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Original article

Synthesis and QSAR evaluation of 2-(substituted phenyl)-1*H*-benzimidazoles and [2-(substituted phenyl)-benzimidazol-1-yl]-pyridin-3-yl-methanones

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Abstract

A series of 2-(substituted phenyl)-1*H*-benzimidazole (**1**–**10**) and [2-(substituted phenyl)-benzimidazol-1-yl]-pyridin-3-yl-methanone (**11**–**19**) derivatives were synthesized and tested in vitro for their antimicrobial activity. The results of QSAR investigation indicated the importance of molecular descriptors, dipole moment (μ), log of octanol water partition coefficient (log *P*) and second order molecular connectivity index ($^2\chi$) in describing the antimicrobial activity of the synthesized compounds.

Keywords: Substituted benzimidazoles; Synthesis; Antimicrobial activity; QSAR

1. Introduction

The resistance to antimicrobial drugs is widespread, the development of new antimicrobial agents and understanding their mechanisms of action are becoming vital nowadays [1]. The incorporation of the benzimidazole nucleus is an important synthetic strategy in studies of antimicrobial drug discovery [2].

Benzimidazole derivatives have attracted continuing interest over the years because of their varied biological activities *viz*. anticancer [3], antihypertensive [4], antiviral [5], anti-inflammatory [6], vasodilator [7] and antimicrobial [8–10]. Nicotinic acid nucleus has been suggested to have antianxiety [11], antifungal [12], anticancer [13,14], antihypertensive [15] and antibacterial activities [16,17].

During the last 20 years quantitative structure–activity relationship (QSAR) models have gained an extensive recognition in physical, organic, analytical, pharmaceutical and medicinal chemistry. The success of the QSAR approach can be explained by the insight offered based on the structural determination of chemical properties, and the possibility to estimate the properties of new chemical compounds without the need to synthesize and test them among the homologous series [18].

In light of the above and in continuation of our efforts in the synthesis and QSAR studies of biologically active molecules [19–32], we hereby report the synthesis, antimicrobial evaluation and QSAR studies of 2-(substituted phenyl)-1*H*-benzimidazoles and [2-(substituted phenyl)-benzimidazol-1-yl]-pyridin-3-yl-methanones.

2. Chemistry

The synthesis of compounds **1**–**19** followed the general pathway elicited in Scheme 1. Compounds **1**–**10** are the key intermediates for the synthesis of compounds **11**–**19**. The key intermediates, 2-(substituted phenyl)-1*H*-benzimidazoles (**1**–**10**), were prepared by the condensation of benzimidazoles with corresponding substituted aryldiazonium chlorides which in turn were prepared by the diazotization of substituted anilines. However, based on our experience, the application of cupric chloride for the condensation of aryldiazonium

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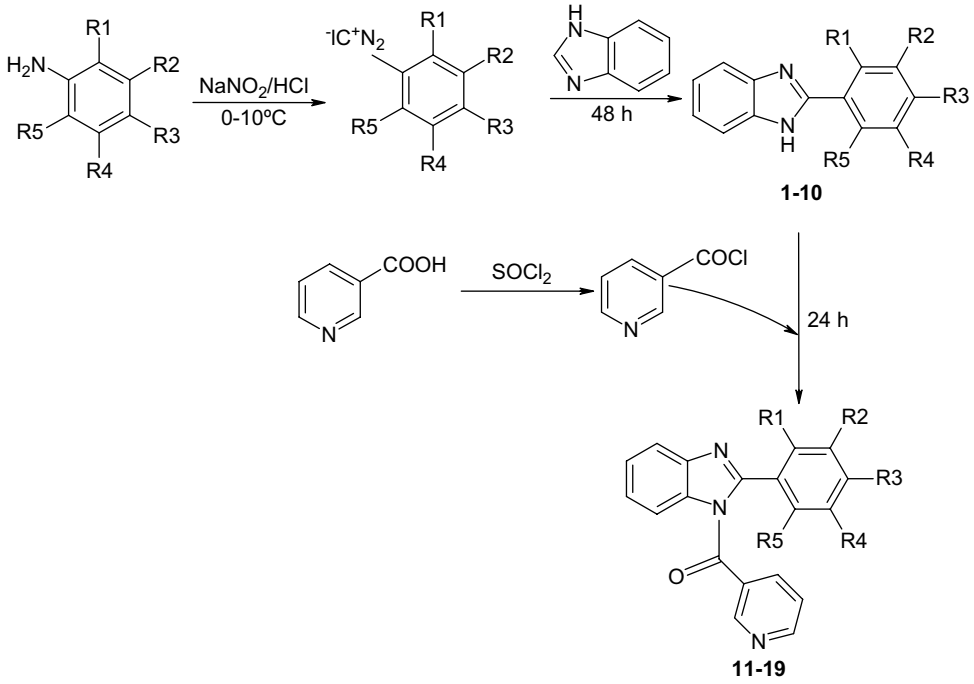

1	$R_2=R_3=R_4=R_5=R_6=H$; $R_1=Cl$	**11**	$R_2=R_3=R_4=R_5=R_6=H$; $R_1=Cl$
2	$R_1=R_2=R_4=R_5=R_6=H$; $R_3=Cl$	**12**	$R_1=R_2=R_4=R_5=R_6=H$; $R_3=Cl$
3	$R_1=R_3=R_4=R_5=R_6=H$; $R_2=Cl$	**13**	$R_1=R_3=R_4=R_5=R_6=H$; $R_2=Cl$
4	$R_1=R_2=R_3=R_4=R_5=R_6=H$	**14**	$R_1=R_2=R_4=R_5=R_6=H$; $R_3=OH$
5	$R_1=R_2=R_4=R_5=R_6=H$; $R_3=NO_2$	**15**	$R_1=R_2=R_4=R_5=R_6=H$; $R_3=NO_2$
6	$R_1=R_3=R_4=R_5=R_6=H$; $R_2=NO_2$	**16**	$R_1=R_3=R_4=R_5=R_6=H$; $R_2=NO_2$
7	$R_2=R_3=R_4=R_5=R_6=H$; $R_1=NO_2$	**17**	$R_2=R_3=R_4=R_5=R_6=H$; $R_1=NO_2$
8	$R_2=R_3=R_4=R_5=R_6=H$; $R_1=COOH$	**18**	$R_2=R_3=R_4=R_5=R_6=H$; $R_1=COOH$
9	$R_2=R_3=R_4=R_5=R_6=H$; $R_1=OCH_3$	**19**	$R_2=R_3=R_4=R_5=R_6=H$; $R_1=OCH_3$
10	$R_1=R_2=R_4=R_5=R_6=H$; $R_3=OH$		

Scheme 1. Scheme for the syntheses of 2-(substituted phenyl)-1*H*-benzimidazole and [2-(substituted phenyl)-benzimidazol-1-yl]-pyridin-3-yl-methanones.

chloride with benzimidazole as suggested by Dahiya and Pathak [33] 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)-benzimidazol-1-yl]-pyridin-3-yl-methanones (**11**–**19**), the key intermediates (**1**–**10**) 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.

The structures of compounds **1**–**19** were assigned by IR and ^{1}H NMR spectroscopic data, which are consistent with the proposed molecular structures (Table 2). The appearance of medium out of plane deformation bands (C–C bending) at 725–680 cm^{-1} indicated the presence of 1,3-disubstituted benzene ring in compound **6**. Similarly the appearance of the C–C out of plane band at 806.19 cm^{-1} indicated the presence of 1,4-disubstituted benzene ring in compound **5**. In contrast, the monosubstituted benzene ring (compound **4**) showed C–C out of plane band deformation at 686.61 cm^{-1}. The presence of 3-substituted pyridine in structures of compounds **11**–**19** was confirmed by strong out of plane deformation bands (C–H bending) at 820–770 cm^{-1} which were visible from their IR spectra. Moreover the presence of NO_2 group in compounds **5**, **15**–**17** was indicated by the appearance of asymmetric and symmetric NO_2 stretching bands at 1550–1510 cm^{-1} and 1365–1335 cm^{-1}, respectively. The appearance of medium bands at 3411 cm^{-1} in the IR spectra of compound **18** indicated the presence of a free OH in the carboxylic acid group. Further, the appearance of strong C=O stretching bands at 1670–1630 cm^{-1} in the IR spectra of [2-(substituted phenyl)-benzimidazol-1-yl]-pyridin-3-yl-methanones (**11**–**19**) demonstrated the presence of tertiary

Table 1
Physicochemical characteristics of 2-(substituted phenyl)-1*H*-benzimidazoles and [2-(substituted phenyl)-benzimidazol-1-yl]-pyridin-3-yl-methanones

Compound	Mol. formula	Mol. wt.	M.p. (°C)	R_f value[a]	Yield (%)
1	$C_{13}H_9N_2Cl$	228.71	Above 250	0.09	36.71
2	$C_{13}H_9N_2Cl$	228.71	179–181	0.18	17.55
3	$C_{13}H_9N_2Cl$	228.71	Above 250	0.29	48.02
4	$C_{13}H_{10}N_2$	194.22	79–81	0.80	14.42
5	$C_{13}H_9N_3O_2$	228.28	Above 250	0.40	49.30
6	$C_{13}H_9N_3O_2$	228.28	Above 250	0.16	15.12
7	$C_{13}H_9N_3O_2$	228.28	239–241	0.81	25.53
8	$C_{14}H_{10}N_2O_2$	238.29	133–135	0.16	15.76
9	$C_{14}H_{10}N_2O$	225.27	119–121	0.36	16.12
10	$C_{13}H_{10}N_2O$	210.25	224–226	0.48	30.02
11	$C_{19}H_{12}N_3OCl$	333.83	84–86	0.95	39.13
12	$C_{19}H_{12}N_3OCl$	333.83	224–226	0.48	47.82
13	$C_{19}H_{12}N_3OCl$	333.83	149–151	0.59	73.91
14	$C_{19}H_{13}N_3O_2$	315.37	64–66	0.15	11.20
15	$C_{19}H_{12}N_4O_3$	344.30	244–246	0.22	48.59
16	$C_{19}H_{12}N_4O_3$	344.30	239–241	0.31	70.09
17	$C_{19}H_{12}N_4O_3$	344.30	49–51	0.90	14.95
18	$C_{20}H_{13}N_3O_3$	343.41	139–141	0.13	45.08
19	$C_{20}H_{15}N_3O_2$	329.39	99–101	0.90	14.20

[a] TLC mobile phase: toluene/chloroform (7:3).

amide linkage between the 3-substituted pyridine and the benzimidazole nucleus.

Compound **5** showed a doublet at δ 8.11–8.14 ppm and 7.85–7.89 ppm corresponding to a proton of the C_3, C_5 and C_2, C_6 of NO_2 substituted benzene ring. Further, the appearance of NMR signals at δ 7.52–7.56 ppm and 6.54–6.56 ppm demonstrated the presence of C_4, C_7 and C_5, C_6 protons of benzimidazole nucleus.

The absence of an additional δ at 7.02–7.06 ppm in compound **5** (in contrast to compound **4**) indicated the absence of proton at C_4 of aromatic ring which has been occupied by the NO_2 group. The appearance of δ at 6.5–7.8 ppm corresponds to the aromatic protons of benzene and benzimidazole 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 11.07 ppm indicated the presence of COOH group in compound **18**. The absence of a singlet at δ 11 ppm in the NMR spectra of compounds **11–17** and **19** indicated the absence of the free COOH group. This confirms that compounds **11–19** are tertiary amides and not the physical mixture of nicotinic acid and compounds **1–10**. Therefore, this assures the reaction of nicotinyl chloride with the secondary nitrogen of benzimidazole nucleus.

3. Results and discussion

3.1. Antimicrobial activity

The synthesized compounds were screened for their in vitro antimicrobial activities against two Gram-positive bacteria – *Staphylococcus aureus*, *Bacillus subtilis*, Gram-negative bacterium – *Escherichia coli* and fungal strains – *Aspergillus niger* and *Candida albicans* by tube dilution method [34] using ciprofloxacin and fluconazole as control drugs for antibacterial and antifungal activities, respectively. The results of antimicrobial studies are presented in Table 3. In general the compounds showed improved antibacterial activity when compared to their antifungal activity. The deduced patterns of antimicrobial activity of substituted benzimidazoles are in the following order.

E. coli > *B. subtilis* > *S. aureus* > *C. albicans* > *A. niger*

Compounds **11–13** are the most effective compounds against *S. aureus* with $pMIC_{sa}$ value of 1.73, 1.73 and 1.79 (Table 3), respectively. For activity against *B. subtilis* compounds **5** and **10** yielded better activity (Table 3) in comparison to other compounds synthesized. The antimicrobial spectrum of substituted benzimidazoles against *E. coli* demonstrated that compounds **12–14** were the most active ones with

Table 2
Spectral characterization of selected compounds of 2-(substituted phenyl)-1*H*-benzimidazoles and [2-(substituted phenyl)-benzimidazol-1-yl]-pyridin-3-yl-methanones

Compound	IR (KBr pellets) cm^{-1}	^1H NMR ($CDCl_3$) δ ppm
4	1557.41, 1411.80 (skeletal bands), 1649.99 (C–H str., Ar), 1518.84 (C–C str., Ar)	7.02–7.06 (t, 1H, CH of C_4 of Ar-H), 7.25–7.29 (t, 2H, CH of C_5 and C_6 of benzimidazole), 7.36–7.39 (d, 2H, CH of C_3 and C_5 of ArH), 7.47–7.48 (d, 2H, CH of C_2 and C_6 of ArH), 7.81–7.82 (d, 2H, CH of C_4 and C_7 of benzimidazole)
5	1558.38, 1416.62 (skeletal bands), 1653.52 (C–H str., Ar), 1520.77 (C–C str., Ar), 1344.29 (NO_2 sym str., Ar)	6.54–6.56 (t, 2H, CH of C_5 and C_6 of benzimidazole), 7.52–7.56 (d, 2H, CH of C_4 and C_7 of benzimidazole), 7.85–7.89 (d, 2H, CH of C_2 and C_6 of Ar-H), 8.11–8.14 (d, 2H, CH of C_3 and C_5 of ArH)
15	1527, 1466 (skeletal bands), 1633.9 (C=O, ter. amide), 799.8 (C–H, 3-sub. pyridine), 1333 (NO_2 sym str., Ar)	9.39 (s, 1H, CH of C_2 of nicotinic acid), 9.00 (d, 1H, CH of C_4 of nicotinic acid), 8.71 (d, 1H, CH of C_6 of nicotinic acid), 8.06–8.43 (m, 4H, CH of Ar-NO_2), 6.9–7.5 (m, 4H, CH of C_4–C_7 of benzimidazole)
16	1523.9, 1467.8 (skeletal bands), 1637.2 (C=O, ter. amide), 832 (C–H, 3-sub. pyridine), 1349.7 (NO_2 sym str., Ar)	9.36 (s, 1H, CH of C_4 of nicotinic acid), 8.82–8.86 (d, 1H, CH of C_6 of nicotinic acid), 9.10 (d, 1H, CH of C_6 of Ar-NO_2), 7.48–8.02 (m, 4H, CH of C_4–C_7 of benzimidazole)
17	1574.0, 1463.2 (skeletal bands), 1637.2 (C=O, ter. amide), 842.2 (C–H, 3-sub. pyridine), 1347.6 (NO_2 sym str., Ar)	8.81 (s, 1H, CH of C_2 of nicotinic acid), 8.11–8.13 (d, 1H, CH of C_6 of nicotinic acid), 7.94–7.96 (m, 4H, CH of C_4–C_7 of Ar-NO_2), 7.77–7.80 (m, 4H, CH of C_4–C_7 of benzimidazole)
18	1527.3, 1438.7 (skeletal bands), 669.2 (ring bend, 3-substituted pyridine), 3411.0 (OH str., COOH)	11.07 (s, 1H, COOH), 9.20 (s, 1H, CH of C_2 of nicotinic acid), 8.91 (d, 1H, CH of C_4 of nicotinic acid), 8.80 (d, 1H, CH of C_6 of nicotinic acid), 7.84–7.86 (d, 1H, CH of C_3 of Ar–COOH), 6.85–6.95 (m, 4H, CH of C_4–C_7 of benzimidazole)

Table 3
Antimicrobial activity of 2-(substituted phenyl)-1*H*-benzimidazoles and [2-(substituted phenyl)-benzimidazol-1-yl]-pyridin-3-yl-methanones

Compound	$pMIC_{sa}$	$pMIC_{bs}$	$pMIC_{ec}$	$pMIC_{ca}$	$pMIC_{an}$
1	1.40	1.49	1.49	1.26	1.26
2	1.66	1.51	1.66	1.56	1.40
3	1.49	1.49	1.65	1.26	1.36
4	1.46	1.68	1.72	1.49	1.49
5	1.01	1.78	1.20	1.58	1.58
6	1.47	1.72	1.41	1.58	1.58
7	1.17	1.73	1.35	1.58	1.58
8	1.50	1.70	1.70	1.48	1.28
9	1.50	1.71	1.65	1.25	1.35
10	1.53	1.79	1.70	1.43	1.33
11	1.73	1.43	1.65	1.73	1.43
12	1.73	1.43	1.73	1.73	1.43
13	1.79	1.50	1.80	1.73	1.43
14	1.70	1.57	1.80	1.70	1.40
15	1.44	1.44	1.44	1.74	1.44
16	1.44	1.44	1.44	1.74	1.44
17	1.50	1.50	1.50	1.74	1.44
18	1.44	1.44	1.44	1.74	1.44
19	1.42	1.42	1.60	1.72	1.42
SD[a]	0.19	0.13	0.16	0.18	0.09
Std. drug	3.33[b]	3.33[b]	3.33[c]	2.64[c]	2.64[c]

[a] Standard deviation.
[b] Ciprofloxacin.
[c] Fluconazole.

$pMIC_{ec}$ value of 1.73, 1.80 and 1.80 (Table 3), respectively. From the above discussion it is evident that compounds **11–13** emerged as most active antibacterial benzimidazoles. Compounds **15–17** have emerged as the most effective antifungal agents against *C. albicans* whereas compounds **5–7** were the most active ones against *A. niger* (Table 3). The results of antimicrobial activity of [2-(substituted phenyl)-benzimidazol-1-yl]-pyridin-3-yl-methanones are summarized in Fig. 1.

3.2. QSAR analysis

In order to identify the substituent effect on antimicrobial activity, quantitative structure–activity relationship (QSAR) studies of title compounds were performed. Biological activity

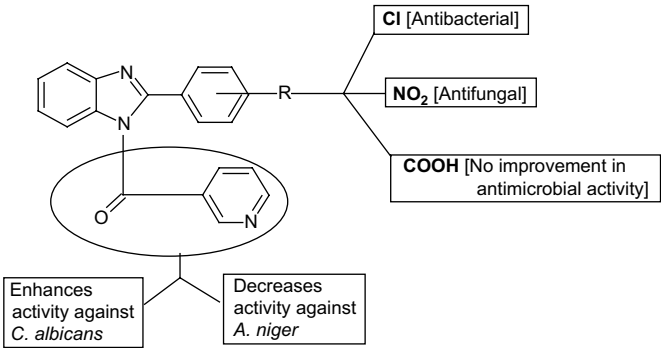

Fig. 1. SAR for the antimicrobial activity of 2-(substituted phenyl)-1*H*-benzimidazoles and [2-(substituted phenyl)-benzimidazol-1-yl]-pyridin-3-yl-methanones.

data (MIC) in their negative logarithmic values (MIC, micromoles) are listed in Table 3. The compounds were analyzed by physicochemical-based QSAR (Hansch) approach using different physicochemical parameters [35–41] (Table 4) as independent and pMIC values as dependent variables. These QSAR descriptors of substituted benzimidazoles were calculated using the molecular package TSAR 3.3 for Windows [42] and the value of the selected descriptors used in the regression analysis is presented in Table 5.

A correlation analysis was performed on all the descriptors, showing the intercorrelation among the independent descriptors and also their individual correlation with antimicrobial activity. The correlation matrix elicited in Table 6 indicated that there is a high autocorrelation ($r > 0.8$) observed between the molecular descriptors except with dipole moment.

The data depicted in Table 7 indicate the correlation of molecular descriptors with antimicrobial activity of substituted benzimidazoles against different representative microorganisms. From the data represented in Table 7, it is evident that in the case of *B. subtilis* and *C. albicans* a significant correlation ($r > 0.6$) was observed almost with all the molecular descriptors except with dipole moment (μ) and Balaban topological index (B). In the case of *S. aureus*, *E. coli* and *A. niger* the electronic parameter, dipole moment (μ) exhibited a significant correlation ($r > 0.7$).

When *E. coli* inhibition data were correlated with physiochemical parameters, the molecular descriptor dipole moment (μ) showed a statistically significant correlation (Eq. (1)).

QSAR model for antibacterial activity against *E. coli*

$$pMIC_{ec} = -0.085\mu + 1.872; \quad n = 19,\ r^2 = 0.884,\ q^2 = 0.859,\ s = 0.058,\ F = 129.47 \qquad (1)$$

Table 4
QSAR descriptors used in the study

S. no.	QSAR descriptor	Type
1	log *P*	Lipophilic
2	Zero order molecular connectivity indices ($^0\chi$)	Topological
3	First order molecular connectivity indices ($^1\chi$)	Topological
4	Second order molecular connectivity indices ($^2\chi$)	Topological
5	Valence zero order molecular connectivity indices ($^0\chi^v$)	Topological
6	Valence first order molecular connectivity indices ($^1\chi^v$)	Topological
7	Valence second order molecular connectivity indices ($^2\chi^v$)	Topological
8	Kier's alpha first order shape indice ($\kappa\alpha_1$)	Topological
9	Kier's alpha second order shape indice ($\kappa\alpha_2$)	Topological
10	Kier's first order shape indice (κ_1)	Topological
11	Randic topological index	Topological
12	Balaban topological index	Topological
13	Wiener's topological index	Topological
14	Kier's second order shape indice (κ_2)	Topological
15	Ionization potential	Electronic
16	Dipole moment (μ)	Electronic
17	Energy of highest occupied molecular orbital (HOMO)	Electronic
18	Energy of lowest unoccupied molecular orbital (LUMO)	Electronic
19	Total energy (Te)	Electronic
20	Molar refractivity (MR)	Steric
21	Solvent accessible surface area (SASA)	Geometrical

Table 5
Values of selected descriptors used in the regression analysis

Compound	log *P*	MR	$^{0}\chi$	$^{0}\chi^{v}$	$^{1}\chi$	$^{1}\chi^{v}$	$^{2}\chi$	$^{2}\chi^{v}$	Te	μ
1	3.63	64.39	10.96	9.26	7.84	5.45	6.96	4.02	−2574.06	3.435
2	3.63	64.39	10.96	9.26	7.83	5.44	7.06	4.08	−2574.16	2.337
3	3.63	64.39	10.96	9.26	7.83	5.44	7.07	4.08	−2574.17	1.900
4	3.11	59.58	10.09	8.14	7.43	4.94	6.43	3.47	−2214.07	2.724
5	3.07	66.91	12.54	9.33	8.74	5.43	7.97	3.91	−3044.85	7.434
6	3.07	66.91	12.54	9.33	8.74	5.43	7.95	3.91	−3044.88	5.781
7	3.07	66.91	12.54	9.33	8.75	5.44	7.89	3.88	−3044.69	6.533
8	2.81	66.34	12.54	9.42	8.75	5.53	7.89	3.95	−2983.47	2.576
9	2.86	66.05	11.66	9.47	8.36	5.46	7.23	3.83	−2689.95	1.899
10	2.83	61.28	10.96	8.51	7.83	5.07	7.06	3.65	−2534.68	1.528
11	4.70	91.94	16.52	13.37	11.75	7.83	10.37	5.72	−3909.44	1.937
12	4.70	91.94	16.52	13.37	11.74	7.83	10.46	5.78	−3909.52	2.313
13	4.70	91.94	16.52	13.37	11.74	7.83	10.46	5.78	−3909.52	2.313
14	3.89	88.83	16.52	12.63	11.74	7.45	10.46	5.35	−3870.04	1.091
15	4.13	94.46	18.10	13.44	12.65	7.82	11.36	5.60	−4380.22	4.847
16	4.13	94.46	18.10	13.44	12.65	7.82	11.37	5.61	−4380.22	4.477
17	4.13	94.46	18.10	13.44	12.66	7.83	11.30	5.57	−4380.11	4.823
18	4.13	94.46	18.10	13.44	12.66	7.83	11.30	5.57	−4380.11	4.823
19	3.93	93.60	17.23	13.59	12.29	7.85	10.56	5.50	−4025.20	3.154

Henceforth, n − number of data points, r − multiple correlation coefficient, r^2 − squared correlation coefficient, q^2 − cross-validated r^2 obtained by leave one out (LOO) technique, s - standard error of the estimate and F − Fischer statistics.

The negative coefficient of μ in Eq. (1) indicates that there is a negative correlation between the antibacterial activity of substituted benzimidazoles and dipole moment (μ). This is evidenced by the antibacterial activity data of substituted benzimidazoles (Table 3) and their μ values (Table 5). Compounds **13** and **14** with minimum μ values of 2.31 and 1.09 (Table 5), respectively, have maximum antibacterial activity against *E. coli* (compound **13**, $pMIC_{ec} = 1.80$; compound **14**, $pMIC_{ec} = 1.80$, Table 3). Compounds **5** and **7** which have maximum μ values showed minimum antibacterial activity against *E. coli* (compound **5**, $\mu = 7.43$; $pMIC_{ec} = 1.20$; compound **7**, $\mu = 6.53$; $pMIC_{ec} = 1.35$, Tables 3 and 5) due to the inverse relationship between μ and antibacterial activity.

The importance of dipole moment in modulating antibacterial activity against *E. coli* may be due to the presence of carbonyl group ($C^{+}-O^{-}$) where permanent polarization is seen due to electronegativity difference between the atoms. The carbonyl oxygen of substituted benzimidazoles may involve in making fruitful binding interactions with amino acid present at the target site, through hydrogen bonding. The molecular property dipole moment thus plays a critical role in modulating antibacterial profile of this class of compounds [43].

Squared correlation coefficient (r^2) of 0.884 in Eq. (1) explains 88.4% variance in antibacterial activity against *E. coli*. Eq. (1) also indicated statistical significance of >99.9% with F value of 129.47. Similarly the cross-validation of Eq. (1) was subsequently checked by employing "leave one out" (LOO) method [44]. The q^2 value of Eq. (1) ($q^2 = 0.859$; $q^2 > 0.5$) qualifies it to be a valid model according to recommendations of Golbraikh and Trophsa [45]. In order to confirm our results, we have predicted the antibacterial activity of substituted benzimidazoles against *E. coli* using Eq. (1). The comparison of the observed and the predicted values (Table 8) demonstrated that they are close to each other

Table 6
Correlation matrix for $pMIC_{ec}$ with molecular descriptors

	$pMIC_{ec}$	log *P*	MR	$^{0}\chi$	$^{0}\chi^{v}$	$^{1}\chi$	$^{1}\chi^{v}$	$^{2}\chi$	$^{2}\chi^{v}$	κ_1	κ_2	Te	μ
pMICec	1.000	0.211	0.037	−0.065	0.069	−0.026	0.093	−0.056	0.122	−0.075	−0.053	0.129	−0.940
log *P*		1.000	0.848	0.768	0.868	0.786	0.878	0.782	0.916	0.760	0.760	−0.750	−0.172
MR			1.000	0.987	0.998	0.993	0.997	0.987	0.986	0.985	0.988	−0.975	0.018
$^{0}\chi$				1.000	0.976	0.999	0.973	0.999	0.953	1.000	0.998	−0.997	0.127
$^{0}\chi^{v}$					1.000	0.984	0.999	0.976	0.992	0.974	0.977	−0.963	−0.020
$^{1}\chi$						1.000	0.982	0.997	0.962	0.998	0.999	−0.991	0.087
$^{1}\chi^{v}$							1.000	0.974	0.995	0.970	0.974	−0.958	−0.041
$^{2}\chi$								1.000	0.957	0.998	0.995	−0.996	0.120
$^{2}\chi^{v}$									1.000	0.949	0.950	−0.938	−0.072
κ_1										1.000	0.999	−0.997	0.136
κ_2											1.000	−0.992	0.115
Te												1.000	−0.189
μ													1.000

Table 7
Correlation of different molecular descriptors with antimicrobial activity of 2-(substituted phenyl)-1*H*-benzimidazole and [2-(substituted phenyl)-benzimidazol-1-yl]-pyridin-3-yl-methanones

	$pMIC_{sa}$	$pMIC_{bs}$	$pMIC_{ec}$	$pMIC_{ca}$	$pMIC_{an}$
log *P*	0.555	−0.876	0.211	0.666	−0.005
MR	0.352	−0.745	0.037	0.831	0.090
$^0\chi$	0.251	−0.667	−0.065	0.851	0.146
$^0\chi^v$	0.385	−0.771	0.069	0.810	0.053
$^1\chi$	0.281	−0.684	−0.026	0.848	0.129
$^1\chi^v$	0.405	−0.774	0.093	0.815	0.047
$^2\chi$	0.267	−0.670	−0.056	0.858	0.148
$^2\chi^v$	0.450	−0.803	0.122	0.800	0.025
κ_1	0.240	−0.661	−0.075	0.850	0.151
κ_2	0.249	−0.667	−0.053	0.845	0.145
$\kappa\alpha_1$	0.284	−0.697	−0.035	0.841	0.120
$\kappa\alpha_2$	0.313	−0.717	0.007	0.831	0.100
R	0.281	−0.684	−0.026	0.848	0.129
B	−0.273	0.116	−0.141	−0.101	−0.161
W	0.262	−0.687	−0.053	0.839	0.131
Te	−0.206	0.648	0.129	−0.850	−0.175
LUMO	0.273	0.136	0.640	−0.662	−0.646
μ	−0.790	0.215	−0.940	0.245	0.710

which is evident by the low residual activity values. Furthermore, it is supported by the plot of $pMIC_{ec}$ observed vs $pMIC_{ec}$ predicted (Fig. 2).

To determine the existence of the systemic error in the model development we have plotted $pMIC_{ec}$ observed against $pMIC_{ec}$ residual values (Fig. 3). The propagation of residuals on both sides of zero indicated that there is no systemic error in the development of QSAR model [46]. The QSAR models elicited in Eqs. (2)–(5) are developed to predict the antimicrobial activity of substituted benzimidazoles against *S. aureus*, *B. subtilis*, *C. albicans* and *A. niger*.

QSAR model for antibacterial activity against *S. aureus*

$$pMIC_{sa} = -0.082\mu + 1.778;$$
$$n = 19, r^2 = 0.625, q^2 = 0.506, s = 0.118, F = 28.32 \quad (2)$$

$$pMIC_{sa} = -0.337\mathrm{LUMO} - 0.133\mu + 1.642;$$
$$n = 19, r^2 = 0.894, q^2 = 0.760, s = 0.080, F = 40.37 \quad (3)$$

QSAR model for antibacterial activity against *B. subtilis*

$$pMIC_{bs} = -0.182\log P + 2.237;$$
$$n = 19, r^2 = 0.767, q^2 = 0.709, s = 0.067, F = 56.25 \quad (4)$$

QSAR model for antifungal activity against *C. albicans*

$$pMIC_{ca} = 0.081\,{}^2\chi + 0.849;$$
$$n = 19, r^2 = 0.735, q^2 = 0.665, s = 0.093, F = 47.31 \quad (5)$$

$$pMIC_{ca} = 0.079\,{}^2\chi + 0.014\mu + 0.815;$$
$$n = 19, r^2 = 0.756, q^2 = 0.622, s = 0.090, F = 24.82 \quad (6)$$

QSAR model for antifungal activity against *A. niger*

$$pMIC_{an} = 0.035\mu + 1.308;$$
$$n = 19, r^2 = 0.504, q^2 = 0.412, s = 0.065, F = 17.32 \quad (7)$$

Antibacterial activity of substituted benzimidazoles against *S. aureus* was significantly correlated with dipole moment (μ). As in the case of *E. coli*, here also a negative correlation was observed between the molecular descriptor (μ) and the antibacterial activity against *S. aureus* (Eq. (2)). Addition of LUMO to dipole moment, μ improved the correlation from 0.625 (Eq. (2)) to 0.894 (Eq. (3)).

Table 8
Observed and predicted antimicrobial activities of 2-(substituted phenyl)-1*H*-benzimidazole and [2-(substituted phenyl)-benzimidazol-1-yl]-pyridin-3-yl-methanones using the best QSAR models

Compound	$pMIC_{sa}$ (Eq. (3))			$pMIC_{bs}$ (Eq. (4))			$pMIC_{ec}$ (Eq. (1))			$pMIC_{ca}$ (Eq. (5))			$pMIC_{an}$ (Eq. (7))		
	Obs.	Pre.	Res.	Obs.	Pre.	Res.	Obs.	Pre.	Res.	Obs.	Pre.	Res.	Obs.	Pre.	Res.
1	1.40	1.33	0.07	1.49	1.58	−0.09	1.49	1.58	−0.09	1.26	1.41	−0.15	1.26	1.42	−0.16
2	1.66	1.58	0.08	1.51	1.58	−0.07	1.66	1.67	−0.01	1.56	1.42	0.14	1.40	1.39	0.01
3	1.49	1.59	−0.10	1.49	1.58	−0.09	1.65	1.71	−0.06	1.26	1.42	−0.16	1.36	1.37	−0.01
4	1.46	1.45	0.01	1.68	1.67	0.01	1.72	1.64	0.08	1.49	1.37	0.12	1.49	1.40	0.09
5	1.01	1.09	−0.08	1.78	1.68	0.10	1.20	1.24	−0.04	1.58	1.50	0.08	1.58	1.57	0.01
6	1.47	1.39	0.08	1.72	1.68	0.04	1.41	1.38	0.03	1.58	1.50	0.08	1.58	1.51	0.07
7	1.17	1.13	0.04	1.73	1.68	0.05	1.35	1.31	0.04	1.58	1.49	0.09	1.58	1.53	0.05
8	1.50	1.55	−0.05	1.70	1.73	−0.03	1.70	1.65	0.05	1.48	1.49	−0.01	1.28	1.39	−0.11
9	1.50	1.54	−0.04	1.71	1.72	−0.01	1.65	1.71	−0.06	1.25	1.44	−0.19	1.35	1.37	−0.02
10	1.53	1.61	−0.08	1.79	1.72	0.07	1.70	1.74	−0.04	1.43	1.42	0.01	1.33	1.36	−0.03
11	1.73	1.66	0.07	1.43	1.38	0.05	1.65	1.71	−0.06	1.73	1.69	0.04	1.43	1.37	0.06
12	1.73	1.64	0.09	1.43	1.38	0.05	1.73	1.67	0.06	1.73	1.70	0.03	1.43	1.38	0.05
13	1.79	1.64	0.15	1.50	1.38	0.12	1.80	1.67	0.13	1.73	1.70	0.03	1.43	1.38	0.05
14	1.70	1.79	−0.09	1.57	1.53	0.04	1.80	1.78	0.02	1.70	1.70	0.00	1.40	1.34	0.06
15	1.44	1.53	−0.09	1.44	1.49	−0.05	1.44	1.46	−0.02	1.74	1.77	−0.03	1.44	1.47	−0.03
16	1.44	1.50	−0.06	1.44	1.49	−0.05	1.44	1.49	−0.05	1.74	1.77	−0.03	1.44	1.46	−0.02
17	1.50	1.44	0.06	1.50	1.49	0.01	1.50	1.46	0.04	1.74	1.77	−0.03	1.44	1.47	−0.03
18	1.44	1.44	0.00	1.44	1.49	−0.05	1.44	1.46	−0.02	1.74	1.77	−0.03	1.44	1.47	−0.03
19	1.42	1.46	−0.04	1.42	1.52	−0.10	1.60	1.60	0.00	1.72	1.71	0.01	1.42	1.41	0.01

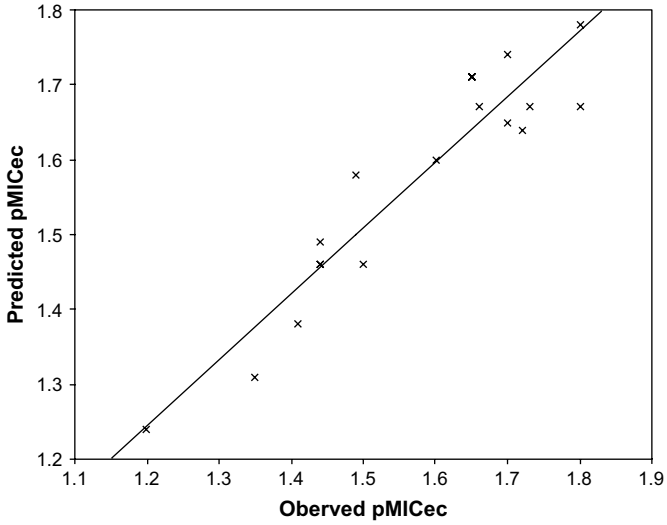

Fig. 2. Plot of predicted $pMIC_{ec}$ activity values against the experimental $pMIC_{ec}$ values for the linear regression developed model by Eq. (1).

The above bi-parametric model was used to predict the antibacterial activity of synthesized compounds against *S. aureus* (Table 8).

In the case of *B. subtilis*, a statistically significant inverse relationship was observed between antibacterial activity of substituted benzimidazoles and lipophilic parameter log *P* (Eq. (4)). This is evidenced by the low log *P* values (2.83–3.11, Table 5) and high $pMIC_{bs}$ values (1.68–1.79, Table 3) of most active compounds **4–10**.

The use of topological index, $^2\chi$ (the second order molecular connectivity index) significantly improved the correlation of antifungal activity of substituted benzimidazoles against *C. albicans* (Eq. (5)). The topological descriptor i.e., the second order molecular connectivity index encodes information related to the degree of stargraph likeness and takes a large value for more linear molecule [32]. The trend of Eq. (5) indicated that higher the $^2\chi$ value, the more will be the antifungal activity (compounds **15–17**; $^2\chi = 11.30–11.37$, Table 5; $pMIC_{ca} = 1.74$, Table 3) whereas the other compounds have minimum activity (Tables 3 and 5) due to their lower $^2\chi$ values. Here also when we attempted bi-parametric model using the least correlated parameter, μ we did not observe any significant improvement in the correlation [$r^2 = 0.735$ (Eq. (5)) to $r^2 = 0.756$ (Eq. (6))] as observed in the case of *S. aureus*.

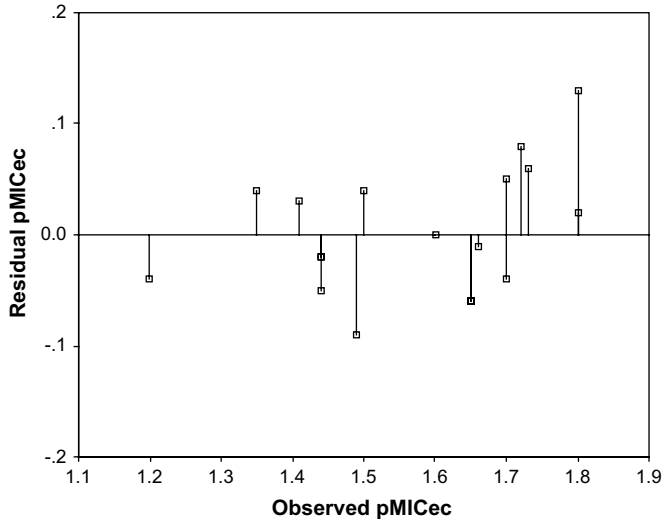

Fig. 3. Plot of residual $pMIC_{ec}$ values against the experimental $pMIC_{ec}$ values.

From Eq. (7) it is evident that the antifungal activity of substituted benzimidazoles against *A. niger* is significantly correlated with dipole moment (μ). In contrast to observations for *E. coli* and *S. aureus*, here a positive correlation with antifungal activity was observed. This further supports the fact that different structural requirements are necessary for antibacterial and antifungal activities.

Similar to Eq. (1) the high q^2 values ($q^2 > 0.5$) obtained by leave one out technique and the observance of low residual values (Table 8) indicated the validity and predictability of Eqs. (3)–(5) and Eq. (7) in the case of *S. aureus*, *B. subtilis*, *C. albicans* and *A. niger*, respectively.

It is important to note that Eqs. (1)–(7) are derived using the entire data set and no outliers were found during model development. Even though the sample size and the 'Rule of Thumb' allowed us to go for development of tetra-parametric model in multiple linear regression analysis, the high autocorrelation (Table 6) among the parameters restricted us for bi-parametric model. The 'rule of thumb' gives information about the number of parameters to be selected for regression analysis in QSAR based on the number of compounds [27]. According to this rule in the QSAR model development one should select one parameter for a five-compound data set.

The multi-collinearity occurs when two independent variables are correlated with each other. One should note that the change in signs of the coefficients, a change in the values of previous coefficient, change of significant variable into insignificant one or an increase in standard error of the estimate on addition of an additional parameter to the model are indications of high interrelationship among descriptors [27].

Generally for QSAR studies, the biological activities of compounds should span 2–3 orders of magnitude. But in the present study the range of antimicrobial activities of the synthesized compounds is within one order of magnitude. It is important to note that the predictability of the QSAR models developed in the present study is high evidenced by the low residual values (Table 8). This is in accordance with results suggested by the Bajaj et al. [47], who stated that the reliability of the QSAR model lies in its predictive ability even though the activity data are in the narrow range. Recent literature reveals that the QSAR has been applied to describe the relationship between narrow range of biological activity and physicochemical properties of the molecules [48–50]. When biological activity data lie in a narrow range, the presence of minimum standard deviation of the biological activity justifies its use in QSAR studies [24,29]. The minimum standard deviation (Table 3) observed in the antimicrobial activity data justifies its use in QSAR studies.

4. Conclusion

Summarizingly, a series of substituted benzimidazole derivatives have been synthesized successfully in appreciable yields and screened for their in vitro antimicrobial activity against bacterial strains *E. coli*, *S. aureus*, *B. subtilis* and fungal strains *A. niger* and *C. albicans*. The SAR studies indicate that the introduction of electron-withdrawing group will improve the antimicrobial activity of substituted benzimidazoles. QSAR analysis carried out to investigate the factors that may be important in describing the antimicrobial activity of substituted benzimidazoles indicated the importance of the electronic parameter, dipole moment (μ) followed by the lipophilic parameter, log of octanol water partition coefficient (log *P*) and the topological parameter, second order molecular connectivity index ($^2\chi$). Further the obtained regression models present a good capacity to explain the observed values of antimicrobial activity, high statistical significance and predictive capacity.

5. 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 on silica gel sheets (Merck silica gel-G) and the purity of the compounds was ascertained by single spot on TLC sheet. ^{1}H nuclear magnetic resonance (^{1}H 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.

*5.1. General procedure for the synthesis of 2-(substituted phenyl)-1H-benzimidazoles (**1–10**)*

Substituted anilines (0.13 mol) in hydrochloric acid/water mixture (1:1) were diazotized using a solution of sodium nitrite at 0–10 °C. To the diazotized mixture, benzimidazole (0.004 mol) was added with vigorous shaking. A solution of sodium acetate (40 g in 100 ml) was added dropwise 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 obtained was filtered, dried and recrystallized from alcohol.

*5.2. General procedure for the synthesis of [2-(substituted phenyl)-benzimidazol-1-yl]-pyridin-3-yl-methanones (**11–19**)*

A solution of 2-(substituted phenyl)-1*H*-benzimidazoles (**1–10**) (0.002 mol) in diethyl ether (50 ml) was added to 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 was isolated by evaporation of ether and purified by recrystallization with methanol.

5.3. Evaluation of antimicrobial activity

The antimicrobial activity was performed against Gram-positive bacteria: *S. aureus*, *B. subtilis*, Gram-negative bacterium: *E. coli* and fungal strains: *C. albicans* and *A. niger*. The standard and test samples were dissolved in DMSO to give a concentration of 100 μg/ml. The minimum inhibitory concentration (MIC) was determined by tube dilution method. Twofold dilutions of test and standard compounds were prepared in double strength nutrient broth – I.P. (bacteria) or Sabouraud dextrose broth I.P. [51] (fungi). The samples were incubated at 37 °C (bacteria) for 24 h, 25 °C for 7 days (*A. niger*) and 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 microorganism).

5.4. QSAR studies

The details of the molecular descriptors are available in literature and can be easily accessed [35–41]. The molecular descriptors for the substituted benzimidazoles were calculated using the TSAR 3.3 software package for windows [42]. Regression analysis was performed using the SPSS software package [52]. The predictive powers of the equation were validated by the determination of cross-validated r^2 (q^2) using the leave one out (LOO) cross-validation method.

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