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RESEARCH ARTICLE

Synthesis, antimicrobial and antiviral activity of substituted benzimidazoles

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Abstract

In the present study we have synthesized (4-nitrophenyl)-[2-(substituted phenyl)-benzimidazol-1-yl]-methanones, (2-bromophenyl)-[2-(substituted phenyl)-benzimidazol-1-yl]-methanone analogues (**1–14**) and evaluated them for their antimicrobial and antiviral potential. The results of antimicrobial screening indicated that none of the synthesized compounds were effective against the tested bacterial strains. Compounds **3**, **11**, **13** and compounds **5**, **11**, **12** were found to be active against *Aspergillus niger* and *Candida albicans* respectively, and may be further developed as antifungal agents. Furthermore, evaluation against a panel of different viruses pointed out the selective activity of compounds **5** and **6** against vaccinia virus and Coxsackie virus B4.

Keywords: Substituted benzimidazoles; antifungal activity; antiviral activity

Introduction

The herpes simplex viruses HSV-1 and HSV-2 are of clinically relevance among the eight known human herpes viruses belongs to the family of the *Herpesviridae* [1]. HSV virions are 180–200 nm in diameter and contain an icosahedral capsid surrounding the linear double-stranded DNA genome. Cellular infection is initiated when the virus binds to heparin sulfate chains on cell surface proteoglycans [2]. HSV-1 is responsible for the lesions at orofacial sites that are commonly known as cold sores. HSV-2 is responsible for mucocutaneous genital infections [3]. Much research has been focused on HSV-1 and HSV-2 as these viruses have a high incidence rate (1.6 million new cases of HSV-2 predicted per year in the United States) and prevalence [50–95% (HSV-1) and 6–50% (HSV-2)] [4].

The alpha viruses are pathogenic viruses with worldwide distribution and causes fever, rash, arthralgia or arthritis, lassitude, headache, and myalgia. There is presently no treatment available. Alphaviruses are transmitted to humans through mosquito bites [5]. VSV is one of the most carefully

studied virus and its replication strategy forms a valid model for the replication of all Mononegavirales viruses and provides important insights for the study of replication of other viruses with negative-sense RNA genomes [6].

Variola, monkeypox, cowpox, and vaccinia viruses are orthopoxviruses, which can infect humans. Among orthopoxviruses, variola virus, that causes smallpox, is of clinical relevance. Smallpox virus was responsible for serious illness and death until the development of successful vaccine [7].

Benzimidazole derivatives are structural isosteres of naturally occurring nucleotides, which allows them to interact easily with the biopolymers of the living systems [8]. Numerous biological activities and functions have been described for benzimidazole nucleus *viz.* antihypertensive [9], antifungal [10], antioxidant [11], antiviral [12] and topoisomerase inhibitory [13] activities. Literature reports revealed that the benzimidazole derivatives also possessed antiviral activity against various strains of pathogenic

viruses *viz.* hepatitis virus [14], human immunodeficiency virus (HIV) [15], enteroviruses [16], respiratory syncytial virus (RSV) [17], human cytomegalovirus virus (HCMV) and varicella-zoster virus (VZV) [18].

Disease-causing microbes that have become resistant to drug therapy are an increasing public health problem nowadays [19]. There is a real need for new chemical entities endowed with antimicrobial activity, possibly acting through mechanisms, which are distinct from the well known classes of antimicrobial agents, to which many clinically relevant pathogens have become resistant [20]. Benzimidazole derivatives have been reported to possess antimicrobial activities [21–23].

Prompted by the chemotherapeutic importance of benzimidazole derivatives especially in treating microbial and viral infections as well in continuation of our ongoing search for novel anti-infective agents [24–34], in the present study we hereby report the synthesis, antimicrobial and antiviral activity of (4-nitrophenyl)-[2-(substituted phenyl)-benzoimidazol-1-yl]-methanones, (2-bromophenyl)-[2-(substituted phenyl)-benzoimidazol-1-yl]-methanone analogues (**1–14**). Further we have mentioned the antiviral potential of [2-(substituted phenyl)-benzoimidazol-1-yl]-pyridin-3-yl-methanones (**15–23**) synthesized in our previous report [35].

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 TLC. ¹H-Nuclear magnetic resonance (¹H NMR) spectra were recorded on a Bruker Avance II 400 NMR spectrometer using appropriate deuterated solvents and are expressed in parts per million (δ , ppm) downfield from tetramethylsilane (internal standard). Infrared (IR) spectra were recorded on a Shimadzu FTIR spectrometer.

General procedure for the synthesis of (4-nitrophenyl)-[2-(substituted phenyl)-benzoimidazol-1-yl]-methanones, (2-bromophenyl)-[2-(substituted phenyl)-benzoimidazol-1-yl]-methanones (1–14)

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 (40g in 100 mL of distilled water) was added drop wise to the above mixture by maintaining the temperature at 5–10 °C. The above solution was stirred initially for 3 h under cold conditions followed by continuation of stirring at room temperature for 48 h. The product thus obtained was filtered, dried and recrystallized from alcohol to yield the intermediate compounds 2-(substituted phenyl)-1-*H*-benzimidazoles. A solution of 2-(substituted phenyl)-1-*H*-benzimidazoles (0.002 mol) in diethyl ether (50 mL) was added to a solution of corresponding acid 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 recrystallisation from methanol (Table 1).

(4-nitrophenyl)-[2-(2-nitrophenyl)-benzoimidazol-1-yl]-methanone (1)

mp (°C) – 154–156; Yield – 52.73 %; ¹H NMR spectra CDCl₃ δ ppm: 7.26–7.70 (m, 4H, ArH of benzimidazole), 7.71–8.05 (m, 4H, ArH of ArNO₂ attached to 2nd position of benzimidazole), 8.07–8.38 (m, 4H, ArH of ArNO₂ attached to N₁ of benzimidazole); IR (KBr pellets): cm⁻¹ 1427.6, 1540.4 (Skeletal bands), 1693.8 (C=O, str. tertiary amide), 1349.4 (NO₂ sym str., ArNO₂), 1492.3 (NO₂ asym. str., ArNO₂); CHN analysis calc.(found): C₂₀H₁₂N₄O₅: C, 61.86 (60.81); H, 3.11 (3.05); N, 14.43 (14.19); O, 20.60 (20.32) %.

2-[1-(4-nitrobenzoyl)-1H-benzoimidazol-2-yl]-benzoic acid (2)

mp (°C) – 209–211; Yield – 14.09 %; ¹H NMR spectra CDCl₃ δ ppm: 7.22–7.61 (m, 4H, ArH of benzimidazole), 7.73–8.02 (m, 4H, ArH of ArCOOH), 8.09–8.33 (m, 4H, ArH of ArNO₂ attached to N₁ of benzimidazole), 11.0 (s, 1H, COOH of ArCOOH); IR (KBr pellets): cm⁻¹ 1427.5, 1540.6 (Skeletal bands), 1693.0 (C=O, str. tertiary amide), 1349.9 (NO₂ sym. str., ArNO₂), 1496.3 (NO₂ asym. str., ArNO₂), 1106.9 (C-O str., COOH); CHN analysis calc.(found): C₂₁H₁₃N₃O₅: C, 65.12 (65.01); H, 3.38 (3.05); N, 10.85 (10.19); O, 20.65 (20.30) %.

[2-(4-Hydroxyphenyl)-benzoimidazol-1-yl]-(4-nitrophenyl) methanone (3)

mp (°C) – 229–231; Yield – 16.09 %; ¹H NMR spectra CDCl₃ δ ppm: 7.24–7.65 (m, 4H, ArH of benzimidazole), 6.79–7.21 (m, 4H, ArH of ArOH), 8.07–8.22 (d, 2H, ArH of ArNO₂ attached to N₁ of benzimidazole), 5.07 (s, 1H, OH of ArOH); IR (KBr pellets): cm⁻¹ 1428, 1541.5 (Skeletal bands), 1692.5 (C=O, str. tertiary amide), 1350.2 (NO₂ sym. str., ArNO₂), 1495.2 (NO₂ asym. str., ArNO₂), 3615.2 (O-H str, ArOH); CHN analysis calc.(found): C₂₀H₁₃N₃O₄: C, 66.85 (66.01); H, 3.65 (3.05); N, 11.69 (11.09); O, 17.81 (17.30) %.

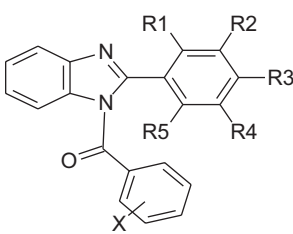
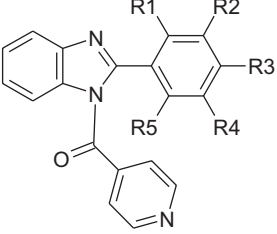
[2-(2-Methoxyphenyl)-benzoimidazol-1-yl]-(4-nitrophenyl) methanone (4)

mp (°C) – 149–151; Yield – 56.72 %; ¹H NMR spectra CDCl₃ δ ppm: 7.30–7.70 (m, 4H, ArH of benzimidazole), 6.83–7.23 (m, 4H, ArOCH₃ attached to 2nd position of benzimidazole), 8.10–8.32 (m, 4H, ArH of ArNO₂ attached to N₁ of benzimidazole), 3.90 (s, 3H, OCH₃); IR (KBr pellets): cm⁻¹ 1427.9, 1539.7 (Skeletal bands), 1694.0 (C=O, str. tertiary amide), 1349.5 (NO₂ sym. str., ArNO₂), 3063.5 (aralkyl ethers); CHN analysis calc.(found): C₂₁H₁₅N₃O₄: C, 67.56 (67.09); H, 4.05 (3.95); N, 11.25 (11.19); O, 17.14 (17.00) %.

(2-Bromophenyl)-[2-(4-nitrophenyl)-benzoimidazol-1-yl]-methanone (11)

mp (°C) – 149–151; Yield – 34.23 %; ¹H NMR spectra CDCl₃ δ ppm: 7.36–7.67 (m, 4H, ArH of benzimidazole), 7.74–8.25 (m,

Table 1. Physicochemical characteristics of synthesized benzimidazole derivatives.

 1-14							 15-23 ^a				
Compound	R ₁	R ₂	R ₃	R ₄	R ₅	X	Mol. formula	Mol. wt.	Mp (°C)	R _f	% yield
1	NO ₂	H	H	H	H	4-NO ₂	C ₂₀ H ₁₂ N ₄ O ₅	388.33	154–156	0.10*	52.73
2	COOH	H	H	H	H	4-NO ₂	C ₂₁ H ₁₃ N ₄ O ₅	387.35	209–211	0.48*	14.09
3	H	H	OH	H	H	4-NO ₂	C ₂₀ H ₁₃ N ₃ O ₄	359.34	229–231	0.47*	16.07
4	OCH ₃	H	H	H	H	4-NO ₂	C ₂₁ H ₁₅ N ₃ O ₄	373.36	149–151	0.35*	56.72
5	H	H	Cl	H	H	4-NO ₂	C ₂₀ H ₁₂ N ₃ O ₃ Cl	377.78	219–221	0.90*	41.56
6	H	H	NO ₂	H	H	4-NO ₂	C ₂₀ H ₁₂ N ₄ O ₅	388.33	139–141	0.12*	45.54
7	H	H	H	H	H	4-NO ₂	C ₂₀ H ₁₂ N ₃ O ₃	343.34	199–201	0.70*	39.80
8	H	NO ₂	H	H	H	4-NO ₂	C ₂₀ H ₁₂ N ₄ O ₅	388.33	209–211	0.90*	48.65
9	H	Cl	H	H	H	4-NO ₂	C ₂₀ H ₁₂ N ₃ O ₃ Cl	377.78	229–231	0.55*	43.98
10	Cl	H	H	H	H	4-NO ₂	C ₂₀ H ₁₂ N ₃ O ₃ Cl	377.78	219–221	0.83*	38.65
11	H	H	NO ₂	H	H	2-Br	C ₂₀ H ₁₂ BrN ₃ O ₃	422.23	149–151	0.21*	34.23
12	H	H	OH	H	H	2-Br	C ₂₀ H ₁₃ BrN ₂ O ₂	393.23	149–151	0.16*	47.65
13	COOH	H	H	H	H	2-Br	C ₂₁ H ₁₃ BrN ₂ O ₃	421.24	139–141	0.28*	57.68
14	NO ₂	H	H	H	H	2-Br	C ₂₀ H ₁₂ BrN ₃ O ₃	422.23	129–131	0.60**	47.39
15	Cl	H	H	H	H	-	C ₁₉ H ₁₂ N ₃ OCl	333.83	84–86	0.95**	39.13
16	H	H	Cl	H	H	-	C ₁₉ H ₁₂ N ₃ OCl	333.83	224–226	0.48**	47.82
17	H	Cl	H	H	H	-	C ₁₉ H ₁₂ N ₃ OCl	333.83	149–151	0.59**	73.91
18	H	H	OH	H	H	-	C ₁₉ H ₁₃ N ₃ O ₂	315.37	64–66	0.15**	11.20
19	H	H	NO ₂	H	H	-	C ₁₉ H ₁₂ N ₄ O ₃	344.30	244–246	0.22**	48.59
20	H	NO ₂	H	H	H	-	C ₁₉ H ₁₂ N ₄ O ₃	344.30	239–241	0.31**	70.09
21	NO ₂	H	H	H	H	-	C ₁₉ H ₁₂ N ₄ O ₃	344.30	49–51	0.90**	14.95
22	COOH	H	H	H	H	-	C ₂₀ H ₁₃ N ₃ O ₃	343.41	139–141	0.13**	45.08
23	OCH ₃	H	H	H	H	-	C ₂₀ H ₁₅ N ₃ O ₂	329.39	99–101	0.90**	14.20

*Benzene (care-carcinogenic). **Toluene:Chloroform (7:3)

^aReported in Sharma D, Narasimhan B, Kumar P, Jalbout A, *Eur. J. Med. Chem.* 2008 (In Press)

4H, ArH of ArNO₂ attached to 2nd position of benzimidazole), 7.09–7.25 (m, 4H, ArH of ArBr); IR (KBr pellets): cm⁻¹ 1430.2, 1540.6 (Skeletal bands), 1684.1 (C=O, str. tertiary amide), 1498.2 (NO₂ asym. str., ArNO₂), 553.2 (C-Br, str.); CHN analysis calc.(found): C₂₀H₁₂N₃O₃Br: C, 56.89 (56.01); H, 2.86 (2.76); N, 9.95 (9.19); O, 11.37 (11.30); Br 18.92 (18.54) %.

(2-Bromophenyl)-[2-(4-hydroxyphenyl)-benzimidazol-1-yl]-methanone (12)

mp (°C) - 149–151; Yield - 47.65 %; ¹H NMR (CDCl₃) δ ppm: 7.39–7.70 (m, 4H, ArH of benzimidazole), 7.05–7.22 (m, 4H, ArH of ArBr), 6.79–6.99 (m, 4H, ArH of ArOH), 5.0 (s, 1H, OH of ArOH); IR (KBr pellets): cm⁻¹ 1432.0, 1535.2 (Skeletal bands), 1684.8 (C=O, str. tertiary amide), 3618.5 (O-H str., ArOH), 552.9 (C-Br, str.); CHN analysis calc.(found): C₂₀H₁₃N₂O₂Br: C, 61.09 (60.91); H, 3.33 (2.96); N, 7.12 (7.02); O, 8.14 (8.07); Br 20.32 (20.06) %.

2-[1-(2-Bromobenzoyl)-1H-benzimidazol-2-yl]-benzoic acid (13)

mp (°C) - 139–141; Yield - 57.68 %; ¹H NMR (CDCl₃) δ ppm: 7.33–7.62 (m, 4H, ArH of benzimidazole), 7.78–8.19 (m, 4H,

ArH of ArCOOH), 7.04–7.25 (m, 4H, ArH of ArBr), 11.0 (s, 1H, COOH of ArCOOH); IR (KBr pellets): cm⁻¹ 1430.9, 1540.2 (Skeletal bands), 1683.6 (C=O str., tertiary amide), 1116.0 (C-O str., COOH), 551.1 (C-Br, str.); CHN analysis calc.(found): C₂₁H₁₃N₂O₃Br: C, 59.88 (59.01); H, 3.11 (2.79); N, 6.65 (6.54); O, 11.39 (11.20); Br 18.97 (18.34) %.

Evaluation of antimicrobial activity

Determination of MIC

The antimicrobial activity was performed against the Gram-positive bacteria *Staphylococcus aureus*, *Bacillus subtilis*, Gram-negative bacterium *Escherichia coli* and the fungal species *Candida albicans* and *Aspergillus niger*. The standard and test samples were dissolved in DMSO and the minimum inhibitory concentration (MIC) was determined by tube dilution method. Dilutions of test and standard compounds were prepared in double strength nutrient broth - I.P. (bacteria) or Sabouraud dextrose broth - I.P. [36] (fungi). The samples were incubated at 37 °C for 24h (bacteria), 25 °C for 7 d (*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).

Determination of MBC/MFC

The minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were determined by subculturing 100 μ L of culture from each tube that remained clear in the MIC determination into fresh agar medium. MBC and MFC values represents the lowest concentration of compound that produces a 99.9% end point reduction of microorganism [37].

Evaluation of antiviral activity

Antiviral assay

The antiviral activity of the (4-nitrophenyl)-[2-(substituted phenyl)-benzimidazol-1-yl]-methanones, (2-bromophenyl)-[2-(substituted phenyl)-benzimidazol-1-yl]-methanones (**1–14**) and [2-(substituted phenyl)-benzimidazol-1-yl]-pyridin-3-yl-methanones (**15–23**) was determined using a CPE reduction assay [38] against herpes simplex virus-1 (KOS), herpes simplex virus-2 (G), vaccinia virus, vesicular stomatitis virus, herpes simplex virus-1 TK⁻ KOS ACV^r in HEL cell cultures, vesicular stomatitis virus (VSV), Coxsackie virus B4, respiratory syncytial virus (RSV) in HeLa cell cultures and parainfluenza-3 virus, reovirus-1, Sindbis virus, Coxsackie virus B4, Punta Toro virus in Vero cell cultures and the results were expressed as the 50% effective concentration (EC_{50}). Cells, grown to confluency in 96-well plates, were infected with 100 CCID₅₀ of virus, one CCID₅₀ being the 50% cell culture infective dose. After an adsorption period of 2 h at 37 °C, the virus was removed and serial dilutions of the compounds were added. The cultures were further incubated at 37 °C for 3 days, until complete CPE was observed in the infected and untreated virus control.

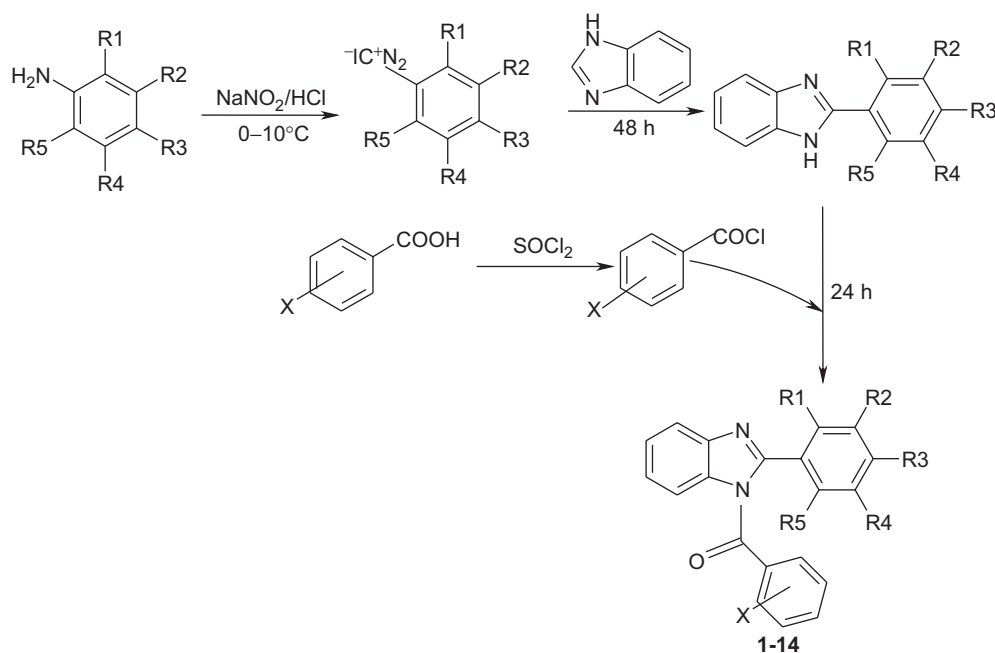
Cytotoxic assay

The cytotoxicity of the compounds was evaluated in parallel with their antiviral activity in uninfected cells, and is expressed as minimum cytotoxic concentration that causes a microscopically detectable alteration of normal cell morphology (HEL cells, HeLa cells, and Vero cells).

Results and discussion

Chemistry

The synthesis of compounds **1–14** followed the general pathway depicted in Scheme 1. The key intermediates, 2-(substituted phenyl)-1*H*-benzimidazoles were prepared by the condensation of benzimidazoles with corresponding substituted aryl diazonium 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 aryl diazonium chloride with benzimidazole as suggested by Dahiya and Pathak [39] resulted in resinous products. Therefore, the coupling was carried out using sodium acetate along with stirring under cold conditions for initial 3 h followed by 48 h stirring at room temperature, which resulted in a solid product. For the synthesis of (4-nitrophenyl)-[2-(substituted phenyl)-benzimidazol-1-yl]-methanones, (2-bromophenyl)-[2-(substituted phenyl)-benzimidazol-1-yl]-methanones (**1–14**), the key intermediates were reacted with substituted benzoyl chloride which was prepared by the reaction of substituted benzoic acid with thionyl chloride. The physicochemical characteristics of the synthesized compounds are presented in Table 1. The synthesized compounds **1–14** were characterized by their IR and ¹H-NMR as well by elemental analysis studies. The elemental analysis results were within $\pm 0.4\%$ of the theoretical values.



Scheme 1. Synthetic scheme for the synthesis of substituted benzimidazole derivatives.

The appearance of IR bands at 3615 cm^{-1} and 3618 cm^{-1} in the spectra of compounds **3** and **12** indicated the presence of an OH group on the aromatic ring substituted at the second position of the benzimidazole. The appearance of IR bands in the range of 555 cm^{-1} to 550 cm^{-1} corresponds to the C-Br stretch of Ar-Br of compounds **11**, **12** and **13**. The presence of aromatic nitro groups in compounds **1**, **2**, **3**, **4** and **11** was indicated by the appearance of symmetric and asymmetric NO_2 stretches in the range of 1350 cm^{-1} - 1345 cm^{-1} and 1500 cm^{-1} - 1495 cm^{-1} , respectively, in their IR spectra. The presence of an aralkyl ether group (Ar-OCH₃) in compound **4** was confirmed by the presence of an IR band at 3063.5 cm^{-1} in its IR spectra. The presence of COOH group in compound **2** and **13** was confirmed by the appearance of C=O stretching and C-O stretching at 1603 cm^{-1} and 1106 cm^{-1} , respectively. Further, the appearance of strong C=O stretching bands at $1695\text{--}1680\text{ cm}^{-1}$ in the IR spectra of (4-nitrophenyl)-[2-(substituted phenyl)-benzimidazol-1-yl]-methanones, (2-bromophenyl)-[2-(substituted phenyl)-benzimidazol-1-yl]-methanones (**1–14**) demonstrated the presence of a tertiary amide linkage between the substituted benzoyl chloride and the N₁ of benzimidazole nucleus.

The ¹H-NMR spectra of the synthesized compounds showed a signal at δ 7.26–7.70 ppm corresponding to Ar-H of the benzimidazole nucleus. The appearance of a multiplet signal at δ 8.07–8.38 ppm indicated the presence of four aromatic protons in Ar-NO₂ (**1–4**) attached to N₁ of benzimidazole. The appearance of signal at δ 5 ppm corresponding to the proton of the OH group confirmed the formation of compound **3** and **12**. The presence of a signal around δ 11 ppm in the ¹H-NMR spectra indicated the presence of a free COOH group in compounds **2** and **13**. Furthermore, the absence of a singlet signal corresponding to the COOH in the NMR spectra of other synthesized compounds supports the complete reaction of acid chlorides with N₁ hydrogen of benzimidazole nucleus and also indicated the absence of free aromatic acids in the final products. The presence of a bromo substituted benzene ring (**11**, **12** and **13**) was identified by the existence of multiplet signals at δ 7.39–7.70 ppm in the NMR spectra. The signal at δ 3.90 ppm in the proton spectra demonstrated the presence of an aralkyl ether group in compound **4**.

Antimicrobial activity

The synthesized compounds were screened for their *in vitro* antimicrobial activities against two Gram positive bacteria - *S. aureus*, *B. subtilis*; Gram negative bacterium - *E. coli* and the fungal species *A. niger* and *C. albicans* by tube dilution method [40], using norfloxacin and fluconazole as control drugs for antibacterial and antifungal activity, respectively. Based on the MIC values observed, the minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were determined by subculturing 100 μL of culture from each tube that remained clear in the MIC determination into fresh medium. The results of antimicrobial studies are presented in Tables 2 and 3. The data shown in Table 2 represents the minimum inhibitory concentration of synthesized substituted benzimidazoles against

Table 2. Antimicrobial activity (MIC) of synthesized benzimidazole derivatives.

Compound	Minimum Inhibitory Concentration ($1 \times 10^{-3} \mu\text{M/mL}$)				
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>A. niger</i>	<i>C. albicans</i>
1	36	18	36	18	36
2	33	266	33	16	33
3	35	35	35	17	35
4	33	33	33	33	8
5	17	33	33	17	4
6	36	36	36	18	9
7	146	146	146	18	9
8	18	18	295	18	18
9	17	33	17	33	17
10	33	33	33	17	17
11	15	15	15	15	7
12	32	32	32	32	4
13	15	30	15	15	30
14	30	30	30	30	15
Std	2*	2*	2*	1**	1**

*Norfloxacin **Fluconazole

Table 3. MBC and MFC values of synthesized benzimidazole derivatives.

Compound	Minimum Bactericidal/fungicidal Concentration ($1 \times 10^{-3} \mu\text{M/mL}$)				
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>A. niger</i>	<i>C. albicans</i>
1	133	66	133	266	295
2	266	-	266	139	133
3	278	278	278	17	69
4	267	267	267	133	33
5	132	264	264	132	17
6	296	296	296	133	36
7	-	-	-	146	18
8	147	147	-	18	147
9	132	264	132	264	132
10	264	264	264	34	132
11	118	118	118	15	14
12	254	254	254	64	16
13	118	236	118	15	118
14	236	236	236	30	58
Std	19*	19*	19*	40**	40**

*Norfloxacin **Fluconazole

the representative organisms. With respect to antibacterial activity none of the synthesized compounds showed activity comparable to the standard norfloxacin in both MIC (Table 2) and MBC (Table 3) determination. Similar results were observed for the antifungal activity of synthesized compounds against *C. albicans* and *A. niger* on the basis of their MIC (Table 2) i.e. none of the synthesized compounds were more active than standard fluconazole. However, in contrast to MBC results, the MFC results yielded better results against *A. niger*.

Compounds **3**, **8**, **10**, **11**, **13** and **14** demonstrated MFC values less than the standard fluconazole (Table 3). Similarly, in the case of *C. albicans* compounds **4–7** and **10–11** expressed MFC values lesser than the standard. Analysis of the structures of most active antifungal compounds indicated the fact that presence of an electron withdrawing group on the

benzene ring substituted at second position as well on the acyl benzene ring substituted at N₁ of benzimidazole favored the antifungal activity of the synthesized compounds. The role of electron withdrawing group in improving antimicrobial activities is supported by the studies of Sharma *et al* [41]. The compounds (**4**, **5**, **6**, **7** and **12**) which are effective against *C. albicans* were found to be inactive against *A. niger* and the reverse is true for compounds (**3**, **8**, **10**, **13** and **14**) which were effective against *A. niger*. This indicated the fact that different structural requirements are needed for the binding of drug to different fungal targets such as *A. niger* and *C. albicans* [42]. In general, the compounds showed better antifungal activity.

The MIC results are different from MFC/MBC results as MIC was determined by the absence of turbidity in the culture tubes which are inoculated with representative microorganism after the addition of test drug. It may be possible that the presence of one or few organisms cannot be detected by determining the turbidity of the solution. In this case, the determination of MBC and MFC may give useful information which involves subculturing of 100 µL of culture from each tube that remained clear in the MIC determination into fresh medium. This is the reason for the fact that MBC/MFC values (Table 3) of test compounds are observed at high concentration than their MIC values (Table 2). In general,

the MFC and MBC values of synthesized benzimidazole derivatives were 3-fold higher than the MIC values which indicated that the synthesized compounds were bacteriostatic and fungistatic in action. (A drug is considered to be bacteriostatic/fungistatic when its MFC and MBC values are 3-fold higher than its MIC value) [43–44].

Antiviral activity

The antiviral evaluation of synthesized compounds (**1–23**) against Herpes simplex virus-1 (KOS) [HSV-1 KOS], Herpes simplex virus-2 (G) [HSV-2G], Vaccinia virus [VV], Vesicular stomatitis virus [VSV], Herpes simplex virus-1 TK⁻ KOS ACV^r [HSV-1 TK⁻ KOS ACV^r] in HEL cell cultures, Vesicular stomatitis virus (VSV), Coxsackie virus B4 [CV-B4], Respiratory syncytial virus [RSV] in HeLa cell cultures and Parainfluenza-3 virus [PI-3V], Reovirus-1 [RV-1], Sindbis virus [SV], Coxsackie virus B4 [CV-B4], Punta Toro virus [PTV] in Vero cell cultures was determined using CPE reduction assay.

With the exception of compounds **5** and **6** against VV, no specific antiviral effects (MCC ≥ 5-fold higher than EC₅₀) were noted for any of the compounds in HEL cells. Compounds **5** and **6** were active against VV at an EC₅₀ of 4 and 2 µg/mL, respectively, while not being cytotoxic at a concentration as high as 100 µg/mL (Table 4).

Table 4. Antiviral activity of synthesized benzimidazole derivatives in HEL cell culture.

Compound	MCC ^a (µg/mL)	EC ₅₀ ^b (µg/mL)				
		Herpes simplex virus-1 (KOS)	Herpes simplex virus-2 (G)	Vaccinia virus	Vesicular stomatitis virus	Herpes simplex virus-1 TK ⁻ KOS ACV ^r
1	>100	>100	>100	>100	>100	>100
2	>100	>100	>100	>100	>100	>100
3	>100	>100	>100	>100	>100	>100
4	>100	>100	>100	>100	>100	>100
5	>100	>100	>100	4	>100	>100
6	>100	100	50	2	45	59
7	100	>20	>20	>20	>20	>20
8	100	>20	>20	10	>20	>20
9	-	-	-	-	-	-
10	>100	>100	>100	>100	>100	>100
11	>100	>100	>100	>100	>100	>100
12	>100	>100	>100	>100	>100	>100
13	>100	>100	>100	>100	>100	>100
14	>100	>100	>100	>100	>100	>100
15	100	>20	>20	>20	>20	>20
16	100	>20	>20	>20	>20	>20
17	>100	>100	>100	>100	>100	>100
18	20	>4	>4	>4	>4	>4
19	>100	>100	>100	>100	>100	>100
20	>100	>100	>100	>100	>100	>100
21	100	>20	>20	>20	>20	>20
22	>100	>100	>100	>100	>100	>100
23	100	>20	>20	>20	>20	>20
Brivudin	>250	0.04	29	5	>250	96
Ribavirin	>250	>250	>250	>250	146	>250
Cidofovir	>250	3	2	7	>250	2
Ganciclovir	>100	0.06	0.06	>100	>100	1

^aRequired to cause a microscopically detectable alteration of normal cell morphology.

^bRequired to reduce virus-induced cytopathogenicity by 50 %.

Table 5. Antiviral activity of synthesized benzimidazole derivatives in HeLa cell culture.

Compound	MCC ^a (µg/mL)	EC ₅₀ ^b (µg/mL)		
		Vesicular stomatitis virus	Coxsackie virus B4	Respiratory syncytial virus
1	>100	>100	>100	>100
2	>100	>100	>100	>100
3	>100	>100	10	>100
4	>100	>100	>100	>100
5	100	>20	>20	>20
6	100	>20	>20	>20
7	100	>20	9	>20
8	100	>20	>20	>20
9	-	-	-	-
10	>100	>100	>100	>100
11	>100	>100	>100	>100
12	>100	>100	>100	>100
13	>100	>100	>100	>100
14	>100	>100	>100	>100
15	100	>20	>20	>20
16	>100	>100	>100	>100
17	>100	>100	>100	>100
18	20	>4	>4	>4
19	>100	>100	>100	>100
20	>100	>100	>100	>100
21	100	>20	>20	>20
22	>100	>100	>100	>100
23	20	>4	>4	>4
DS-5000	>100	>100	9	0.8
(S)-DHPA	>250	>250	>250	>250
Ribavirin	>250	29	146	10

^aRequired to cause a microscopically detectable alteration of normal cell morphology.

^bRequired to reduce virus-induced cytopathogenicity by 50 %.

In HeLa cell cultures, compounds **3** and **7** showed an EC₅₀ of 9–10 µg/mL (Table 5) for Coxsackie B4 virus, but in Vero cells compound **3** was inactive against Coxsackie B4, and compound **7** was not found to be active (MCC < 5-fold higher than EC₅₀) (Table 6). In fact, none of the compounds emerged as specifically antiviral compounds in Vero cell cultures (Table 6).

Conclusion

In an effort to discover new (4-nitrophenyl)-[2-(substituted phenyl)-benzoimidazol-1-yl]-methanone, (2-bromophenyl)-[2-(substituted phenyl)-benzoimidazol-1-yl]-methanone (**1–14**) analogues as antimicrobial agents we found that none of the synthesized compounds emerged as effective antibacterial compound. Compounds **3**, **11** and **13** were found to be effective against *A. niger* and compounds **5**, **11** and **12** were found to be effective against *C. albicans*. These compounds were effective at less than half the concentration of the standard drug fluconazole. Further, the antiviral evaluation results of the synthesized compounds indicated that compounds **5** and **6** have the

Table 6. Antiviral activity of synthesized benzimidazole derivatives in Vero cell culture.

Compound	MIC ^a (µg/mL)	EC ₅₀ ^b (µg/mL)				
		Para-influenza -3 virus	Reovirus -1	Sindbis virus	Coxsackie virus B4	Punta Toro virus
1	>100	>100	>100	>100	>100	>100
2	>100	>100	>100	>100	>100	>100
3	>100	>100	>100	>100	>100	>100
4	>100	>100	>100	>100	>100	>100
5	>100	>100	>100	>100	>100	>100
6	>100	>100	>100	>100	59	100
7	20	>4	>4	>4	>4	>4
8	>100	>100	>100	>100	>100	>100
9	-	-	-	-	-	-
10	>100	>100	>100	>100	>100	>100
11	>100	>100	>100	>100	>100	>100
12	>100	>100	>100	>100	>100	>100
13	>100	>100	>100	>100	>100	>100
14	>100	>100	>100	>100	>100	>100
15	100	>20	>20	>20	>20	>20
16	100	>20	>20	>20	>20	>20
17	>100	>100	>100	>100	>100	>100
18	20	>4	>4	>4	>4	>4
19	>100	>100	>100	>100	>100	>100
20	>100	>100	>100	>100	>100	>100
21	100	>20	>20	>20	>20	>20
22	>100	>100	>100	>100	>100	>100
23	20	>4	>4	>4	>4	>4
DS-5000	>100	>100	>100	59	>100	100
(S)-DHPA	>250	>250	>250	>250	>250	>250
Ribavirin	>250	45	>250	>250	>250	146

^aRequired to cause a microscopically detectable alteration of normal cell morphology.

^bRequired to reduce virus-induced cytopathogenicity by 50 %.

potential to be selected as lead compounds for the development of novel antiviral agent against vaccinia virus.

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