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Original article

Synthesis, physicochemical properties and antimicrobial activity of some new benzimidazole derivatives

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ABSTRACT

Some derivatives of benzimidazole were synthesized by nucleophilic substitution of 2-substituted-1*H*-benzimidazole. The resulting ethyl (2-substituted-1*H*-benzimidazol-1-yl) acetate on treatment with hydrazine hydrate yielded 2-(2-substituted-1*H*-benzimidazol-1-yl) acetohydrazide, which on further reaction with one equivalent of different aliphatic or aromatic carboxylic acids in the presence of phosphoryl chloride afforded the corresponding target compounds, 2-substituted-1-[{(5-substituted alkyl/aryl)-1,3,4-oxadiazol-2-yl} methyl]-1*H*-benzimidazole. The structures of the synthesized compounds were evaluated by spectral and elemental methods of analyses. All the synthesized compounds were screened for their antimicrobial activities. All of the derivatives showed good activity towards Gram-positive bacteria and negligible activity towards Gram-negative bacteria. Some of the synthesized compounds showed moderate activity against tested fungi.

1. Introduction

Benzimidazoles are remarkably effective compounds both with respect to their inhibitory activity and their favorable selectivity ratio. Extensive biochemical and pharmacological studies have confirmed that benzimidazole molecules are effective against various strains of microorganisms [1–8]. Benzimidazoles are regarded as a promising class of bioactive heterocyclic compounds that exhibit a range of biological activities. Specifically, this nucleus is a constituent of vitamin-B_{12} [9]. This ring system is present in numerous antioxidant [10–12], antiparasitic [13,14], antihelmintics [15], antiproliferative [16], anti-HIV [17], anticonvulsant [18], anti-inflammatory [19–22], antihypertensive [23,24], antineoplastic [25,26], and antitrichinellosis [27] activities. Owing to the immense importance and varied bioactivities exhibited by benzimidazoles, efforts have been made from time to time to generate libraries of these compounds and screened them for potential biological activities. Also it is well documented that oxadiazole nucleus is associated with a variety of pharmacological actions [28,29]. It displays pronounced anticonvulsant [30], antifungal [31] and antimycobacterial [32] activities. Looking at the importance of benzimidazole and oxadiazole nucleus, it was thought that it would be worthwhile to design and synthesize some new benzimidazole derivatives bearing oxadiazole moiety and screen them for potential biological activities. We have previously reported the synthesis of some new biologically active benzimidazoles [33]. In continuation of our work on biologically active benzimidazoles, we have synthesized some 1-{[5-(alkyl/aryl)-1,3,4-oxadiazol-2-yl]methyl}-2-alkyl-1*H*-benzimidazoles for their antimicrobial activities.

2. Results and discussion

2.1. Chemistry

The reaction sequence for different title compounds is outlined in Scheme 1. The starting material 2-substituted-1*H*-benzimidazole **2** was prepared according to a reported procedure through the reaction of *o*-phenylenediamine with appropriate carboxylic acid [34]. Structure of compound **2** was confirmed by comparison of its physical and spectral data with the reported ones [35]. Nucleophilic substitution of compound **2** yielded ethyl (2-substituted-1*H*-benzimidazol-1-yl) acetate **3**. The structure of compound **3** was confirmed by its IR, ^{1}H NMR as well as elemental analysis. The IR spectra of compound **3a** showed broad band at 1745 cm^{-1} (–C=O ester) and at 1632 cm^{-1} (–C=N). ^{1}H NMR spectra revealed the multiplet at δ 7.72–7.90 corresponding to the four aromatic protons. Mass spectrum of compound **3b** revealed the molecular ion peak M^+ at *m*/*z* 218 (R = Me) (100%), corresponding to molecular mass of this compound. Compound **3** on treatment with hydrazine hydrate resulted in the

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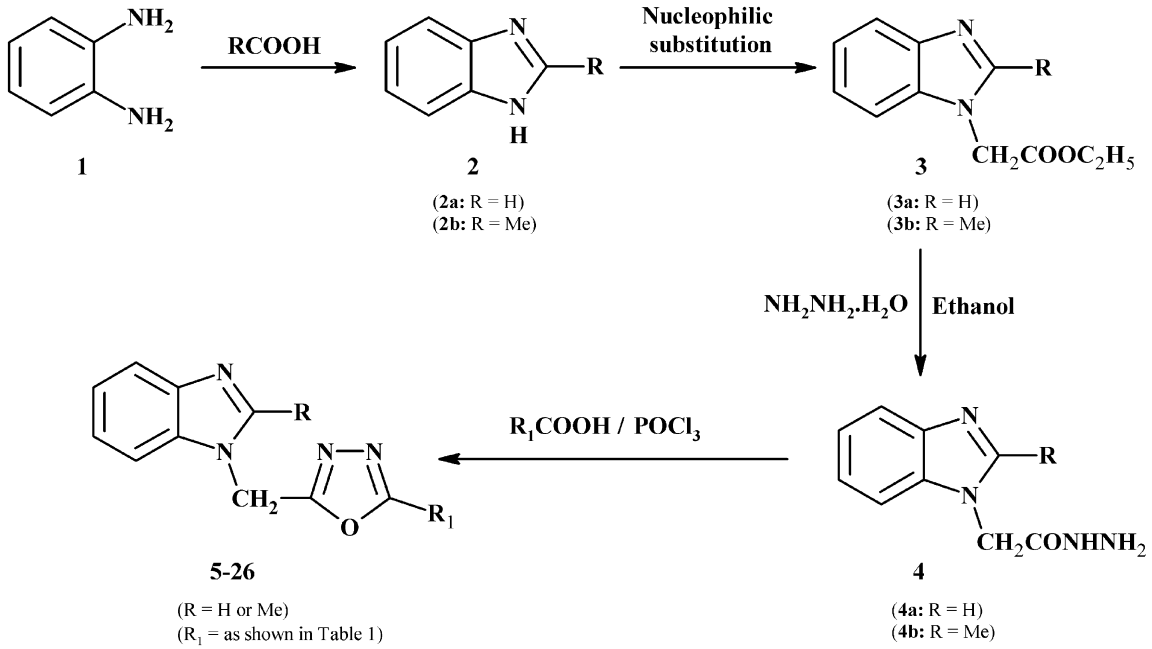

Scheme 1. Synthesis of benzimidazole derivatives.

formation of 2-(2-substituted-1*H*-benzimidazol-1-yl)acetohydrazide **4**. IR spectrum of compound **4a** showed a broad band at 1682 cm^{-1} (–C═O amide) and C═N stretching at 1640 cm^{-1}. Compound **4** on treatment with different aliphatic or aromatic carboxylic acids in the presence of phosphoryl chloride yielded the 2-methyl-1-[(5-substituted-1,3,4-oxadiazol-2-yl)methyl]-1*H*-benzimidazole **5**–**26**. All the spectral data of compounds **2a**, **2b**, **3a**, **3b**, **4a**, **4b** and **5**–**26** were in accordance with assumed structures. The purity of the synthesized compounds was monitored by TLC. Physical and analytical data of compounds **5**–**26** are shown in Table 1.

2.2. Antimicrobial evaluation

The in vitro antibacterial activity was performed against Gram-positive bacteria including *Staphylococcus aureus* (ATCC 29213), *Bacillus subtilis* (MTCC 121) and *Streptococcus mutans* (MTCC 890) and Gram-negative bacteria including *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (MTCC 741) and *Salmonella typhi* (MTCC 733). Yeast including *Candida albicans* (MTCC 1637), and fungi *Aspergillus flavus* (AIIMS) and *Aspergillus niger* (AIIMS) were used to test the antifungal activity. To evaluate the activity of synthesized compounds against bacteria minimum inhibitory concentrations (MICs) were determined and for yeast and fungi zone of inhibition was determined. Known antibiotics like Ciprofloxacin and Ampicillin (the reference antibacterial drugs) and Amphotericin B (the reference antifungal drug) were used for comparison.

By comparing the antimicrobial activity of the synthesized compounds, it was found that the tested compounds are more effective against the Gram-positive bacteria. It is believed that the strong lipophilic character of the molecule plays an essential role in

Table 1
Physical and analytical data of compounds **5**–**26**.

Compounds	R	R_1	Yield (%)	M.p. (°C)	Mol. formula	Analysis % calculated (Found)		
						C	H	N
5	H	$-CH_3$	79	181–83	$C_{11}H_{10}N_4O$	61.67 (61.65)	4.71 (4.69)	26.15 (26.12)
6	H	$-C_2H_5$	81	187–89	$C_{12}H_{12}N_4O$	63.15 (63.12)	5.30 (5.28)	24.55 (24.52)
7	H	$-CH_2Cl$	76	176–78	$C_{11}H_9N_4OCl$	53.13 (53.11)	3.65 (3.64)	22.53 (22.51)
8	H	$-CH_2CH_2Cl$	87	172–74	$C_{12}H_{11}N_4OCl$	54.87 (54.84)	4.22 (4.20)	21.33 (21.30)
9	H	$-C_6H_5$	80	176–78	$C_{16}H_{12}N_4O$	69.55 (69.52)	4.38 (4.35)	20.28 (20.26)
10	H	2-ClC_6H_4	83	197–99	$C_{16}H_{11}N_4OCl$	61.84 (61.81)	3.57 (3.56)	18.03 (18.02)
11	H	4-ClC_6H_4	80	165–67	$C_{16}H_{11}N_4OCl$	61.84 (61.81)	3.57 (3.54)	18.03 (18.00)
12	H	2-OHC_6H_4	84	166–68	$C_{16}H_{12}N_4O_2$	65.75 (65.72)	4.14 (4.12)	19.17 (19.15)
13	H	4-OHC_6H_4	88	158–60	$C_{16}H_{12}N_4O_2$	65.75 (65.73)	4.14 (4.11)	19.17 (19.16)
14	H	2-$OCH_3C_6H_4$	72	153–55	$C_{17}H_{14}N_4O_2$	66.66 (66.63)	4.61 (4.59)	18.29 (18.26)
15	H	4-$OCH_3C_6H_4$	71	167–69	$C_{17}H_{14}N_4O_2$	66.66 (66.64)	4.61 (4.59)	18.29 (18.26)
16	$-CH_3$	$-CH_3$	84	178–80	$C_{12}H_{12}N_4O$	63.15 (63.12)	5.30 (5.28)	24.55 (24.53)
17	$-CH_3$	$-C_2H_5$	68	167–69	$C_{13}H_{14}N_4O$	64.45 (64.42)	5.82 (5.81)	23.13 (23.11)
18	$-CH_3$	$-CH_2Cl$	75	194–99	$C_{12}H_{11}N_4OCl$	54.87 (54.84)	4.22 (4.20)	21.33 (21.30)
19	$-CH_3$	$-CH_2CH_2Cl$	69	172–74	$C_{13}H_{13}N_4OCl$	56.42 (56.40)	4.74 (4.72)	20.25 (20.22)
20	$-CH_3$	$-C_6H_5$	77	191–93	$C_{17}H_{14}N_4O$	70.33 (70.30)	4.86 (4.83)	19.30 (19.27)
21	$-CH_3$	2-ClC_6H_4	81	182–84	$C_{17}H_{13}N_4OCl$	62.87 (62.84)	4.03 (4.00)	17.25 (17.23)
22	$-CH_3$	4-ClC_6H_4	75	174–76	$C_{17}H_{13}N_4OCl$	62.87 (62.85)	4.03 (4.01)	17.25 (17.22)
23	$-CH_3$	2-OHC_6H_4	76	156–58	$C_{17}H_{14}N_4O_2$	66.66 (66.63)	4.61 (4.60)	18.29 (18.26)
24	$-CH_3$	4-OHC_6H_4	65	150–52	$C_{17}H_{14}N_4O_2$	66.66 (66.63)	4.61 (4.59)	18.29 (18.27)
25	$-CH_3$	2-$OCH_3C_6H_4$	79	178–80	$C_{18}H_{16}N_4O_2$	67.49 (67.46)	5.03 (5.02)	17.49 (17.48)
26	$-CH_3$	4-$OCH_3C_6H_4$	82	167–69	$C_{18}H_{16}N_4O_2$	67.49 (67.47)	5.03 (5.01)	17.49 (17.47)

producing antimicrobial effect. These properties are seen as an important parameter related to membrane permeation in biological system. Many of the processes of drug disposition depend on the capability to cross membranes and hence there is a high correlation with measures of lipophilicity. Hydrophobic drugs with high partition coefficients are preferentially distributed to hydrophobic compartments such as lipid bilayers of cells while hydrophilic drugs (low partition coefficients) preferentially are found in hydrophilic compartments such as blood serum. Hydrophobicity/lipophilicity play a major role in determining where drugs are distributed within the body after adsorption and as a consequence in how rapidly they are metabolized and excreted. In this context the presence of the hydrophobic moiety would be important for such activity. Moreover, many of the proteins involved in drug disposition have hydrophobic binding sites further adding to the importance of lipophilicity.

The lipophilicity of the compounds, expressed as log *P*, explains the main predictor for the activity. The octanol/water partition coefficient *C* log *P* being a measure of hydrophobicity/lipophilicity was calculated using ChemDraw Ultra 11.0 software integrated with Cambridgesoft Software (Cambridgesoft Corporation) [36]. The results obtained are given in Table 2. The calculated values of log *P* for 2-methyl-1-(1,3,4-oxadiazol-2-ylmethyl)-1*H*-benzimidazole derivatives **16**–**26** were about 0.27 higher than for the corresponding 1-(1,3,4-oxadiazol-2-ylmethyl)-1*H*-benzimidazole derivatives. The lipophilic power of compounds increased with increasing log *P*. The activity observed for compounds **16**–**26**, having higher values of log *P* was slightly higher than that of the corresponding compounds **5**–**15** having no methyl group at C-2 position of benzimidazole.

Regarding the correlation of the antimicrobial activity of substituted benzimidazoles with the planarity of their molecules, the *ortho*-substitution on benzene ring of 2-substituted-1-(1,3,4-oxadiazol-2-ylmethyl)-1*H*-benzimidazole generates steric hindrance, hence it is less active than the corresponding *para*-substitution on same nucleus which shows marginal steric effect. The molar refractivity (MR), which represents size and polarizability of a molecule describing steric effects, was calculated (using ChemDraw Ultra 11.0 software) to explain the activity behavior of the synthesized compounds. From Table 2, it can be inferred that the higher value of molar refractivity favors the activity ratio.

Table 2
Calculated log *P* and molar refractivity of compounds **3a**, **b**, **4a**, **b** and **5**–**26**.

Compounds	*C* log *P*	MR
3a	1.88	55.96
3b	2.15	60.34
4a	−0.12	52.54
4b	0.15	56.92
5	0.07	60.24
6	0.59	64.87
7	0.36	64.76
8	0.31	69.36
9	1.89	80.92
10	2.39	85.52
11	2.64	85.52
12	1.21	82.73
13	1.52	82.73
14	1.41	88.14
15	1.97	88.17
16	0.34	64.65
17	0.87	69.25
18	0.63	69.14
19	0.58	73.74
20	2.16	85.30
21	2.66	89.90
22	2.91	89.90
23	1.49	87.11
24	1.79	87.11
25	1.68	92.55
26	2.24	92.55

Table 3
Antibacterial activity of compounds **5**–**26** (MIC in μg/mL).

Compounds	Microorganisms					
	S. aureus	*B. subtilis*	*S. mutans*	*E. coli*	*P. aeruginosa*	*S. typhi*
5	16	16	16	16	>128	32
6	32	32	16	16	>128	64
7	16	32	16	NT	32	64
8	16	16	NT	NT	32	64
9	16	16	32	64	NT	>128
10	32	16	16	>128	NT	32
11	32	32	16	32	NT	NT
12	32	NT	NT	64	64	16
13	NT	16	32	32	64	NT
14	16	16	NT	32	>128	NT
15	16	32	16	64	32	32
16	4	4	8	64	64	16
17	4	8	4	32	>128	32
18	16	16	32	16	NT	>128
19	4	8	8	32	NT	16
20	8	8	8	32	NT	64
21	NT	NT	16	>128	NT	32
22	2	4	4	>128	NT	NT
23	NT	16	8	32	NT	32
24	8	8	4	>128	NT	NT
25	8	4	8	64	64	16
26	2	8	4	16	64	16
Ampicillin	2	2	2	4	>128	>128
Ciprofloxacin	≤1	≤1	NT	≤1	NT	NT

NT = Not tested.

The values of the MIC against microorganisms tested are reported in Table 3. The investigations showed significant inhibitory effects, with the majority of the compounds with the MIC values 4–64 μg/mL. The antibacterial data indicated that 1-{[5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl]methyl}-2-methyl-1*H*-benzimidazole **22** and 1-{[5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl]methyl}-2-methyl-1*H*-benzimidazole **26** illustrated appreciable antibacterial activity (MIC 2 μg/mL) against *S. aureus*. All the synthesized compounds showed significant antibacterial activity against all the Gram-positive strains of bacteria. Among them

Table 4
Antifungal activity of compounds **5**–**26**.

Compounds[a]	Microorganisms		
	C. albicans	*A. niger*	*A. flavus*
5	++	++	++
6	+	−	+
7	++	+	+
8	++	++	+
14	++	−	−
15	++	−	−
16	+++	++	++
17	++	+	++
18	+	++	+
19	++	+	+
21	++	++	++
22	+++	+	+++
24	+++	++	++
25	++	++	++
26	+++	++	++
Amphotericin B[a]	+++	+++	+++

Symbols: zone diameter of growth inhibition: (−) = inactive (<10 mm); (+) = weakly active (10–15 mm); (++) = moderately active (16–21 mm); (+++) = highly active (22–28 mm); (+++) = amphotericin B.

[a] Amphotericin B (10 μg/disc) was used as positive reference; synthesized compounds (300 μg/disc).

compounds **16**, **17**, **19**, **20**, **22**, **24**, **25** and **26** were found to be more potent. As shown in Table 3, none of the compounds have inhibitory effect against *E. coli*. Less activity was noted against *P. aeruginosa* and *S. typhi*. According to the investigations, 2-methyl benzimidazole derivatives having *para*-substituted benzene ring on oxadiazole moiety yielded increased activity.

The in vitro antifungal activity of the derivatives of 2-methyl-1-(1,3,4-oxadiazol-2-ylmethyl)-1*H*-benzimidazole **5**–**15** and 1-(1,3,4-oxadiazol-2-ylmethyl)-1*H*-benzimidazole **16**–**26** is characterized in Table 4. Some of the synthesized compounds tested were endowed with a medium activity against *A. flavus*. Of these, 1-{[5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl]methyl}-2-methyl-1*H*-benzimidazole **22** was found to be most potent. The compounds with *para*-substitution on the benzene ring of 1-{[5-(4-substituted phenyl)-1,3,4-oxadiazol-2-yl]methyl}-2-methyl-1*H*-benzimidazole (**22**, **24** and **26**) also showed better activity against *C. albicans*. For *A. niger*, the tested compounds showed low to moderate antifungal activity. The compounds which have no antifungal activity are not included in the table.

3. Conclusions

In conclusion, several substituted 2-substituted-1-[{(5-substituted alkyl/aryl)-1,3,4-oxadiazol-2-yl} methyl]-1*H*-benzimidazoles **5**–**26** were synthesized. The pharmacological study was undertaken to evaluate the effects of substituents on the antibacterial and antifungal activities. All the synthesized compounds exhibited good antibacterial activity towards Gram-positive bacteria and some of the synthesized compounds showed good to moderate antifungal activity. These compounds however did not show any promising activity towards Gram-negative bacteria. In conclusion, antimicrobial activity of the synthesized compounds increases with increasing log *P* and molar refractivity. From Tables 2–4, it can be inferred that as the number of carbon atom increases in side chain at 2-position of oxadiazole heterocyclic ring causes an increase in the intensity of the activity against *S. aureus*, *B. subtilis* and *C. albicans* and also the *para*-substitution on benzene nucleus at oxadiazole moiety supports the chemotherapeutic activity.

4. Experimental section

All melting points were determined in open capillary tube and are uncorrected. Infrared spectra were recorded in KBr on Perkin–Elmer RX1 spectrophotometer. The ^{1}H NMR spectra were measured in dimethyl sulfoxide-d_6 or $CDCl_3$ solutions on a Brucker 400 MHz spectrometer using TMS as an internal reference (chemical shift in δ ppm). The mass spectra were recorded on a Jeol SX-102 instrument. All the synthesized compounds were microanalyzed satisfactorily for C, H and N by Elementar Vario EL III elemental analyzer.

4.1. Methods of synthesis

4.1.1. Synthesis of 1H-benzimidazole (**2a**)

Take 0.24 mol of *o*-phenylenediamine in round-bottomed flask and add 0.48 mol of 90% formic acid. Heat the mixture on water bath at 100 °C for 2 h. Cool, add 10% NaOH solution slowly with constant rotation of the flask until the mixture is just alkaline to litmus. Filter out the crude benzimidazole at the pump, wash with ice-cold water. Dissolve the crude product in 400 mL of boiling water, add 2 g of decolourizing carbon and digest for 15 min, filter, cool the filtrate to about 10 °C, filter off the benzimidazole, wash with 25 mL of cold water and dry at 100 °C. Yield 85%, mp 171–173 °C (Found: C, 71.15; H, 5.10; N, 23.70. Calcd for $C_7H_6N_2$: C, 71.17; H, 5.12; N, 23.71%).

IR (KBr): 1148 (–C–N), 1670 (–C=N ester) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ ppm: 2.51 (s, 3H, –CH_3), 5.0 (s, 1H, –NH), 7.22–7.59 (m, 4H, Ar–H); EI-MS: 119 ($M^+ + 1$).

4.1.2. Synthesis of 2-methyl-1H-benzimidazole (**2b**)

Heat together the mixture of 0.03 mol of *o*-phenylenediamine dihydrochloride, 20 mL of water and 0.09 mol of acetic acid under reflux for 45 min, make the cooled reaction mixture distinctly basic by gradual addition of conc. ammonia solution, collect the precipitate product and recrystallized it from 10% aqueous ethanol. Yield 60%, mp 176–178 °C (Found: C, 72.68; H, 6.08; N, 21.17. Calcd for $C_8H_8N_2$: C, 72.70; H, 6.10; N, 21.20%).

IR (KBr): 1150 (–C–N), 1675 (–C=N ester) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ ppm: 2.51 (s, 3H, –CH_3), 5.0 (s, 1H, –NH), 7.22–7.59 (m, 4H, Ar–H); EI-MS: 133 ($M^+ + 1$).

4.1.3. Synthesis of ethyl 1H-benzimidazol-1-ylacetate (**3a**)

A mixture of equimolar alkaline solution (0.5 mL, 4 N NaOH) of 1*H*-benzimidazole **2a** (0.01 mol, 1.18 g) in MeOH (50 mL) and ethylchloroacetate (0.01 mol, 1 mL) in MeOH (30 mL) was heated gently on boiling water bath for 0.5 h. The solid thus obtained on cooling was recrystallized from chloroform to give **3a**. Yield 76%, mp 178–180 °C (Found: C, 64.67; H, 5.91; N, 13.70. Calcd for $C_{11}H_{12}N_2O_2$: C, 64.69; H, 5.92; N, 13.72%).

IR (KBr): 1632 (–C=N), 1150 (–C–N), 1745 (–C=O ester), 1460 (–N–CH_2) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ ppm: 3.70 (s, 2H, –N–CH_2), 4.21 (q, 2H, –COOCH_2CH$_3$), 1.32 (t, 3H, –COOCH$_2$$CH_3$), 8.50 (s, 1H, –N=CH), 7.72–7.90 (m, 4H, Ar–H); EI-MS: 205 ($M^+ + 1$).

4.1.4. Synthesis of ethyl (2-methyl-1H-benzimidazol-1-yl)acetate (**3b**)

Ethylchloroacetate (0.01 mol, 1.06 mL) was added to a solution of 2-methyl-1*H*-benzimidazole (0.01 mol, 1.32 g) in dry acetone (20 mL). To that mixture, anhydrous K_2CO_3 (1 g) was added and the reaction mixture was refluxed for 10 h. Acetone was removed after completion of reaction and the residue crystallized from ethanol to give compound **3b**. Yield 78%, mp 184–186 °C (Found: C, 66.01; H, 6.45; N, 12.81. Calcd for $C_{12}H_{14}N_2O_2$: C, 66.04; H, 6.47; N, 12.84%).

IR (KBr): 1624 (–C=N), 1145 (–C–N), 1730 (–C=O ester), 1455 (–N–CH_2), 2850 (–C–CH_3) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ ppm: 3.67 (s, 2H, –N–CH_2), 4.24 (q, 2H, –COOCH_2CH$_3$), 1.26 (t, 3H, –COOCH$_2$$CH_3$), 2.25 (s, 3H, –$CH_3$), 7.75–7.94 (m, 4H, Ar–H); EI-MS: 219 ($M^+ + 1$).

4.1.5. Synthesis of 2-(2-substituted-1H-benzimidazol-1-yl) acetohy-drazides (**4a**) and (**4b**)

General method. To a solution of compounds **3a** and **3b** (0.01 mol) dissolved in dry methanol (50 mL) 99% hydrazine hydrate (1 mL) was added and the mixture was refluxed for 4–5 h. The reaction mixture was cooled and the solid obtained was filtered, washed with small quantity of cold methanol to give **4a** and **4b** respectively.

*4.1.5.1. 2-(1H-Benzimidazol-1-yl)acetohydrazide (**4a**).* Yield 80%, mp 200–202 °C (Found: C, 56.82; H, 5.29; N, 29.43. Calcd for $C_9H_{10}N_4O$: C, 56.83; H, 5.30; N, 29.46%).

IR (KBr): 1640 (–C=N), 1122 (–C–N), 1682 (–C=O amide), 1461 (–N–CH_2) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ ppm: 3.66 (s, 2H, –N–CH_2), 8.45 (s, 1H, –N=CH), 8.10 (m, 3H, –CONHNH_2), 7.60–8.00 (m, 4H, Ar–H); EI-MS: 191 ($M^+ + 1$).

*4.1.5.2. 2-(2-Methyl-1H-benzimidazol-1-yl)acetohydrazide (**4b**).* Yield 77%, mp 248–250 °C (Found: C, 58.79; H, 5.90; N, 27.40. Calcd for $C_{10}H_{12}N_4O$: C, 58.81; H, 5.92; N, 27.43%).

IR (KBr): 1635 (–C=N), 1130 (–C–N), 1775 (–C=O amide), 1457 (–N–CH_2), 2862 (–C–CH_3) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ ppm: 3.70 (s, 2H,

–N–CH_2), 2.25 (s, 3H, –CH_3), 8.05 (m, 3H, –$CONHNH_2$), 7.70–8.05 (m, 4H, Ar–H); EI-MS: 205 ($M^+ + 1$).

*4.1.6. Synthesis of 2-substituted-1-[{(5-substituted alkyl/aryl)-1,3,4-oxadiazol-2-yl} methyl]-1H-benzimidazole (**5**–**26**)*

General method. An equimolar mixture of compound **4** (0.001 mol) and substituted carboxylic acid in phosphoryl chloride was refluxed for 10–16 h. Then reaction mixture was cooled, poured into ice-cold water and neutralized with 20% $NaHCO_3$ solution. The resultant solid was filtered, washed with water and recrystallized from ethanol to give the title compounds.

*4.1.6.1. 1-[(5-Methyl-1,3,4-oxadiazol-2-yl)methyl]-1H-benzimidazole (**5**).* IR (KBr): 1680 (–C═N), 1134 (–C–N), 1457 (–N–CH_2), 2870 (–C–CH_3) cm^{-1}; ^1H NMR ($CDCl_3$) δ ppm: 3.65 (s, 2H, –N–CH_2), 4.10 (s, 1H, –N–CH), 2.30 (s, 3H, –CH_3), 7.22–7.85 (m, 4H, Ar–H); EI-MS: 215 ($M^+ + 1$).

*4.1.6.2. 1-[(5-Ethyl-1,3,4-oxadiazol-2-yl)methyl]-1H-benzimidazole (**6**).* IR (KBr): 1672 (–C═N), 1155 (–C–N), 1473 (–N–CH_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ ppm: 3.70 (s, 2H, –N–CH_2), 8.42 (s, 1H, –N═CH), 4.12 (q, 2H, –CH_2CH_3), 1.02 (t, 3H, –CH_2CH_3), 7.22–7.89 (m, 4H, Ar–H); EI-MS: 229 ($M^+ + 1$).

*4.1.6.3. 1-{[5-(Chloromethyl)-1,3,4-oxadiazol-2-yl]methyl}-1H-benzimidazole (**7**).* IR (KBr): IR (KBr): 1660 (–C═N), 1140 (–C–N), 1469 (–N–CH_2), 760 (–C–Cl) cm^{-1}; ^1H NMR ($CDCl_3$) δ ppm: 3.61 (s, 2H, –N–CH_2), 8.55 (s, 1H, –N═CH), 5.25 (s, 2H, –CH_2Cl), 7.35–7.92 (m, 4H, Ar–H); EI-MS: 249 ($M^+ + 1$).

*4.1.6.4. 1-{[5-(2-Chloroethyl)-1,3,4-oxadiazol-2-yl]methyl}-1H-benzimidazole (**8**).* IR (KBr): 1662 (–C═N), 1160 (–C–N), 1463 (–N–CH_2), 752 (–C–Cl) cm^{-1}; ^1H NMR ($CDCl_3$) δ ppm: 3.59 (s, 2H, –N–CH_2), 8.46 (s, 1H, –N═CH), 5.12 (q, 2H, –CH_2CH_3Cl), 5.36 (t, 3H, –CH_2CH_3Cl), 7.10–7.84 (m, 4H, Ar–H); EI-MS: 263 ($M^+ + 1$).

*4.1.6.5. 1-[(5-Phenyl-1,3,4-oxadiazol-2-yl)methyl]-1H-benzimidazole (**9**).* IR (KBr): 1670 (–C═N), 1152 (–C–N), 1475 (–N–CH_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ ppm: 3.69 (s, 2H, –N–CH_2), 8.59 (s, 1H, –N═CH), 6.69–7.50 (m, 9H, Ar–H); EI-MS: 277 ($M^+ + 1$).

*4.1.6.6. 1-{[5-(2-Chlorophenyl)-1,3,4-oxadiazol-2-yl]methyl}-1H-benzimidazole (**10**).* IR (KBr): 1665 (–C═N), 1138 (–C–N), 1453 (–N–CH_2), 792 (–C–Cl) cm^{-1}; ^1H NMR ($CDCl_3$) δ ppm: 3.67 (s, 2H, –N–CH_2), 8.40 (s, 1H, –N═CH), 6.65–7.74 (m, 8H, Ar–H); EI-MS: 311 ($M^+ + 1$).

*4.1.6.7. 1-{[5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl]methyl}-1H-benzimidazole (**11**).* IR (KBr): 1645 (–C═N), 1145 (–C–N), 1446 (–N–CH_2), 780 (–C–Cl) cm^{-1}; ^1H NMR ($CDCl_3$) δ ppm: 3.65 (s, 2H, –N–CH_2), 8.54 (s, 1H, –N═CH), 6.62–7.78 (m, 8H, Ar–H); EI-MS: 311 ($M^+ + 1$).

*4.1.6.8. 2-[5-(1H-Benzimidazol-1-ylmethyl)-1,3,4-oxadiazol-2-yl]phenol (**12**).* IR (KBr): 1660 (–C═N), 1140 (–C–N), 1452 (–N–CH_2), 3384 (–C–OH aromatic) cm^{-1}; ^1H NMR ($CDCl_3$) δ ppm: 3.64 (s, 2H, –N–CH_2), 8.62 (s, 1H, –N═CH), 5.50 (s, 1H, Ar–OH), 6.78–7.93 (m, 8H, Ar–H); EI-MS: 293 ($M^+ + 1$).

*4.1.6.9. 4-[5-(1H-Benzimidazol-1-ylmethyl)-1,3,4-oxadiazol-2-yl]phenol (**13**).* IR (KBr): 1667 (–C═N), 1130 (–C–N), 1457 (–N–CH_2), 3360 (–C–OH aromatic) cm^{-1}; ^1H NMR ($CDCl_3$) δ ppm: 3.67 (s, 2H, –N–CH_2), 8.65 (s, 1H, –N═CH), 5.50 (s, 1H, Ar–OH), 6.78–7.93 (m, 8H, Ar–H); EI-MS: 293 ($M^+ + 1$).

*4.1.6.10. 1-{[5-(2-Methoxyphenyl)-1,3,4-oxadiazol-2-yl]methyl}-1H-benzimidazole (**14**).* IR (KBr): 1647 (–C═N), 1138 (–C–N), 1458 (–N–CH_2), 2810 (–C–OCH_3) cm^{-1}; ^1H NMR ($CDCl_3$) δ ppm: 3.59 (s, 2H, –N–CH_2), 8.61 (s, 1H, –N═CH), 3.73 (s, 3H, –OCH_3), 6.61–7.85 (m, 8H, Ar–H); EI-MS: 307 ($M^+ + 1$).

*4.1.6.11. 1-{[5-(4-Methoxyphenyl)-1,3,4-oxadiazol-2-yl]methyl}-1H-benzimidazole (**15**).* IR (KBr): 1650 (–C═N), 1135 (–C–N), 1455 (–N–CH_2), 2816 (–C–OCH_3) cm^{-1}; ^1H NMR ($CDCl_3$) δ ppm: 3.50 (s, 2H, –N–CH_2), 8.67 (s, 1H, –N═CH), 3.75 (s, 3H, –OCH_3), 6.67–7.90 (m, 8H, Ar–H); EI-MS: 307 ($M^+ + 1$).

*4.1.6.12. 2-Methyl-1-[(5-methyl-1,3,4-oxadiazol-2-yl)methyl]-1H-benzimidazole (**16**).* IR (KBr): 1642 (–C═N), 1150 (–C–N), 1466 (–N–CH_2), 2852 (–C–CH_3) cm^{-1}; ^1H NMR ($CDCl_3$) δ ppm: 3.75 (s, 2H, –N–CH_2), 2.22 (s, 3H, –CH_3), 7.20–7.81 (m, 4H, Ar–H); EI-MS: 229 ($M^+ + 1$).

*4.1.6.13. 1-[(5-Ethyl-1,3,4-oxadiazol-2-yl)methyl]-2-methyl-1H-benzimidazole (**17**).* IR (KBr): 1650 (–C═N), 1139 (–C–N), 1451 (–N–CH_2), 2859 (–C–CH_3) cm^{-1}; ^1H NMR ($CDCl_3$) δ ppm: 3.65 (s, 2H, –N–CH_2), 2.19 (s, 3H, –CH_3), 4.45 (q, 2H, –CH_2CH_3), 1.00 (t, 3H, –CH_2CH_3), 7.25–7.84 (m, 4H, Ar–H); EI-MS: 243 ($M^+ + 1$).

*4.1.6.14. 1-{[5-(Chloromethyl)-1,3,4-oxadiazol-2-yl]methyl}-2-methyl-1H-benzimidazole (**18**).* IR (KBr): 1657 (–C═N), 1150 (–C–N), 1442 (–N–CH_2), 2822 (–C–CH_3) 765 (–C–Cl) cm^{-1}; ^1H NMR ($CDCl_3$) δ ppm: 3.71 (s, 2H, –N–CH_2), 2.28 (s, 3H, –CH_3), 4.20 (s, 2H, –CH_2Cl), 7.52–8.00 (m, 4H, Ar–H); EI-MS: 263 ($M^+ + 1$).

*4.1.6.15. 1-{[5-(2-Chloroethyl)-1,3,4-oxadiazol-2-yl]methyl}-2-methyl-1H-benzimidazole (**19**).* IR (KBr): 1648 (–C═N), 1145 (–C–N), 1457 (–N–CH_2), 2830 (–C–CH_3) 760 (–C–Cl) cm^{-1}; ^1H NMR ($CDCl_3$) δ ppm: 3.75 (s, 2H, –N–CH_2), 2.25 (s, 3H, –CH_3), 4.13 (t, 2H, –CH_2CH_2Cl), 1.08 (t, 2H, –CH_2CH_2Cl), 7.62–8.15 (m, 4H, Ar–H); EI-MS: 277 ($M^+ + 1$).

*4.1.6.16. 2-Methyl-1-[(5-phenyl-1,3,4-oxadiazol-2-yl)methyl]-1H-benzimidazole (**20**).* IR (KBr): 1660 (–C═N), 1152 (–C–N), 1439 (–N–CH_2), 2828 (–C–CH_3) cm^{-1}; ^1H NMR ($CDCl_3$) δ ppm: 3.69 (s, 2H, –N–CH_2), 2.27 (s, 3H, –CH_3), 7.58–8.12 (m, 9H, Ar–H); EI-MS: 291 ($M^+ + 1$).

*4.1.6.17. 1-{[5-(2-Chlorophenyl)-1,3,4-oxadiazol-2-yl]methyl}-2-methyl-1H-benzimidazole (**21**).* IR (KBr): 1662 (–C═N), 1148 (–C–N), 1435 (–N–CH_2), 2832 (–C–CH_3), 767 (–C–Cl) cm^{-1}; ^1H NMR ($CDCl_3$): δ ppm: 3.73 (s, 2H, –N–CH_2), 2.20 (s, 3H, –CH_3), 7.64–8.15 (m, 8H, Ar–H); EI-MS: 325 ($M^+ + 1$).

*4.1.6.18. 1-{[5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl]methyl}-2-methyl-1H-benzimidazole (**22**).* IR (KBr): 1670 (–C═N), 1132 (–C–N), 1440 (–N–CH_2), 2839 (–C–CH_3) 782 (–C–Cl) cm^{-1}; ^1H NMR ($CDCl_3$): δ ppm: 3.70 (s, 2H, –N–CH_2), 2.24 (s, 3H, –CH_3), 7.60–8.11 (m, 8H, Ar–H); EI-MS: 325 ($M^+ + 1$).

*4.1.6.19. 2-{5-[(2-Methyl-1H-benzimidazol-1-yl)methyl]-1,3,4-oxadiazol-2-yl}phenol (**23**).* IR (KBr): 1672 (–C═N), 1145 (–C–N), 1457 (–N–CH_2), 2852 (–C–CH_3), 3350 (–C–OH aromatic) cm^{-1}; ^1H NMR ($CDCl_3$) δ ppm: 3.68 (s, 2H, –N–CH_2), 2.19 (s, 3H, –CH_3), 5.58 (s, 1H, Ar–OH), 7.61–8.12 (m, 8H, Ar–H); EI-MS: 307 ($M^+ + 1$).

*4.1.6.20. 4-{5-[(2-Methyl-1H-benzimidazol-1-yl)methyl]-1,3,4-oxadiazol-2-yl}phenol (**24**).* IR (KBr): 1665 (–C═N), 1132 (–C–N), 1449 (–N–CH_2), 2847 (–C–CH_3), 3362 (–C–OH aromatic) cm^{-1}; ^1H NMR ($CDCl_3$) δ ppm: 3.62 (s, 2H, –N–CH_2), 2.15 (s, 3H, –CH_3), 5.62 (s, 1H, Ar–OH), 7.58–8.11 (m, 8H, Ar–H); EI-MS: 307 ($M^+ + 1$).

*4.1.6.21. 1-{[5-(2-Methoxyphenyl)-1,3,4-oxadiazol-2-yl]methyl}-2-methyl-1H-benzimidazole (**25**).* IR (KBr) δ ppm: 1658 (–C═N), 1124 (–C–N), 1453 (–N–CH_2), 2830 (–C–CH_3), 2792 (–C–OCH_3) cm^{-1}; ^1H NMR ($CDCl_3$): 3.66 (s, 2H, –N–CH_2), 2.22 (s, 3H, –CH_3), 3.84 (s, 3H, –OCH_3), 7.55–7.97 (m, 8H, Ar–H); EI-MS: 321 ($M^+ + 1$).

*4.1.6.22. 1-{[5-(4-Methoxyphenyl)-1,3,4-oxadiazol-2-yl]methyl}-2-methyl-1H-benzimidazole (**26**).* IR (KBr): 1666 (–C═N), 1135 (–C–N), 1461 (–N–CH_2), 2827 (–C–CH_3), 2788 (–C–OCH_3) cm^{-1}; ^1H NMR ($CDCl_3$) δ ppm: 3.60 (s, 2H, –N–CH_2), 2.15 (s, 3H, –CH_3), 3.89 (s, 3H, –OCH_3), 7.51–7.93 (m, 8H, Ar–H); EI-MS: 321 ($M^+ + 1$).

4.2. Antimicrobial activity test

All the synthesized compounds **5**–**26** were screened for their in vitro antimicrobial activity against the standard strains: *S. aureus* (ATCC 29213), *B. subtilis* (MTCC 121), *S. mutans* (MTCC 890), *E. coli* (ATCC 25922), *P. aeruginosa* (MTCC 741) and *S. typhi* (MTCC 733) and the yeasts *C. albicans* (MTCC 1637), *A. flavus* (AIIMS) and *A. niger* (AIIMS).

The twofold dilution technique [37] was followed to determine the minimum inhibitory concentration (MIC) of the synthesized compounds. The test compounds were dissolved in dimethyl sulfoxide (DMSO) and then diluted with culture medium (Mueller–Hinton agar medium) at the required final concentration with variation of 128–1 μg/mL. A plate containing only the culture medium and DMSO in the same dilution was used as negative control. The final amount applied was 10^4 CFU/plate. The MIC values were recorded after incubation at 35 °C for a period of 20 h. The lowest concentration of the test substance that completely inhibited the growth of the microorganism was reported as MIC expressed in terms of μg/mL. Ampicillin and Ciprofloxacin were used as reference drugs. All experiments were performed three times.

Antifungal activity of the synthesized compounds was tested by the disc diffusion method under standard conditions using Mueller–Hinton agar medium as described by NCCLS [38]. Sterile filter paper discs (6 mm diameter) containing specific amounts of an antifungal agent (300 μg for the synthesized compounds) were placed on the surface of an agar plate inoculated with a standardized suspension of the microorganisms tested. The plates were incubated at 28 ± 2 °C for 7 days for evaluating antifungal activity. The diameters of inhibition zones (in mm) of triplicate sets were measured and the results are reported in Table 3. Paper discs with dimethyl sulfoxide (DMSO) only were utilized as negative controls.

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