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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)-benzoimidazol-1-yl]-methanones, (2-bromophenyl)-[2-(substituted phenyl)-benzoimidazol-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

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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)-benzimidazol-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. $^1$H-Nuclear magnetic resonance ($^1$H 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 %; $^1$H NMR spectra $CDCl_3$ δ ppm: 7.26–7.70 (m, 4H, ArH of benzimidazole), 7.71–8.05 (m, 4H, ArH of $ArNO_2$ attached to 2$^{nd}$ position of benzimidazole), 8.07–8.38 (m, 4H, ArH of $ArNO_2$ attached to $N_1$ of benzimidazole).; IR (KBr pellets): cm$^{-1}$ 1427.6, 1540.4 (Skeletal bands), 1693.8 (C=O, str. tertiary amide), 1349.4 ($NO_2$ sym str., $ArNO_2$), 1492.3 ($NO_2$ asym. str., $ArNO_2$); CHN analysis calc.(found): $C_{20}H_{12}N_4O_5$: 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 %; $^1$H NMR spectra $CDCl_3$ 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 $ArNO_2$ attached to $N_1$ 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 ($NO_2$ sym. str., $ArNO_2$), 1496.3 ($NO_2$ asym. str., $ArNO_2$), 1106.9 (C-O str., COOH); CHN analysis calc.(found): $C_{21}H_{13}N_3O_5$: 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 %; $^1$H NMR spectra $CDCl_3$ 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 $ArNO_2$ attached to $N_1$ 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 ($NO_2$ sym. str., $ArNO_2$), 1495.2 ($NO_2$ asym. str., $ArNO_2$), 3615.2 (O-H str, ArOH); CHN analysis calc.(found): $C_{20}H_{13}N_3O_4$: C, 66.85 (66.01); H, 3.65 (3.05); N, 11.69 (11.09); O, 17.81 (17.30) %.

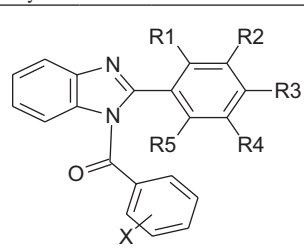
**[2-(2-Methoxyphenyl)-benzoimidazol-1-yl]-(4-nitrophenyl) methanone (4)**

mp (°C) – 149–151; Yield –56.72 %; $^1$H NMR spectra $CDCl_3$ δ ppm: 7.30–7.70 (m, 4H, ArH of benzimidazole), 6.83–7.23 (m, 4H, $ArOCH_3$ attached to 2$^{nd}$ position of benzimidazole), 8.10–8.32 (m, 4H, ArH of $ArNO_2$ attached to $N_1$ of benzimidazole), 3.90 (s, 3H, $OCH_3$).; IR (KBr pellets): cm$^{-1}$ 1427.9, 1539.7 (Skeletal bands), 1694.0 (C=O, str. tertiary amide), 1349.5 ($NO_2$ sym. str., $ArNO_2$), 3063.5 (aralkyl ethers); CHN analysis calc.(found): $C_{21}H_{15}N_3O_4$: C, 67.56 (67.09); H, 4.05 (3.95); N, 11.25 (11.19); O, 17.14 (17.00) %.

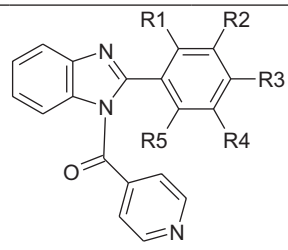
**(2-Bromophenyl)-[2-(4-nitrophenyl)-benzoimidazol-1-yl]-methanone (11)**

mp (°C) – 149–151; Yield – 34.23 %; $^1$H NMR spectra $CDCl_3$ 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-$NO_2$ | $C_{20}H_{12}N_4O_5$ | 388.33 | 154–156 | 0.10* | 52.73 |
| **2** | COOH | H | H | H | H | 4-$NO_2$ | $C_{21}H_{13}N_4O_5$ | 387.35 | 209–211 | 0.48* | 14.09 |
| **3** | H | H | OH | H | H | 4-$NO_2$ | $C_{20}H_{13}N_3O_4$ | 359.34 | 229–231 | 0.47* | 16.07 |
| **4** | $OCH_3$ | H | H | H | H | 4-$NO_2$ | $C_{21}H_{15}N_3O_4$ | 373.36 | 149–151 | 0.35* | 56.72 |
| **5** | H | H | Cl | H | H | 4-$NO_2$ | $C_{20}H_{12}N_3O_3Cl$ | 377.78 | 219–221 | 0.90* | 41.56 |
| **6** | H | H | $NO_2$ | H | H | 4-$NO_2$ | $C_{20}H_{12}N_4O_5$ | 388.33 | 139–141 | 0.12* | 45.54 |
| **7** | H | H | H | H | H | 4-$NO_2$ | $C_{20}H_{12}N_3O_3$ | 343.34 | 199–201 | 0.70* | 39.80 |
| **8** | H | $NO_2$ | H | H | H | 4-$NO_2$ | $C_{20}H_{12}N_4O_5$ | 388.33 | 209–211 | 0.90* | 48.65 |
| **9** | H | Cl | H | H | H | 4-$NO_2$ | $C_{20}H_{12}N_3O_3Cl$ | 377.78 | 229–231 | 0.55* | 43.98 |
| **10** | Cl | H | H | H | H | 4-$NO_2$ | $C_{20}H_{12}N_3O_3Cl$ | 377.78 | 219–221 | 0.83* | 38.65 |
| **11** | H | H | $NO_2$ | H | H | 2-Br | $C_{20}H_{12}Br\ N_3O_3$ | 422.23 | 149–151 | 0.21* | 34.23 |
| **12** | H | H | OH | H | H | 2-Br | $C_{20}H_{13}Br\ N_2O_2$ | 393.23 | 149–151 | 0.16* | 47.65 |
| **13** | COOH | H | H | H | H | 2-Br | $C_{21}H_{13}BrN_2O_3$ | 421.24 | 139–141 | 0.28* | 57.68 |
| **14** | $NO_2$ | H | H | H | H | 2-Br | $C_{20}H_{12}BrN_3O_3$ | 422.23 | 129–131 | 0.60** | 47.39 |
| **15** | Cl | H | H | H | H | - | $C_{19}H_{12}N_3OCl$ | 333.83 | 84–86 | 0.95** | 39.13 |
| **16** | H | H | Cl | H | H | - | $C_{19}H_{12}N_3OCl$ | 333.83 | 224–226 | 0.48** | 47.82 |
| **17** | H | Cl | H | H | H | - | $C_{19}H_{12}N_3OCl$ | 333.83 | 149–151 | 0.59** | 73.91 |
| **18** | H | H | OH | H | H | - | $C_{19}H_{13}N_3O_2$ | 315.37 | 64–66 | 0.15** | 11.20 |
| **19** | H | H | $NO_2$ | H | H | - | $C_{19}H_{12}N_4O_3$ | 344.30 | 244–246 | 0.22** | 48.59 |
| **20** | H | $NO_2$ | H | H | H | - | $C_{19}H_{12}N_4O_3$ | 344.30 | 239–241 | 0.31** | 70.09 |
| **21** | $NO_2$ | H | H | H | H | - | $C_{19}H_{12}N_4O_3$ | 344.30 | 49–51 | 0.90** | 14.95 |
| **22** | COOH | H | H | H | H | - | $C_{20}H_{13}N_3O_3$ | 343.41 | 139–141 | 0.13** | 45.08 |
| **23** | $OCH_3$ | H | H | H | H | - | $C_{20}H_{15}N_3O_2$ | 329.39 | 99–101 | 0.90** | 14.20 |

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

[a]Reported in Sharma D, Narasimhan B, Kumar P, Jalbout A, *Eur. J. Med. Chem.* 2008 (In Press)

4H, ArH of $ArNO_2$ 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 ($NO_2$ asym. str., $ArNO_2$), 553.2 (C-Br, str.); CHN analysis calc.(found): $C_{20}H_{12}N_3O_3Br$: 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)-benzoimidazol-1-yl]-methanone (12)**

mp (°C) - 149–151; Yield – 47.65 %; $^1$H NMR ($CDCl_3$) 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): $C_{20}H_{13}N_2O_2Br$: 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-benzoimidazol-2-yl]-benzoic acid (13)**

mp (°C) – 139–141; Yield – 57.68 %; $^1$H NMR ($CDCl_3$) 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): $C_{21}H_{13}N_2O_3Br$: 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 24 h (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 μL 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)-benzoimidazol-1-yl]-methanones, (2-bromophenyl)-[2-(substituted phenyl)-benzoimidazol-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 $CCID_{50}$ of virus, one $CCID_{50}$ 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.

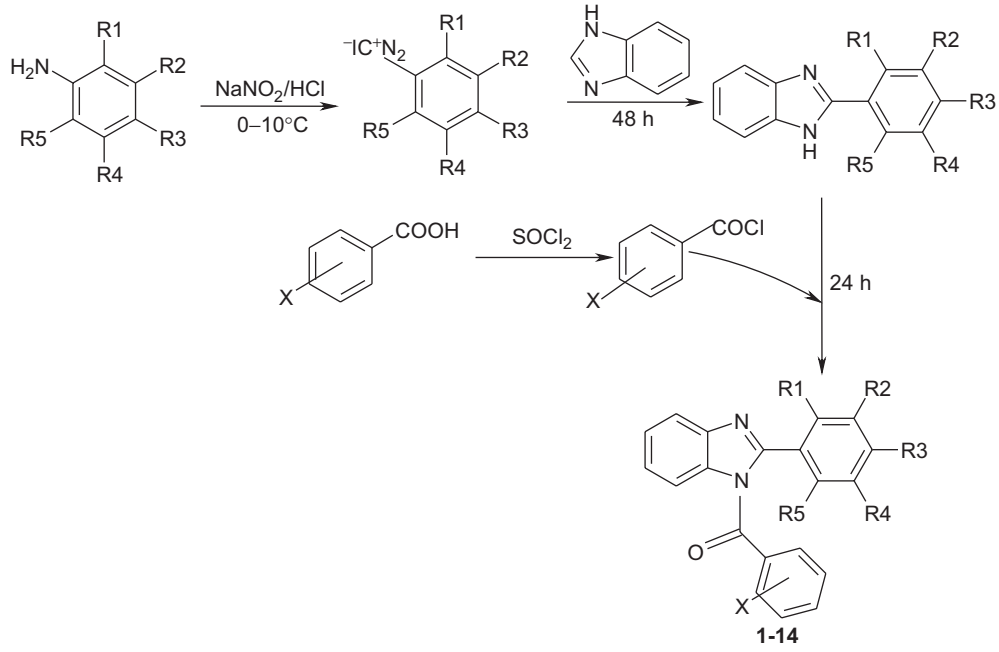
**Cytotoxic assay**

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 aryldiazonium 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)-benzoimidazol-1-yl]-methanones, (2-bromophenyl)-[2-(substituted phenyl)-benzoimidazol-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 $^1$H-NMR as well by elemental analysis studies. The elemental analysis results were within ± 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-$OCH_3$) 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- 1680 $cm^{-1}$ in the IR spectra of (4-nitrophenyl)-[2-(substituted phenyl)-benzoimidazol-1-yl]-methanones, (2-bromophenyl)- [2-(substituted phenyl)-benzoimidazol-1-yl]-methanones **(1–14)** demonstrated the presence of a tertiary amide linkage between the substituted benzoyl chloride and the $N_1$ of benzimidazole nucleus.

The $^1$H- 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-$NO_2$ (**1–4**) attached to $N_1$ 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 $^1$H-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 $N_1$ 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 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

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

| | Minimum Inhibitory Concentration ( $1\times10^{-3}$ μM/mL) | | | | |
|---|---|---|---|---|---|
| Compound | *S. aureus* | *B. subtilis* | *E. coli* | *A. niger* | *C. albicans* |
| **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.

| | Minimum Bactericidal/fungicidal Concentration ($1\times10^{-3}$ μM/mL) | | | | |
|---|---|---|---|---|---|
| Compound | *S. aureus* | *B. subtilis* | *E. coli* | *A. niger* | *C. albicans* |
| **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

benzene ring substituted at second position as well on the acyl benzene ring substituted at $N_1$ 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 $EC_{50}$) were noted for any of the compounds in HEL cells. Compounds **5** and **6** were active against VV at an $EC_{50}$ 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.

| | | $EC_{50}$[b] (μg/mL) | | | | |
|---|---|---|---|---|---|---|
| Compound | MCC[a] (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 |

[a]Required to cause a microscopically detectable alteration of normal cell morphology.
[b]Required to reduce virus-induced cytopathogenicity by 50 %.

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

| | | $EC_{50}$[b] (μg/mL) | | |
|---|---|---|---|---|
| **Compound** | MCC[a] (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 |

[a]Required to cause a microscopically detectable alteration of normal cell morphology.
[b]Required to reduce virus-induced cytopathogenicity by 50 %.

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

| | | $EC_{50}$[b] (μg/mL) | | | | |
|---|---|---|---|---|---|---|
| **Compound** | MIC[a] (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 |

[a]Required to cause a microscopically detectable alteration of normal cell morphology.
[b]Required to reduce virus-induced cytopathogenicity by 50 %.

In HeLa cell cultures, compounds **3** and **7** showed an $EC_{50}$ 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 $EC_{50}$) (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 potential to be selected as lead compounds for the development of novel antiviral agent against vaccinia virus.

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