitis*, Gram-negative bacterium: *E. coli*, and fungal strains: *C. albicans* and *A. niger* by tube dilution method[17]. Dilutions of test and standard compounds [ciprofloxacin (antibacterial) and fluconazole (antifungal)] were prepared in double strength nutrient broth—I.P. (bacteria) and Sabouraud dextrose broth I.P.[18] (fungi). The samples were incubated at 37°C for 24 h (bacteria), at 25°C for 7 days (*A. niger*), and at 37°C for 48 h (*C. albicans*), respectively, and the results were recorded in terms of MIC (the lowest concentration of test substance which inhibited the growth of microorganisms).

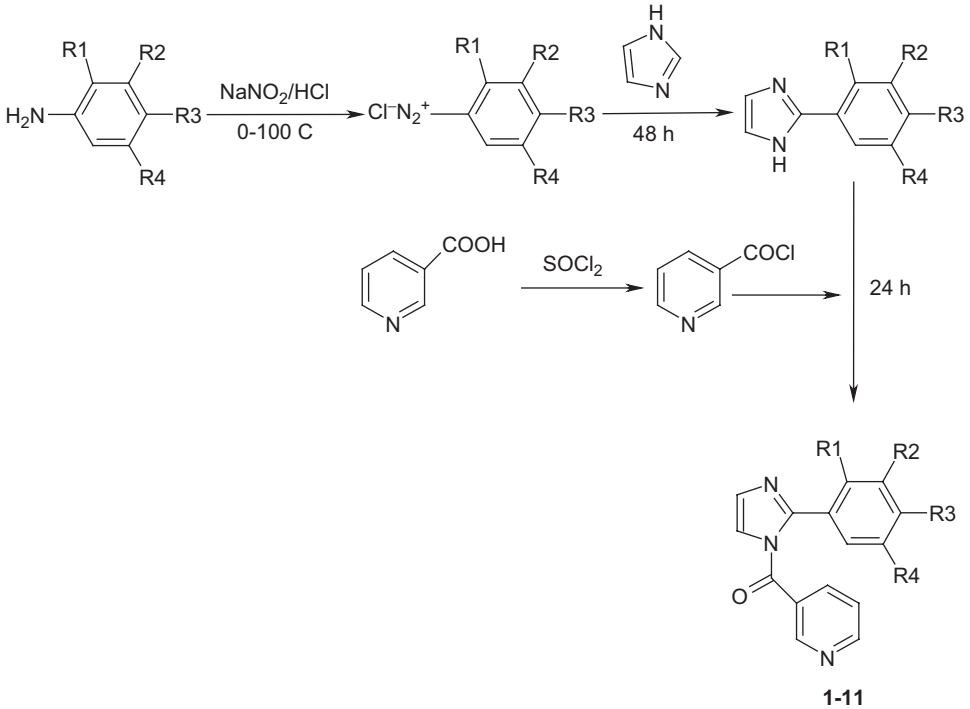
Determination of minimum bactericidal/fungicidal concentration (MBC/MFC)

The minimum bactericidal concentration (MBC) and fungicidal concentration (MFC) were determined by sub-culturing 100 µL of culture from each tube that remained clear in the MIC determination into fresh medium. MBC and MFC values represent the lowest concentration of compound that produces a 99.9% end point reduction[19].

Results and discussion

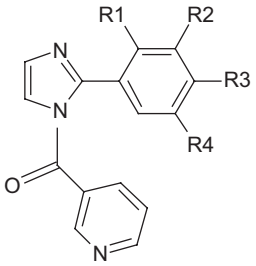
Chemistry

The general procedure for the preparation of target compounds **1–11** is described in Scheme 1. The key intermediates, 2-(substituted phenyl)-1H-imidazoles, were prepared by the condensation of imidazoles with corresponding substituted aryl diazonium chlorides which in turn was prepared by the diazotization of substituted anilines. However, based on our experience, the application of the cupric chloride for the condensation of aryl-diazonium chloride with benzimidazole as suggested by Dahiya et al.[20] resulted in resinous products. Therefore, the coupling was carried out by using sodium acetate along with stirring at cold conditions for the initial 3 h followed by 48 h stirring at room temperature, which resulted in a solid product. For the synthesis of [2-(substituted phenyl)-imidazol-1-yl]-pyridin-3-yl-methanones **(1–11)**, the key intermediates, 2-(substituted phenyl)-1-H-imidazoles, have been reacted with nicotinyl chloride which was formed by the reaction of nicotinic acid with thionyl chloride. The physicochemical characteristics of the synthesized compounds are presented in Table 1. It is important to note here that the % yield of most of the synthesized compounds are below 50%. The low yield of synthetic compounds may be attributed to any one or more of the following reasons[21]: (i) the reaction may be reversible and position of equilibrium is unfavorable to the product; (ii) the incursion of side reactions leading to

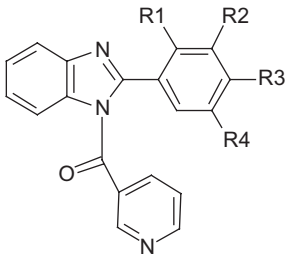


Scheme 1. Synthetic route followed to obtain target compounds

Table 1. Physicochemical characteristics and antimycobacterial activities of synthesized [2-(substituted phenyl)-imidazol-1-yl/benzimidazole-1-yl]-pyridin-3-yl-methanones.



1-11



12-20[a]

Comp.	R_1	R_2	R_3	R_4	Mol. formula	M. wt.	m.p. (°C)	Rf value*	% yield	MIC (μg/ml)
1	H	H	H	H	$C_{15}H_{11}N_3O$	249.27	79–81	0.83	50.76	>25.0
2	CH_3	CH_3	H	H	$C_{17}H_{15}N_3O$	277.33	219–221	0.32	42.50	25.0
3	CH_3	H	H	CH_3	$C_{17}H_{15}N_3O$	277.33	209–211	0.15	56.80	25.0
4	Cl	H	H	H	$C_{15}H_{10}ClN_3O$	283.72	94–96	0.15	68.75	12.5
5	H	H	Cl	H	$C_{15}H_{10}ClN_3O$	283.72	119–121	0.80	54.76	6.25
6	H	Cl	H	H	$C_{15}H_{10}ClN_3O$	283.72	99–101	0.74	36.80	12.5
7	H	H	Br	H	$C_{15}H_{10}BrN_3O$	328.17	>260	0.32	35.78	6.25
8	H	H	NO_2	H	$C_{15}H_{10}N_4O_3$	294.27	49–51	0.58	45.98	3.13
9	NO_2	H	H	H	$C_{15}H_{10}N_4O_3$	294.27	189–191	0.53	65.70	6.25
10	H	NO_2	H	H	$C_{15}H_{10}N_4O_3$	294.27	219–221	0.18	43.76	6.25
11	COOH	H	H	H	$C_{16}H_{11}N_3O_3$	293.28	145–147	0.15	56.30	>25.0
12	Cl	H	H	H	$C_{19}H_{12}N_3OCl$	333.83	84–86	0.95	39.13	3.13
13	H	H	Cl	H	$C_{19}H_{12}N_3OCl$	333.83	224–226	0.48	47.82	3.13
14	H	Cl	H	H	$C_{19}H_{12}N_3OCl$	333.83	149–151	0.59	73.91	6.25
15	H	H	OH	H	$C_{19}H_{13}N_3O_2$	315.37	64–66	0.15	11.20	25
16	H	H	NO_2	H	$C_{19}H_{12}N_4O_3$	344.30	244–246	0.22	48.59	1.56
17	H	NO_2	H	H	$C_{19}H_{12}N_4O_3$	344.30	239–241	0.31	70.09	3.13
18	NO_2	H	H	H	$C_{19}H_{12}N_4O_3$	344.30	49–51	0.90	14.95	3.13
19	COOH	H	H	H	$C_{20}H_{13}N_3O_3$	343.41	139–141	0.13	45.08	25
20	OCH_3	H	H	H	$C_{20}H_{15}N_3O_2$	329.39	99–101	0.90	14.20	25
Isoniazid										0.10
Ethambutol										1.56
Ciprofloxacin										3.13

MIC, minimum inhibitory concentration.
*Toluene:Chloroform (7:3).
[a]Reported in Reference 16.

the formation of by-products; (iii) the premature work-up of the reaction before its completion; (iv) the volatilization of products during reaction or work-up; (v) the loss of product due to incomplete extraction, inefficient crystallization, or other work-up procedures; (vi) the presence of contaminants in the reactants or reagents leading to a less efficient reaction.

The structures of compounds **1–11** were assigned by IR and ^{1}H-NMR spectroscopic data, which are consistent with the proposed molecular structures. The appearance of medium out of plane deformation bands (C-C bending) at 746.4 cm^{-1} and 756.6 cm^{-1} indicated the presence of 1,2-disubstituted benzene ring in compound **4** and **11**. Similarly, the appearance of the C-C out of plane band at 679.1 cm^{-1} indicated the presence of 1,3-disubtituted benzene ring in compound **6**. In contrast, the monosubstituted benzene ring (compound **1**) showed C-C out of plane band deformation at 684.6 cm^{-1}. The presence of 3-substituted pyridine in structures of compounds (**1–11**) was confirmed by strong out of plane deformation bands (C-H bending) at 820–770 cm^{-1} which were visible from their IR spectra. The appearance of medium bands at 3500 cm^{-1} in the IR spectra of compound **11** indicated the presence of a free OH in the carboxylic acid group. Further, the appearance of strong C=O stretching bands at 1735–1710 cm^{-1} in the IR spectra of [2-(substituted phenyl)-imidazol-1-yl]-pyridin-3-yl-methanones **(1–11)** demonstrated the presence of tertiary amide linkage between the 3-substituted pyridine and the benzimidazole nucleus. The presence of an additional IR band

C=O stretching at 1702 cm^{-1} indicated the presence of carboxylic acid group in compound **11**. The appearance of IR bands at 1602–1594 cm^{-1} and 1699– 1653 cm^{-1} in the IR spectra synthesized compounds (**1–11**) indicated the presence of C=C and C=N stretching of pyridine nucleus of the nicotinic acid. The ring breathing mode of imidazoles nucleus was indicated by the appearance of IR bands at 1019–1030 cm^{-1} of the synthesized compounds (**1–11**). Further, the presence of halogens in compound **4** and **6** was indicated by the presence of Ar-Cl stretching vibrations at 735.7 cm^{-1} and 710.2 cm^{-1}, respectively.

The appearance of δ at 6.9–7.8 ppm corresponds to the aromatic protons of benzene and imidazole nucleus, whereas the nicotinic acid nucleus showed δ at 8–9 ppm. Further, it is important to note that the appearance of a singlet at δ 10.50 ppm indicated the presence of COOH group in compound **11**. The absence of a singlet around δ 11 ppm in the NMR spectra of compounds **1–10** indicated the absence of the free COOH group. This confirms that the compounds **1–11** are tertiary amides and not the physical mixture of nicotinic acid. Therefore, this assures the reaction of nicotinyl chloride with the secondary nitrogen of imidazole nucleus.

Antimycobacterial activity

The *in vitro* antimycobacterial activity of synthesized compounds against MTB was carried out in Middlebrook 7H11agar medium supplemented with OADC by agar dilution method and the results are presented in Table 1[22]. At the commencement of this study in the preliminary screening, compound (**1**) imidazole nucleus with an unsubstituted phenyl ring displayed poor antimycobacterial activity with an MIC of > 25 μM/mL. So we have taken compound **1** as a lead compound and planned to improve the antimycobacterial activity by making substitutions on phenyl ring. The first step toward lead optimization was incorporation of electron donating group (CH_3) on phenyl ring. The addition of electron donating group does not improve the antitubercular activity evidenced by the slight increase in the activity of compound **2**, **3** (MIC = 25 μM/ml) in comparison to **1**. Based on these results, we have dropped the idea of introducing electron donating groups to the phenyl ring of the imidazole nucleus. The next structural modification was introduction of electron withdrawing halogen group *viz.* Cl and Br. The presence of *p*-chloro (**5**) phenyl ring improved the antimycobacterial potential of imidazole derivative in comparison to its presence in *ortho* (**4**) and *meta* (**6**) position as evidenced by their MIC values presented in Table 1. This fact is true with the *p*-bromo derivative (**7**) also. Enhancement in activity proves that this modification is step up toward synthesis of a pharmacophore. On the basis of biological data, improvement in biological activity was observed with increase in electronegativity of molecule. Hence, we planned to introduce more electron withdrawing nitro group to the phenyl ring of imidazole nucleus. This modification produced excellent improvement in the antitubercular activity as evidenced by the fact that the 50% improvement in MIC values of *p*, *o*, *m*– nitro substituted phenyl imidazoles (**8**, **9**, and **10**) in comparison to *o*, *p*, *m*– chloro substituted phenyl imidazoles (**4**, **5**, and **6**). Among the different nitro substitutions, the *p*-nitro phenyl group (**8**) conferred the antimycobacterial activity equivalent to ciprofloxacin. Further, to study the effect of electron withdrawing carboxylic acid group, we have designed and synthesized compound **11** and tested for its antitubercular activity. This change resulted in substantial loss in activity (Compound **11**, MIC = > 25.0 μM/ml). This is similar to the results observed by Gill et al.[23].

The antimycobacterial activity results of benzimidazole derivatives (**12–20**) are presented in Table 1. All the synthesized compounds were found to be less active than the standard drug isoniazid. Among the halo substituted benzimidazole derivatives, *ortho* (**12**) and *para* chloro (**13**) derivatives are more effective than the *meta* chloro (**14**) derivative. This is in contrast to the results obtained for imidazole derivatives in case of *ortho* chloro derivative, which is not equipotent to *p*-chloro derivative. The improved antimycobacterial activity of *o*-chloro benzimidazole derivative (**12**) may be attributed to the presence of a fused benzene ring to the imidazole nucleus which may favor the binding of *o*-chloro derivative to its target site. As in case of imidazole derivatives, the presence of electron donating group does not favor the antimycobacterial activity of the benzimidazole derivatives also (**15** and **20**). Among the *p*, *o*, *m*– nitro substituted phenyl benzimidazoles (**16–18**), the *p*-nitro phenyl group (**16**) (Compound **16**, MIC = 1.56 μM/ml) conferred the antimycobacterial activity equivalent to the reference compound ethambutol. As in case of imidazoles, the presence of electron withdrawing carboxylic acid group in benzimidazole (**19**) resulted in substantial loss in activity (Compound **19**, MIC = 25.0 μM/ml). In general, the benzimidazole derivatives are more potent than the imidazole derivatives against *M. tuberculosis* strain as they are active at half the concentration of imidazole derivatives i.e., fusion of benzene ring to imidazole nucleus greatly improved the antimycobacterial activity.

From the aforementioned results, we can draw following conclusions:

1. Electron withdrawing groups improve the antimycobacterial activity compared to the electron donating groups. The presence of halo groups at *para* position improved the antitubercular activity of the synthesized compounds. This is similar to the results observed by Mamolo et al.[24], who stated that the presence of bromo, chloro, and phenyl substituents at the *para* position of the benzene ring attached to imidazole improved the antitubercular activity.
2. Among the different electron withdrawing groups, nitro group is most effective in conferring the antimycobacterial activity to the imidazole derivative. This is supported by the finding of Satyajit Dutta, who stated that nitrophenyl group at the 2nd position in

Table 2. Antimicrobial activity of synthesized [2-(substituted phenyl)-imidazol-1-yl]-pyridin-3-yl-methanones.

	MIC (μM/ml)				
Comp.	*S. aureus*	*B. subtilis*	*E. coli*	*C. albicans*	*A. niger*
1	0.012	0.003	0.003	0.025	0.050
2	ND	ND	ND	0.022	0.005
3	ND	ND	ND	0.005	0.005
4	0.044	0.044	0.044	0.022	0.044
5	0.022	0.044	0.006	0.022	0.044
6	0.044	0.044	0.011	0.342	0.044
7	ND	ND	ND	0.004	0.019
8	0.010	0.010	0.040	0.020	0.040
9	0.040	0.002	0.040	0.020	0.040
10	0.013	0.005	0.002	0.025	0.025
11	0.002	0.002	0.002	0.020	0.040
Ciprofloxacin	0.004	0.004	0.004	—	—
Fluconazole	—	—	—	0.005	0.005

MIC, minimum inhibitory concentration; ND, not detected.

imidazole derivatives is responsible for high biological activity[19].

3. Fusion of benzene ring to imidazole nucleus greatly improves the antimycobacterial activity.

Antimicrobial activity

The synthesized imidazole derivatives were evaluated for their *in vitro* antibacterial activity against Gram-positive *S. aureus*, *B. subtilis* and Gram-negative *E. coli* and *in vitro* antifungal activity against *C. albicans* and *A. niger* by tube dilution method[17]. Double strength Nutrient broth I.P. and Sabouraud dextrose broth I.P.[18] have been employed as media for growth of bacterial and fungal species, respectively. The results of antimicrobial activity are presented in Table 2.

In case of *S. aureus*, compound **11** was found to be more active than the standard ciprofloxacin with an MIC value of 0.002 μM/ml (Table 2). Against *B. subtilis*, compounds **1**, **9**, and **11** were emerged as most active ones with MIC value of 0.003, 0.002, and 0.002 μM/ml, respectively, in comparison to the standard compound ciprofloxacin (MIC = 0.004 μM/ml, Table 2). Compounds **1**, **10**, and **11** demonstrated high antibacterial activity against the Gram negative bacteria, *E. coli* (Table 2).

In case of antifungal activity against *C. albicans*, compound **7** (MIC = 0.004 μM/ml, Table 2) was found to be more active than the reference compound fluconazole (MIC = 0.005 μM/ml, Table 2) and compound **3** was equipotent to the reference drug fluconazole. For antifungal activity against *A. niger*, compounds **2** and **3** exhibited their antifungal potential at MIC values of 0.005 μM/ml, which is same as the reference, fluconazole.

From the aforementioned antimicrobial activity results, following structure activity relationship (SAR) can be deduced:

1. The antibacterial activity of compounds **9**, **10**, and **11** may be due to the presence of electron withdrawing COOH and NO_2 group. Role of electron withdrawing group in improving antimicrobial activity is similar to the results of Sharma et al.[25].
2. The compound **11** demonstrated high antibacterial activity compared to the reference compound ciprofloxacin against all the tested bacterial species. The higher activity of compound **11** can be attributed to the presence of imidazole as well as the pyridine nucleus which was absent in the ciprofloxacin, even though both compound **11** and ciprofloxacin have the common free carboxylic acid group.
3. It is interesting to note an important observation that the compound **11** demonstrated excellent antibacterial activity but exhibited poor antifungal activity. The lower antifungal activity of **11** is supported by the work of Goker et al.[26] who reported that the presence of trifluoromethyl, carboxylic, ester, and amide groups does not improve antifungal activity.
4. The presence of electron donating CH_3 group is responsible for the high antifungal activity of the imidazole derivative compound **3** against the tested fungal strains. The role of electron donating group in

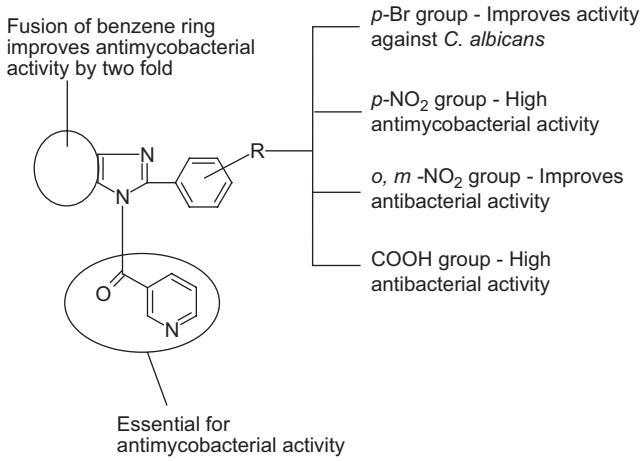


Figure 1. Structural requirements for the antimicrobial and antimycobacterial activity of [2-(substituted phenyl)-imidazol-1-yl]-pyridin-3-yl-methanones.

Table 3. MBC/MFC of [2-(substituted phenyl)-imidazol-1-yl]-pyridin-3-yl-methanones.

	MBC/MFC(μM/ml)				
Comp.	*S. aureus*	*B. subtilis*	*E. coli*	*C. albicans*	*A. niger*
1	0.025	0.025	0.025	0.100	0.400
2	ND	ND	ND	0.090	0.360
3	ND	ND	ND	0.045	ND
4	0.352	0.352	0.352	0.352	ND
5	0.176	0.352	0.044	0.088	0.176
6	0.352	0.352	0.088	ND	ND
7	ND	ND	ND	0.076	0.019
8	ND	ND	0.169	0.169	0.169
9	0.169	0.169	0.084	0.169	ND
10	0.338	0.006	0.003	0.169	ND
11	0.021	0.021	0.021	0.09	ND

MBC/MFC, minimum bactericidal/fungicidal concentration; ND, not detected.

improving antifungal activity of imidazole derivatives is supported by the studies of Emami et al.[27].

5. In contradiction, the compound **7** which contains an electron withdrawing bromo group showed an appreciable antifungal activity against *C. albicans*. This result indicated that the presence of the electron withdrawing Br substituent on the phenyl ring attached to the 2nd position of imidazole nucleus may be favorable for its binding with the fungal target of *C. albicans.* This is similar to the results of one of our previous study of antimicrobial activity of imidazole derivatives[28].
6. The presence of electron withdrawing groups and electron donating groups conferred antibacterial and antifungal activity respectively to the synthesized imidazole derivatives. This fact is also supported by the antitubercular results, where the most active compounds against the mycobacterium are the ones which have the electron withdrawing groups in their structure.
7. The aforementioned results indicated the fact that different structural requirements are essential for a compound to be selected as antibacterial or antifungal agent. This is similar to the results obtained by Sortino et al.[29].

The SAR of antimicrobial and antitubercular activity of synthesized substituted imidazole derivatives are summarized in Figure 1.

Based on the MIC values observed, the MBC and MFC were determined by subculturing 100 μL of culture from each tube that remained clear in the MIC determination into fresh medium. It may be possible that the presence of one or few organisms cannot be detected by determining the turbidity of the solution. In this case, the determination of MBC and MFC may give useful information which involves subculturing of 100 μL of culture from each tube that remained clear in the MIC determination into fresh medium. This is the reason for the fact that MBC/MFC values (Table 3) of test compounds are observed at high concentration than their MIC values (Table 2). In general, the MFC and MBC values of synthesized imidazole derivatives were 3-fold higher than the MIC values, which indicated that the synthesized compounds were bacteriostatic and fungistatic in action. (A drug is considered to be bacteriostatic/fungistatic when its MFC and MBC values are 3-fold higher than its MIC value)[30].

Conclusion

A series of [2-(substituted phenyl)-imidazol-1-yl]-pyridin-3-yl-methanones (**1–11**) and [2-(substituted phenyl)-benzimidazol-1-yl]-pyridin-3-yl-methanones (**12–20**) were screened for their antimycobacterial activity. The antimycobacterial activity results indicated that [2-(4-Nitro-phenyl)-imidazol-1-yl]-pyridin-3-yl-methanone (**8**) and [2-(4-Nitro-phenyl)-benzimidazol-1-yl]-pyridin-3-yl-methanone (**16**) were the most effective ones. The results of antimicrobial screening of [2-(substituted phenyl)-imidazol-1-yl]-pyridin-3-yl-methanones (**1–11**) demonstrated that 2-[1-(Pyridine-3-carbonyl)-1H-imidazol-2-yl]-benzoic acid (**11**) was two times more effective than standard drug ciprofloxacin against the tested bacterial strains and [2-(2,5-Dimethyl-phenyl)-imidazol-1-yl]-pyridin-3-yl-methanone (**3**) was equipotent to the reference compound, fluconazole, against tested fungal strains. Further, the comparison of antimycobacterial results of imidazole and benzimidazole derivatives indicated that the benzimidazole derivatives are more effective in fighting against *M. tuberculosis* and can be selected as lead compounds for the development of NCEs.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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