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Synthesis and characterization of antibacterial 2-(pyridin-3-yl)-1*H*-benzo[*d*]imidazoles and 2-(pyridin-3-yl)-3*H*-imidazo[4,5-*b*]pyridine derivatives

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Abstract The increasing clinical importance of drug-resistant fungal and bacterial pathogens has stimulated microbiological research into and development of new antimicrobial agents. Hence, in this study, we synthesized novel series of 2-(pyridin-3-yl)-1*H*-benzo[*d*]imidazoles and 2-(pyridin-3-yl)-3*H*-imidazo[4,5-*b*]pyridine derivatives. The structures of the compounds were confirmed by spectral and CHN analysis. The compounds were examined for in-vitro antimicrobial activity against Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), and the fungus *Candida albicans*.

Keywords Benzimidazole · Imidazo[4,5-*b*]pyridine · 1,2,3-Triazole · Antimicrobial

Introduction

In the last few decades, microbes have developed strong resistance to antimicrobial drugs [1, 2]. Development of this resistance has recently accelerated substantially, leading to an increase in the number of infections. As a result there is a constant need to develop antimicrobial drugs [3]. One major objective of organic and

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medicinal chemistry is to design and synthesize new molecules with high therapeutic indices which can overcome resistant microorganisms. Despite significant progress in antimicrobial therapy there is still much demand for novel antimicrobial drugs [4]. Because infectious disease [3] is a major global health problem [5], the resistance acquired by microbes may be because of increasing use and misuse of antimicrobial drugs [6, 7].

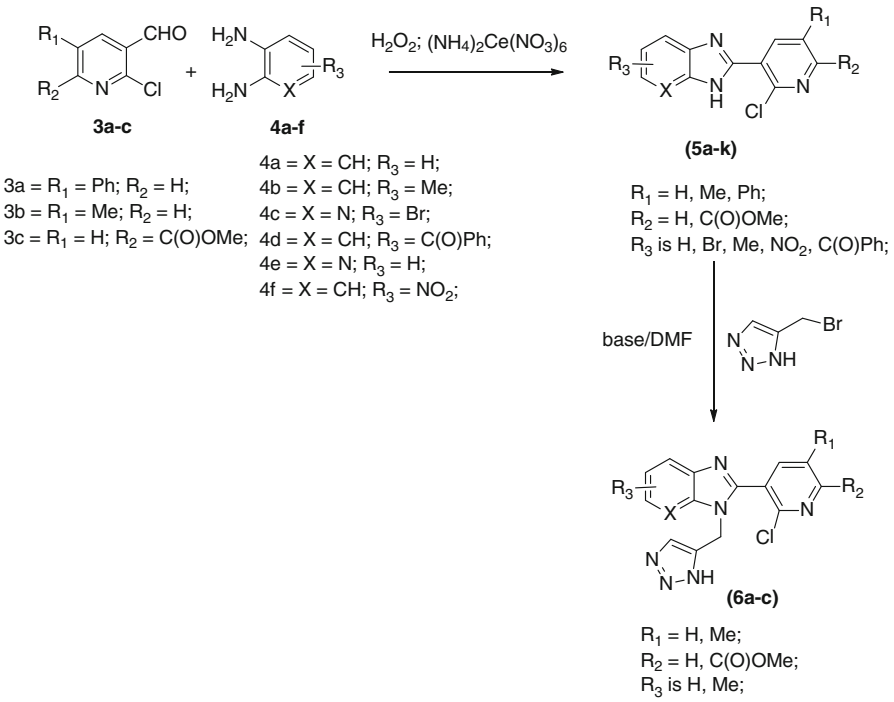
In this study we synthesized benzimidazole and imidazo[4,5-*b*]pyridine derivatives, because these structures are known to have a wide range of pharmacological activity [8–13]. Incorporation of an imidazole nucleus, a biologically well-established pharmacophore [13, 14], in 2-(pyridin-3-yl)-1*H*-benzo[*d*]imidazoles and 2-(pyridin-3-yl)-3*H*-imidazo[4,5-*b*]pyridine derivatives has resulted in versatile heterocyclic systems with a wide range of biological activity [13, 15–17].

Benzmidazole and imidazo[4,5-*b*]pyridine groups readily interact with the biopolymers of living organisms [18]. Because this type of structure is known to have a wide range of biological activity, for example antibacterial [19], antiviral [20], antimicrobial [21], antiulcer proton-pump inhibiting [22, 23], and anticancer [24] activity, and because, in the pharmaceutical sciences, new drugs are usually discovered on the basis of molecular modification of lead compounds or already established pharmacophores, we synthesized fourteen novel derivatives of 2-(pyridin-3-yl)-1*H*-benzo[*d*]imidazole and 2-(pyridin-3-yl)-3*H*-imidazo[4,5-*b*]pyridine.

Recent investigation of a variety of triazoles has revealed the importance of this pharmacophore to antibacterial activity [12, 13, 19]. Interestingly, the 1,2,3-triazole ring had been reported to mimic peptide bonds (amide bond surrogate) [25, 26]. 1,2,3-Triazoles can be regarded as antibacterial agents, because they can inhibit synthesis of the cell membrane, cell wall, and nucleic acids of bacteria [27]. We therefore synthesized 2-(pyridin-3-yl)-1*H*-benzo[*d*]imidazoles and 2-(pyridin-3-yl)-3*H*-imidazo[4,5-*b*]pyridine derivatives with or without methyl-1,2,3-triazole substitution (Scheme 1) and characterized the compounds by spectral and CHN analysis. The compounds were screened for antimicrobial activity, and minimum inhibitory concentration (MIC) was determined by use of the repeated twofold serial dilution technique (Table 4).

Result and discussion

A general multistep synthetic procedure used for preparation of 2-(pyridin-3-yl)-1*H*-benzo[*d*]imidazoles and 2-(pyridin-3-yl)-3*H*-imidazo[4,5-*b*]pyridine derivatives. A pyridine aldehyde (**3a–c**) was reacted with a benzene-1,2-diamine or pyridine-2, 3-diamine (**4a–f**) in the presence of CAN/H_2O_2 to give 2-(pyridin-3-yl)-1*H*-benzo[*d*]imidazoles or 2-(pyridin-3-yl)-3*H*-imidazo[4,5-*b*]pyridine derivatives, respectively (**5a–5k**), in good yield and purity. The pyridine aldehyde intermediates (**3a–c**) were prepared directly or via enamide formation starting from pent-3-en-2-one oxime, 4-phenyl-3-butene-2-one-oxime, or methyl 2-acetamidoacrylate (Table 1) [28–31].



Scheme 1 Synthesis of 2-(pyridine-3-yl)-1*H*-benzo[*d*]imidazoles and 2-(pyridine-3-yl)-3*H*-imidazo[4,5-*b*]pyridine derivatives

We then synthesized 1-((3*H*-1,2,3-triazol-4-yl)methyl)-2-(pyridin-3-yl)-1*H*-benzo[*d*]imidazole and 3-((3*H*-1,2,3-triazol-4-yl)methyl)-2-(pyridin-3-yl)-3*H*-imidazo[4,5-*b*]pyridine derivatives (**6a–c**) by C–N coupling of the *N*-alkylated methyltriazole compounds 2-(pyridin-3-yl)-1*H*-benzo[*d*]imidazoles and 2-(pyridin-3-yl)-3*H*-imidazo[4,5-*b*]pyridine derivatives (**5a–k**) in the presence of a base in DMF [32]. Details of the synthesis of all these new compounds are given in Scheme 1 and Table 2. All compounds were characterized by use of spectral techniques, for example ^{1}H NMR, ^{13}C NMR, and mass spectroscopy, and CHN analysis.

Biological evaluation

Because heterocyclic systems, especially benzimidazole and imidazo[4,5-*b*]pyridine derivatives, are known to be pharmacologically important [19–21], the novel 2-(pyridin-3-yl)-1*H*-benzo[*d*]imidazole and 2-(pyridin-3-yl)-3*H*-imidazo[4,5-*b*]pyridine compounds (**5a–k**) and their methyltriazole derivatives (**6a–c**) were screened for antibacterial and antifungal activity, in comparison with streptomycin and gentamicin, respectively, as reference standards. The results are summarized in Tables 3 and 4.

Table 1 Synthesis of 2-(pyridine-3-yl)11*H*-benzo[*d*]imidazoles and 2-(pyridine-3-yl)-3*H*-imidazo[4,5-*b*]pyridine derivatives (**5a–k**)

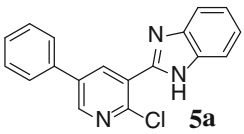
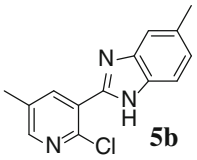
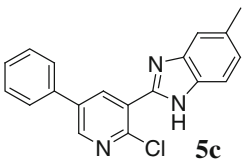
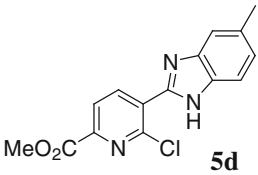
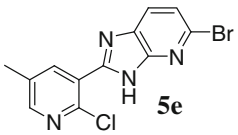
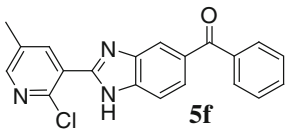
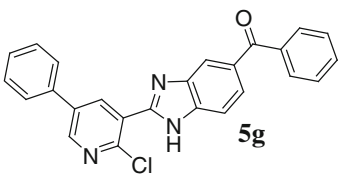
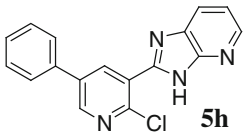
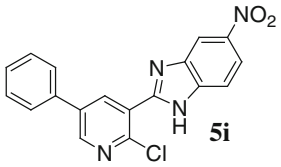
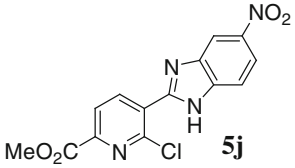
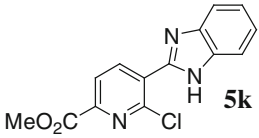
Entry	Aldehyde, **3**	Diamine, **4**	Product, **5**	Time (h)	Yield (%)
1	**3a**	**4a**	 **5a**	2	90
2	**3b**	**4b**	 **5b**	2.5	88
3	**3a**	**4b**	 **5c**	2.2	86
4	**3c**	**4b**	 **5d**	3.0	89
5	**3b**	**4c**	 **5e**	2.5	91
6	**3b**	**4d**	 **5f**	2.0	85
7	**3a**	**4d**	 **5g**	2.5	87

Table 1 continued

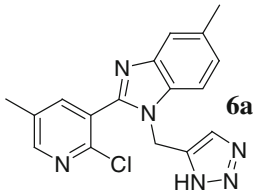
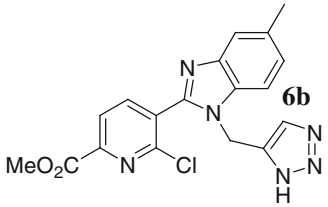
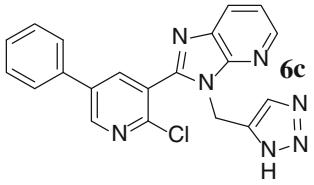
Entry	Aldehyde, **3**	Diamine, **4**	Product, **5**	Time (h)	Yield (%)
8	**3a**	**4e**	 **5h**	3.5	85
9	**3a**	**4f**	 **5i**	3.0	89
10	**3c**	**4f**	 **5j**	3.2	88
11	**3c**	**4a**	 **5k**	2.5	86

Reaction conditions: reactant **3** (1.0 mmol); reactant **4** (1.0 mmol); reagents: $(NH_4)_2Ce(NO_3)_6$ (CAN) (0.1 mmol) and H_2O_2 (30 % 4 mmol) heated at 50 °C for 2–4 h

Antibacterial activity

Perusal of the results from antibacterial screening revealed that, compared with streptomycin, most of the compounds were effective against the Gram-positive bacteria *Staphylococcus aureus* (NCIM 5022) and *Bacillus subtilis* (NCIM 2545) and the Gram-negative bacteria *Escherichia coli* (NCIM 2036) and *Pseudomonas aeruginosa* (NCIM 2036). In particular, compounds **5b** and **5c** had promising activity, compounds **5d**, **5e**, **5g–k**, and **6a–c** had moderate activity, and compounds **5a** and **5f** had activity at high concentrations only. The MIC of all the compounds were also determined, by use of the broth-dilution method; the results are given in Table 4. Except for compounds **5a** and **5f** the MIC values were within the range 100–500 $\mu g/ml^{-1}$ against all the strains evaluated.

Table 2 Synthesis of 1-((3*H*-1,2,3-triazol-4-yl)methyl)-1*H*-benzo[*d*]imidazole and 3-((3*H*-1,2,3-triazol-4-yl)methyl)-3*H*-imidazo[4,5-*b*]pyridine derivatives (**6a–c**)

Entry	NH compound, **5**	Product, **6**	Time (h)	Yield (%)
1	**5b**	 **6a**	10	88
2	**5d**	 **6b**	12	85
3	**5h**	 **6c**	11	80

Reaction conditions: NH compound **5** (1.0 mmol), 5-(bromomethyl)-1*H*-1,2,3-triazole (1.1 mmol), NaH (60 %, 2.0 mmol) in DMF stirred overnight

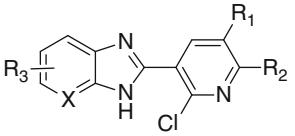
Antifungal activity

The antifungal activity of the compounds (125 μg/ml) against *Candida albicans* (NCIM 3102), compared with gentamicin as standard, were evaluated by use of the cup plate method. The results obtained (Tables 3 and 4) revealed that compounds **5b** and **5g** had good antifungal activity whereas the activity of the other compounds was similar to or less than that of gentamicin.

Experimental

All reagents were purchased from Sigma–Aldrich, Lancaster, and Qualigens and were used without further purification. ^{1}H NMR spectra were recorded on a Bruker Spectrospin Avance DPX400 Ultrashield (400 and 300-MHz) spectrometer, at room temperature; coupling constants, *J*, are in Hz. ^{13}C NMR spectra were recorded on Bruker Spectrospin Avance DPX400 Ultrashield (100-MHz) spectrometer. Samples (2–3 mg) were dissolved in 0.8 ml deuterated $CDCl_3$. Mass spectra were obtained by use of LC–MS with ESI for ionization. All reactions were monitored by thin layer chromatography (TLC) on silica gel with chloroform–acetone as mobile phase. The newly synthesized products were also separated and purified by column chromatography.

General procedure for synthesis of 2-(pyridin-3-yl)-1*H*-benzo[*d*]imidazoles and 2-(pyridin-3-yl)-3*H*-imidazo[4,5-*b*]pyridine derivatives



R_1 = H, Me, Ph;
R_2 = H, C(O)OMe;
R_3 is H, Br, Me, NO_2, C(O)Ph;

(5a-k)

A mixture of benzene-1,2-diamine or pyridine-2,3-diamine (**4a–f**, 1 mmol), pyridine aldehyde (**3a–c**, 1 mmol), H_2O_2 (30 %, 4 mmol), and $(NH_4)_2Ce(NO_3)_6$ (CAN) (0.1 mmol) was heated to 50 °C and maintained at this temperature for 2–4 h. Completion of the reactions was monitored by TLC (mobile phase chloroform–acetone 3:7; the starting materials and products are highly polar). On completion of the reaction, the mixture was dissolved in ethanol (10 ml) then poured into ice-cold water (30 ml). The solid product which precipitated was isolated by filtration, washed with ice-cold water, then dried under vacuum. The solvent was removed under reduced pressure to furnish off-white to yellow solids (**5a–k**, yield 82–91 %). The compounds were characterized by use of spectral techniques, for example ^{1}H NMR, ^{13}C NMR, and mass spectroscopy, and by CHN analysis.

Table 3 In-vitro antimicrobial activity as zones of inhibition

Entry	Antibacterial				Antifungal
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*	*C. albicans*
5a	6	6	7	8	00
5b	17	15	18	16	16
5c	14	18	13	17	7
5d	12	12	16	19	00
5e	10	08	9	11	06
5f	00	00	00	00	00
5g	13	16	10	11	19
5h	12	11	18	16	15
5i	14	16	11	12	07
5j	12	12	14	10	08
5k	13	16	12	11	ND
6a	16	13	14	12	10
6b	13	14	14	12	14
6c	12	10	14	13	15
Std. 1	18	17	20	15	–
Std. 2	–	–	–	–	16

Values are zones of inhibition (mm) obtained by use of 250 μg/ml of the text compound

Std. 1 streptomycin, *Std. 2* gentamicin

Table 4 In vitro antimicrobial activity as minimum inhibitory concentration ($\mu g/ml^{-1}$)

Entry	Antibacterial				Antifungal
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*	*C. albicans*
5a	>1,000	>1,000	>1,000	>1,000	500
5b	125	62.5	62.5	125	125
5c	62.5	62.5	62.5	125	500
5d	250	250	125	125	>1,000
5e	500	500	500	500	>1,000
5f	>1,000	>1,000	>1,000	>1,000	>1,000
5g	250	125	250	250	500
5h	250	250	125	125	500
5i	250	250	250	250	500
5j	250	250	250	500	Not calculated
5k	250	125	250	250	ND
6a	125	250	250	250	Not calculated
6b	250	250	250	250	500
6c	250	500	250	250	500
Std. 1	<31.25	<31.25	<31.25	<31.25	–
Std. 2	–	–	–	–	62.5

Std. 1 streptomycin, *Std. 2* gentamicin

*2-(2-Chloro-5-phenylpyridin-3-yl)-1H-benzo[d]imidazole (**5a**)*

Yellow solid, ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 8.69–8.62 (2H, m), 8.41–8.36 (1H, m), 8.06–8.04 (1H, d, $J = 7.6$ Hz), 7.62–7.60 (2H, d, $J = 8.0$ Hz), 7.53–7.51 (1H, d, $J = 8.0$ Hz), 7.47–7.37 (4H, m), 7.23–7.21 (1H, m). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 166.62, 149.41, 148.58, 146.88, 144.98, 139.10, 138.75, 136.21, 135.50, 129.26, 128.88, 127.11, 127.00, 125.87, 118.79. ESI–MS: *m/z* 307.0, $C_{18}H_{12}ClN_3$ requires Mol. Wt.: 305.76. Elemental analysis, calculated: C, 70.71; H, 3.96; Cl, 11.60; N, 13.74 %. Found: C, 70.75; H, 3.98; N, 13.72 %.

*2-(2-Chloro-5-methylpyridin-3-yl)-5-methyl-1H-benzo[d]imidazole (**5b**)*

Off white solid, ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 8.28 (1H, s), 8.11 (1H, s), 7.51 (1H, bs), 7.07–7.05 (2H, d, $J = 6.9$ Hz), 2.63 (3H, s), 2.43 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 169.31, 166.08, 164.81, 160.74, 152.28, 145.26, 142.72, 142.49, 131.61, 115.20, 35.84, 36.45. ESI–MS; *m/z*; 258.00, $C_{14}H_{12}ClN_3$ requires Mol. Wt.: 257.72. Elemental analysis, calculated: C, 65.25; H, 4.69; Cl, 13.76; N, 16.30 %. Found: C, 65.70; H, 4.81; N, 16.74 %.

*2-(2-Chloro-5-phenylpyridin-3-yl)-5-methyl-1H-benzo[d]imidazole (**5c**)*

Yellow solid, ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 8.68–8.67 (2H, d, $J = 2.1$ Hz), 7.70–7.68 (2H, d, $J = 7.2$ Hz), 7.52–7.41 (4H, m), 7.21–7.16 (1H, d, $J = 7.5$ Hz),

7.08–7.06 (1H, d, $J = 7.5$ Hz), 2.68 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$, ppm): δ 167.00, 165.92, 158.35, 155.39, 154.50, 148.40, 148.01, 146.14, 145.56, 142.80, 142.66, 35.92. ESI–MS: *m/z* 320.00, $C_{19}H_{14}ClN_3$ requires Mol. Wt.: 319.79. Elemental analysis, calculated: C, 71.36; H, 4.41; Cl, 11.09; N, 13.14 %. Found: C, 71.77; H, 4.61; N, 13.54 %.

*Methyl 6-chloro-5-(5-methyl-1H-benzo[d]imidazol-2-yl)picolinate (**5d**)*

Light yellow solid, ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 9.05–9.03 (1H, d, $J = 8.0$ Hz), 8.22–8.20 (1H, d, $J = 8.0$ Hz), 7.54–7.53 (1H, d, $J = 7.6$ Hz), 7.29–7.26 (1H, t, $J = 7.2$ Hz), 7.17–7.15 (1H, d, $J = 7.2$ Hz), 4.05 (3H, s), 2.69 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 163.97, 147.73, 146.55, 144.86, 141.82, 128.76, 124.32, 53.38, 29.71. ESI–MS: *m/z* 302.00, $C_{15}H_{12}ClN_3O_2$ requires Mol. Wt.: 301.73. Elemental analysis, calculated: C, 59.71; H, 4.01; Cl, 11.75; N, 13.93; O, 10.61 %. Found: C, 59.64; H, 4.86; N, 13.48 %.

*5-Bromo-2-(2-chloro-5-methylpyridin-3-yl)-3H-imidazo[4,5-b]pyridine (**5e**)*

Yellow solid, ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 8.87 (1H, s), 8.30 (1H, s), 7.95 (1H, bs), 7.44–7.47–7.44 (1H, m), 2.44 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 147.53, 146.55, 144.86, 141.76, 128.08, 124.36, 29.76. Mass: ESI–MS: *m/z* 325.00 $(M+H)^+$, $C_{12}H_8BrClN_4$ requires Mol. Wt.: 323.58. Elemental analysis, calculated: C, 44.54; H, 2.49; Br, 24.69; Cl, 10.96; N, 17.31. Found: C, 44.46; H, 3.69; N, 17.29.

*(2-(2-Chloro-5-methylpyridin-3-yl)-1H-benzo[d]imidazol-5-yl)(phenyl)methanone (**5f**)*

Yellow solid, ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 11.38 (1H, bs, NH), 8.48 (1H, bs), 8.25 (1H, bs), 8.15 (1H, bs), 7.84–7.78 (3H, m), 7.73–7.68 (1H, bs), 7.58–7.54 (1H, t, $J = 7.2$ Hz), 7.47–7.43 (2H, t, $J = 7.6$ Hz), 2.35 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 196.88, 150.99, 144.76, 141.26, 138.01, 133.38, 132.27, 130.96, 130.02, 128.27, 125.70, 124.48, 17.53. ESI–MS: *m/z* 348.0, $C_{20}H_{14}ClN_3O$ requires Mol. Wt.: 347.80. Elemental analysis, calculated: C, 69.07; H, 4.06; Cl, 10.19; N, 12.08; O, 4.60 %. Found: C, 69.02; H, 4.16; N, 10.19 %.

*(2-(2-Chloro-5-phenylpyridin-3-yl)-1H-benzo[d]imidazol-5-yl)(phenyl)methanone (**5g**)*

Light yellow solid, ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 9.12 (1H, s), 8.72 (1H, s), 8.23 (1H, s), 7.94–7.92 (1H, d, $J = 8.0$ Hz), 7.86–7.84 (3H, d, $J = 8.4$ Hz), 7.71–7.69 (2H, d, $J = 6.8$ Hz), 7.66–7.60 (1H, m), 7.55–7.44 (5H, m). ^{13}C NMR

(100 MHz, $CDCl_3$, ppm): δ 196.35, 145.76, 139.17, 137.91, 136.73, 135.11, 133.67, 132.38, 130.06, 129.34, 129.11, 128.36, 127.23, 126.31. ESI–MS: *m/z* 410.00, $C_{25}H_{16}ClN_3O$ requires Mol. Wt.: 409.87. Elemental analysis, calculated: C, 73.26; H, 3.93; Cl, 8.65; N, 10.25; O, 3.90 %. Found: C, 73.11; H, 4.10; N, 10.33 %.

*2-(2-Chloro-5-phenylpyridin-3-yl)-3H-imidazo[4,5-b]pyridine (**5h**)*

Yellow solid, ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 8.69–8.62 (2H, m), 8.41–8.36 (1H, m), 8.06–8.04 (1H, d, $J = 7.6$ Hz), 7.62–7.60 (2H, d, $J = 8.0$ Hz), 7.53–7.51 (1H, d, $J = 8.0$ Hz), 7.47–7.37 (3H, m). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 149.41, 148.58, 146.88, 144.98, 139.10, 138.75, 136.21, 135.50, 129.26, 128.88, 127.11, 127.00, 125.87, 118.79. LC–MS: *m/z* 307.0, $C_{17}H_{11}ClN_4$ requires Mol. Wt.: 306.75. Elemental analysis, calculated: C, 66.56; H, 3.61; Cl, 11.56; N, 18.26 %. Found: C, 66.33; H, 3.97; N, 18.52 %.

*2-(2-Chloro-5-phenylpyridin-3-yl)-5-nitro-1H-benzo[d]imidazole (**5i**)*

Yellow solid, ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 8.75–8.56 (3H, m), 8.25 (1H, bs), 7.72–7.69 (2H, d, $J = 7.2$ Hz), 7.55–7.47 (4H, m). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 148.41, 139.05, 136.20, 129.06, 128.76, 126.71, 118.98, 115.57, 111.76, 95.81. ESI–MS: *m/z* 351.00, $C_{18}H_{11}ClN_4O_2$ requires Mol. Wt.: 350.76. Elemental analysis, calculated: C, 61.64; H, 3.16; Cl, 10.11; N, 15.97;O, 9.12 %. Found: C, 61.49; H, 3.48; N, 15.77 %.

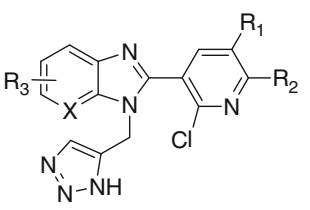
*Methyl 6-chloro-5-(5-nitro-1H-benzo[d]imidazol-2-yl)picolinate (**5j**)*

Yellow solid, ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 9.05–9.03 (1H, d, $J = 8.0$ Hz), 8.22–8.20 (1H, d, $J = 8.0$ Hz), 7.54–7.53 (1H, d, $J = 7.6$ Hz), 7.29–7.26 (1H, t, $J = 7.2$ Hz), 7.17–7.15 (1H, d, $J = 7.2$ Hz), 4.05 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 163.97, 147.73, 146.55, 144.86, 141.82, 128.76, 124.32, 114.22, 53.38. LC–MS: *m/z* 333.50, $C_{14}H_9ClN_4O_4$ requires Mol. Wt.: 332.70. Elemental analysis, calculated: C, 50.54; H, 2.73; Cl, 10.66; N, 16.84; O, 19.24. Found: C, 50.38; H, 3.18; N, 16.72 %.

*Methyl 5-(1H-benzo[d]imidazol-2-yl)-6-chloropicolinate (**5k**)*

Yellow solid, ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 8.90–8.88 (1H, d, $J = 8.0$ Hz), 8.18–8.16 (1H, d, $J = 8.0$ Hz), 7.72–7.69 (2H, m), 7.36–7.34 (2H, m), 4.02 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 167.89, 163.91, 147.83, 147.01, 145.58, 141.80, 138.40, 132.38, 130.94, 128.79, 124.24, 115.83, 53.37. LC–MS: *m/z* 287.90, $C_{14}H_{10}ClN_3O_2$ requires Mol. Wt.: 287.70. Elemental analysis, calculated: C, 58.45; H, 3.50; Cl, 12.32; N, 14.61; O, 11.12 %. Found: C, 58.30; H, 3.81; N, 14.58 %.

General procedure for alkylation of 2-(pyridin-3-yl)-1*H*-benzo[*d*]imidazoles and 2-(pyridin-3-yl)-3*H*-imidazo[4,5-*b*]pyridine derivatives



(6a-c)

R_1 = H, Me;
R_2 = H, C(O)OMe;
R_3 is H, Me;

A suspension of 60 % sodium hydride (2.0 equiv.) and dry *N*,*N*-dimethylformamide (eightfold volume excess) was stirred for 5–10 min under a nitrogen atmosphere. A solution of compound **5a–k** (1 mmol) in dry *N*,*N*-dimethylformamide (fivefold volume excess) was slowly added, and stirring was continued for an additional 30–60 min. A solution of 5-(bromomethyl)-1*H*-1,2,3-triazole (1.1 equiv.) in *N*,*N*-dimethylformamide (fivefold volume excess) was then added dropwise, and the mixture was left overnight at room temperature. Completion of the reactions (10–14 h) was monitored by TLC (mobile phase chloroform–acetone 3:7; the starting materials and products are highly polar). On completion, the reaction mixture was quenched with saturated ammonium chloride solution, concentrated under reduced pressure to remove volatile materials, then extracted with ethyl acetate. The organic extract was filtered through Celite and washed with water then saturated brine solution. The organic layer was dried over anhydrous Na_2SO_4, then the solvent was removed by evaporation under reduced pressure to give **6a–c** as yellow solids (yield: 80–90 %). The compounds were characterized by use of spectral techniques, for example ^{1}H NMR, ^{13}C NMR, and mass spectroscopy, and by CHN analysis.

*1-((3H-1,2,3-Triazol-4-yl)methyl)-2-(2-chloro-5-methylpyridin-3-yl)-5-methyl-1H-benzo[d] imidazole (**6a**)*

Yellow solid, ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 11.84 (1H, bs), 9.11 (1H, s), 8.84–8.72 (1H, m), 8.33 (1H, bs), 7.82–7.81 (1H, bs), 7.72–7.71 (2H, d, $J = 6.0$ Hz), 4.11 (2H, s), 2.42 (3H, s), 2.32 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 157.37, 149.83, 149.22, 147.73, 146.55, 144.86, 141.82, 134.21, 133.27, 131.55, 128.61, 124.42, 122.82, 116.75, 49.28, 22.70, 20.05. ESI–MS: *m/z* 339.90, $C_{17}H_{15}ClN_6$ requires Mol. Wt.: 338.79. Elemental analysis, calculated: C, 60.27; H, 4.46; Cl, 10.46; N, 24.81 %. Found: C, 60.54; H, 4.96; N, 24.90 %.

*Methyl 5-(1-((3H-1,2,3-triazol-4-yl)methyl)-5-methyl-1H-benzo[d]imidazol-2-yl)-6-chloro pyridine-2-carboxylate (**6b**)*

Yellow solid, ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 11.84 (1H, bs), 9.11 (1H, s), 8.84–8.72 (1H, m), 8.33–8.31 (1H, bs), 7.82–7.81 (1H, bs), 7.72–7.71 (2H, d,

$J = 6.0$ Hz), 4.12 (2H, s), 3.94 (3H, s), 2.42 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 163.97, 157.27, 149.23, 147.73, 146.55, 144.86, 141.82, 134.17, 133.27, 131.55, 128.76, 124.32, 122.82, 116.85, 53.38, 22.70. ESI–MS: *m/z* 383.00, $C_{18}H_{15}ClN_6O_2$requires Mol. Wt.: 382.80. Elemental analysis, calculated: C, 56.48; H, 3.95; Cl, 9.26; N, 21.95; O, 8.36 %. Found: C, 56.82; H, 4.46; N, 21.98 %.

*3-((3H-1,2,3-Triazol-4-yl)methyl)-2-(2-chloro-5-phenylpyridin-3-yl)-3H-imidazo[4,5-b]pyridine (**6c**)*

Yellow solid, ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 11.71 (1H, bs), 8.69–8.62 (2H, m), 8.41–8.36 (1H, m), 8.06–8.04 (1H, d, $J = 7.6$ Hz), 7.47–7.32 (5H, m), 7.72–7.71 (2H, d, $J = 6.0$ Hz), 3.72 (2H, s). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 151.62, 149.41, 148.84, 148.58, 146.88, 144.98, 139.10, 138.75, 136.21, 135.50, 135.42, 129.26, 128.88, 128.77, 127.46, 127.11, 127.00, 125.88, 118.79, 50.95. ESI–MS: *m/z* 388.00, $C_{20}H_{14}ClN_7$ requires Mol. Wt.:387.83. Elemental analysis, calculated: C, 61.94; H, 3.64; Cl, 9.14; N, 25.28 %. Found: C, 62.68; H, 4.18; N, 25.18 %.

Minimum inhibitory concentration (MIC)

Sterile nutrient agar (Sabourd dextrose agar) plates were prepared by pouring the sterile agar into sterile Petri dishes under aseptic conditions and the test organism (0.1 ml) was spread on the plates. Holes (5 mm diameter) were made in the agar plates by use of a sterile bore. Test compound, standard drug, and the DMSO (as control) were placed in separate holes. The plates were maintained at +4 °C for 1 h to enable diffusion of the solutions into the agar medium. Plates containing bacteria were incubated at 37 °C for 24 h; those containing fungi were incubated at 28 °C for 48 h.

Determination of MIC in liquid medium

A series of test tubes were prepared containing the same volume of medium inoculated with the test organism (the inoculum may vary from 103 to 106 cells per milliliter). Decreasing concentrations of the test compounds were added to the tubes; stepwise dilution by a factor of 2 (twofold serial dilution) was usually used (500 μg/ml, 250 μg/ml, 125 μg/ml, etc.) [33, 34]. One tube was left without test compound, to serve as a positive control for growth of the organism. The cultures were incubated. The tubes were inspected visually to monitor growth of the organism (indicated by turbidity);tubes containing the antimicrobial agent at a concentration sufficient to inhibit growth remained clear. Experimentally, the MIC is the concentration of the test compound present in the last clear tube, i.e. the tube containing the lowest concentration of test compound in which growth is not observed.

The synthesized compounds were checked, in vitro, for inhibitory activity against five microorganisms—the bacteria *S. aureus*, *B. subtilis*, *E. coli*, and *P. aeruginosa*

and the fungus *C. albicans*. Streptomycin (125 μg/ml) and gentamicin (125 μg/ml) were used as controls.

Conclusion

In this study we synthesized 2-(pyridin-3-yl)-1*H*-benzo[*d*]imidazoles and 2-(pyridin-3-yl)-3*H*-imidazo[4,5-*b*]pyridine derivatives by use of a CAN/H_2O_2 catalytic system and characterized the compounds by spectral and CHN analysis. We also synthesized *N*-alkylated 1,2,3-triazolemethyl derivatives. All the compounds were screened for antibacterial and antifungal activity, and found to have moderate to good activity.

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