Synthesis and biological profile of substituted benzimidazoles

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Abstract
Background: A series of benzimidazole derivatives was developed and its chemical scaffolds were authenticated by NMR, IR, elemental analyses and physicochemical properties. The synthesized compounds were screened for their antimicrobial and antiproliferative activities.

Results and discussion: The synthesized benzimidazole compounds were evaluated for their antimicrobial activity using the tube dilution method and were found to exhibit good antimicrobial potential against selected Gram negative and positive bacterial and fungal species. The compounds were also assessed for their anticancer activity exhibited using the SRB assay and were found to elicit antiproliferative activity against MCF7 breast cancer cell line, which was comparable to the standard drug.

Conclusion: Antimicrobial screening results indicated that compounds 1, 2 and 19 to be promising antimicrobial agents against selected microbial species and comparable to standard drugs which included norfloxacin and fluconazole. The anticancer screening results revealed that compounds, 12, 21, 22 and 29 to show the highest activity against MCF7 and their IC50 values were more potent than 5-fluorouracil.

Keywords: Benzimidazoles, Synthesis, Antimicrobial activity, Anticancer activity

Background
The emergence of antibiotic-resistant microorganisms such as fluoroquinolone-resistant Escherichia coli, Streptococcus pneumonia, carbapenem-resistant Klebsiella pneumonia, vancomycin-resistant enterococci and methicillin-resistant Staphylococcus aureus is becoming a serious health issue worldwide. There is a critical need to develop new chemotherapeutic agents with different mechanism of action [1].

Cancer is a deadly disease prevalent in both the developing as well as the developed countries. In spite of significant improvements in recognition and treatment of cancer, the incidence of certain types of malignancy is still on the rise. Current treatments such as cytotoxic chemotherapy and radiotherapy yielded only transient therapeutic aids that are accompanied by severe adverse effects. This is due to their toxic effects against normal growing cells. Concerted effort is, therefore, required to eliminate or at least reduce these incidences significantly [2].

Recent findings suggest that substituted benzimidazole derivatives possess potential chemotherapeutic activity with reduced toxic effects. Antibacterial activity of substituted benzimidazole derivatives can be explained by their competition with purines, an integral part of bacterial strain, resulting in inhibition of bacterial nucleic acids and proteins synthesis [3]. Compounds containing benzimidazole moiety such as thiabendazole, parbendazole, mebendazole, albendazole, cambendazole and flubendazole had also been reported for their antihelminthic activity. Similarly, the proton pump inhibitors, omeprazole, lansoprazole, rabeprazole, pantoprazole, had been reported for their use in the management of acid related disorders. In fact, benzimidazole derivatives had found their applications as antioxidant [4], antimicrobial [5], antihelmintic...
antitumour potential was observed for compound 22 and 27.

The synthesis of the benzimidazole derivatives was performed by condensation of the benzimidazoles with various aroyl, aryalkyl and benzylationdehydes. The reactions were carried out in ethanol at reflux for 4-5 h. The formed Schiff bases were isolated and characterized by IR, 1H-NMR and mass spectral analysis. The elemental analyses of the compounds were in agreement with the theoretical values. The target compounds were screened for their antimicrobial and antitumour activities against a panel of microorganisms.

**Antitumour activity**

The synthesized benzimidazole derivatives were screened for their antitumour activity against MCF7 (ATCC HTB-22), an oestrogen receptor positive human breast adenocarcinoma cell line. Antitumour screening results (Table 2) indicated that compound 22 (IC50 = 0.9 µM) was found to be the most potent when compared to the standard drug, 5-fluorouracil (IC50 = 35.4 µM). Other compounds which included 12, 21 and 29 also exhibited more potent antiproliferative results (IC50 = 7.0, 5.4 and 5.5 µM, respectively) when compared to the standard drug. These compounds may be used as drug leads for discovery of new antitumour agents.

**Antimicrobial activity**

Antimicrobial activity results (Table 3) indicated that the compounds possessed good antimicrobial activity against the tested bacterial and fungal strains. Compound 1 showed good antibacterial activity against E. coli (MICec = 5.4 µM) and B. subtilis (MICbs = 10.7 µM), whereas compound 19 was found to be more potent against S. aureus (MICsa = 12.4 µM). The reference drug, norfloxacin, yielded MIC of 4.7 µM against the tested microorganisms. The antifungal activity results indicated that compound 2 showed good activity against C. albicans (MICca = 5.4 µM). Compound 19, on the other hand, was the most potent antifungal agent against A. niger (MICan = 3.1 µM) in comparison to fluconazole (MIC = 5.0 µM), the reference drug. Thus, compound 19 may serve as a potential lead compound for the design of novel antifungal agents.

**Structure activity relationship**

The following structure activity relationship may be drawn from the antimicrobial and antitumour activities of the benzimidazole derivatives (Fig. 1):

- It has been noticed that the antibacterial activity of Schiff bases against E. coli enhanced due to the presence of vinyl group between benzimidazole amine and N-benzylidene moiety and the substitution of electron releasing group at phenyl nucleus as in the compound 1 and the same moiety improved antitumour activity of methanone derivatives as in compound 22.
- The electron donating group placed at phenyl ring attached to N-acyliden/arylidene moiety along with presence of electron withdrawing group on phenyl ring attached to methanone moiety improved antibacterial and antifungal activity of synthesized benzimidazole derivatives against bacterial and fungal strains as in compound 19.

**Experimental**

**Materials and methods**

All the laboratory reagents were procured from Sigma Aldrich and were used without any purification. Melting points were determined on Sonar melting point apparatus in an open capillary tube and are uncorrected. Purity of the compound was ascertained by commercialized (E-Merck Kieselgel 60 F254) TLC plates. The Infrared spectrum was recorded in KBr discs on a Shimadzu FTIR 8400S spectrometer (νmax in cm⁻¹).
Scheme 1  Synthesis of benzimidazole derivatives (1–30). Reaction condition:  
Step i: 2-Aminobenzimidazole, substituted aldehyde, ethanol, glacial acetic acid, reflux for 4–5 h (RT),  
Step ii: Schiff’s base, different acylchlorides, dimethylformamide, triethylamine, stir for 24 h (RT)
| Comp. | Molecular structures with stereochemistry | M. formula and CHN analyses | M. wt. | Rf value | % Yield | M. Pt. (°C) |
|-------|----------------------------------------|-----------------------------|--------|----------|---------|-------------|
| 1     | (E)-N-[(E)-3-(4-(Dimethylamino)phenyl) allylidene]-1H-benzo[d]imidazol-2-amine | C_{18}H_{18}N_{4}: Anal calcd: C, 74.46; H, 6.25; N, 19.30; Found: C, 74.43; H, 6.27; N, 19.33 | 290.40 | 0.76<sup>a</sup> | 76 | 228–230 |
| 2     | (E)-1-{[(1H-Benzo[d]imidazol-2-yl)imino]methyl}naphthalen-2-ol | C_{18}H_{13}N_{3}O: Anal calcd: C, 75.25; H, 4.56; N, 14.63; Found: C, 75.27; H, 4.59; N, 14.60 | 287.34 | 0.79<sup>a</sup> | 74 | 255–257 |
| 3     | (E)-N-(3,4-Dimethoxybenzylidene)-1H-benzo[d]imidazol-2-amine | C_{16}H_{15}N_{3}O_{2}: Anal calcd: C, 68.31; H, 5.37; N, 14.94; Found: C, 68.34; H, 5.35; N, 14.97 | 281.34 | 0.77<sup>a</sup> | 78 | 225–227 |
| 4     | (E)-4-{[(1H-Benzo[d]imidazol-2-yl)imino]methyl}phenol | C_{14}H_{11}N_{3}O: Anal calcd: C, 70.87; H, 4.67; Cl, 17.71; Found: C, 70.88; H, 4.65; Cl, 17.73 | 237.28 | 0.75<sup>a</sup> | 67 | 220–222 |
| 5     | (E)-N-(4-Nitrobenzylidene)-1H-benzo[d]imidazol-2-amine | C_{14}H_{10}N_{4}O_{2}: Anal calcd: C, 63.15; H, 3.79; N, 21.04; Found: C, 63.13; H, 3.77; N, 21.07 | 266.28 | 0.72<sup>a</sup> | 72 | 236–238 |
| 6     | (E)-N-(3-Dimethylamino)benzylidene)-1H-benzo[d]imidazol-2-amine | C_{14}H_{10}N_{4}O_{2}: Anal calcd: C, 63.15; H, 3.79; N, 21.04; Found: C, 63.18; H, 3.77; N, 21.05 | 264.36 | 0.79<sup>a</sup> | 82 | 238–240 |
| 7     | (E)-N-(3-Nitrobenzylidene)-1H-benzo[d]imidazol-2-amine | C_{14}H_{10}N_{4}O_{2}: Anal calcd: C, 63.15; H, 3.79; N, 21.04; Found: C, 63.18; H, 3.77; N, 21.05 | 266.28 | 0.76<sup>a</sup> | 76 | 190–192 |
| 8     | (E)-N-Ethylidene-1H-benzo[d]imidazol-2-amine | C_{6}H_{13}N_{2}: Anal calcd: C, 74.53; H, 5.70; N, 19.77; Found: C, 74.43; H, 5.72; N, 19.75 | 159.21 | 0.72<sup>a</sup> | 80 | 172–175 |
| 9     | (E)-N-(4-Methoxybenzylidene)-1H-benzo[d]imidazol-2-amine | C_{14}H_{13}N_{3}O: Anal calcd: C, 71.70; H, 5.21; N, 16.72; Found: C, 71.73; H, 5.22; N, 16.74 | 251.31 | 0.71<sup>a</sup> | 75 | 198–200 |
Table 1 (continued)

| Comp. | Molecular structures with stereochemistry | M. formula and CHN analyses | M. wt. | Rf value | % Yield | M. Pt. (°C) |
|-------|------------------------------------------|----------------------------|--------|----------|----------|-------------|
| 10    | (E)-5-((1H-Benzo[d]imidazol-2-yl)imino)pentanal | C_{12}H_{13}N_{3}O: Anal calcd: C, 66.96; H, 6.09; N, 19.52; Found: C, 66.94; H, 6.11; N, 19.55 | 215.28 | 0.75^a | 78 | 265–267 |
| 11    | (E)-N-(3,4,5-Trimethoxybenzylidene)-1H-benzo[d]imidazol-2-amine | C_{17}H_{17}N_{3}O_{3}: Anal calcd: C, 65.58; H, 5.50; N, 13.50; Found: C, 65.61; H, 5.53; N, 13.52 | 311.37 | 0.72^a | 84 | 242–245 |
| 12    | (E)-1-(2-(Ethylideneamino)-1H-benzo[d]imidazol-1-yl)ethanone | C_{11}H_{11}N_{3}O: Anal calcd: C, 65.66; H, 5.51; N, 20.88; Found: C, 65.65; H, 5.54; N, 20.86 | 201.22 | 0.63^b | 74 | 262–265 |
| 13    | (E)-(3,5-Dinitrophenyl)(2-(4-methoxybenzylidene)amino)-1H-benzo[d]imidazol-1-yl)methanone | C_{22}H_{15}N_{5}O_{6}: Anal calcd: C, 59.33; H, 3.39; N, 15.72; Found: C, 59.35; H, 3.42; N, 15.75 | 445.38 | 0.58^b | 68 | 243–245 |
| 14    | (E)-5-((1-(3,5-Dinitrobenzoyl)-1H-benzo[d]imidazol-2-yl)imino)pentanal | C_{14}H_{11}N_{3}O_{2}: Anal calcd: C, 55.75; H, 3.69; N, 17.11; Found: C, 55.78; H, 3.71; N, 17.14 | 253.26 | 0.66^b | 65 | 162–164 |
| 15    | (E)-5-((1-Acetyl-1H-benzo[d]imidazol-2-yl)imino)pentanal | C_{14}H_{15}N_{3}O_{2}: Anal calcd: C, 65.96; H, 5.88; N, 16.33; Found: C, 65.37; H, 5.90; N, 16.36 | 257.29 | 0.62^b | 72 | 226–228 |
| 16    | (E)-(3,5-Dinitrophenyl)(2-(4-hydroxybenzylidene)amino)-1H-benzo[d]imidazol-1-yl)methanone | C_{21}H_{13}N_{3}O_{6}: Anal calcd: C, 58.47; H, 3.04; N, 16.24; Found: C, 58.49; H, 3.05; N, 16.25 | 431.36 | 0.64^b | 70 | 175–177 |
| 17    | (E)-2-(2,4-Dimethoxybenzylideneamino)-1H-benzo[d]imidazol-1-yl)(naphthalen-2-yl)methanone | C_{23}H_{21}N_{3}O_{6}: Anal calcd: C, 74.47; H, 4.86; N, 9.65; Found: C, 74.49; H, 4.88; N, 9.68 | 435.47 | 0.54^b | 67 | 120–122 |
| Comp. | Molecular structures with stereochemistry | M. formula and CHN analyses | M. wt. | Rf value | % Yield | M. Pt. (°C) |
|-------|-----------------------------------------|-----------------------------|--------|----------|---------|-------------|
| 18    | ![Molecular structure 18](image1) (E)-Naphthalen-2-yl(2-[(3,4,5-trimethoxybenzylidene)amino]-1H-benzo[d]imidazol-1-yl) methanone | C_{28}H_{23}N_{3}O_{4}: Anal calcd: C, 72.24; H, 4.98; N, 9.03; Found: C, 72.27; H, 4.95; N, 9.05 | 465.5 | 0.65b | 75 | 210–212 |
| 19    | ![Molecular structure 19](image2) (E)-(3,5-Dinitrophenyl)(2-[(3,4,5-trimethoxybenzylidene)amino]-1H-benzo[d]imidazol-1-yl) methanone | C_{24}H_{19}N_{5}O_{8}: Anal calcd: C, 57.03; H, 3.79; N, 13.86; Found: C, 57.07; H, 3.76; N, 13.88 | 505.44 | 0.66b | 66 | 141–143 |
| 20    | ![Molecular structure 20](image3) (E)-1-(2-[(3,4,5-trimethoxybenzylidene)amino]-1H-benzo[d]imidazol-1-yl)hexadecan-1-one | C_{33}H_{47}N_{3}O_{4}: Anal calcd: C, 72.10; H, 8.62; N, 7.64; Found: C, 72.11; H, 8.65; N, 7.67 | 549.74 | 0.62b | 78 | 136–138 |
| 21    | ![Molecular structure 21](image4) (E)-(3-Nitrophenyl)(2-[(4-(pyridin-2-yl)benzylidene)amino]-1H-benzo[d]imidazol-1-yl)methanone | C_{26}H_{17}N_{5}O_{3}: Anal calcd: C, 69.79; H, 3.83; N, 15.65; Found: C, 69.77; H, 3.86; N, 15.68 | 447.44 | 0.57b | 67 | 142–144 |
| 22    | ![Molecular structure 22](image5) (2-[(E)-(E)-3-(4-Dimethylamino)phenyl)allylidene)amino)-1H-benzo[d]imidazol-1-yl(3-nitrophenyl)methanone | C_{24}H_{19}N_{5}O_{3}: Anal calcd: C, 68.33; H, 4.82; N, 15.94; Found: C, 68.37; H, 4.80; N, 15.97 | 439.47 | 0.59b | 82 | 126–128 |
| 23    | ![Molecular structure 23](image6) (2-((E)-(E)-3-(4-Dimethylamino)benzylidene)amino)-1H-benzo[d]imidazol-1-yl(3-nitrophenyl)methanone | C_{26}H_{19}N_{5}O_{3}: Anal calcd: C, 66.82; H, 4.63; N, 16.94; Found: C, 66.83; H, 4.66; N, 16.97 | 413.43 | 0.63b | 76 | 131–133 |
| Comp. | Molecular structures with stereochemistry | M. formula and CHN analyses | M. wt. | Rf value | % Yield | M. Pt. (°C) |
|-------|------------------------------------------|-----------------------------|--------|----------|---------|-------------|
| 24    | (E)-(2-((2-hydroxynaphthalen-1-yl)-methylene)amino)-1H-benzo[d]imidazol-1-yl)(3-nitrophenyl)methanone | C_{25}H_{16}N_{4}O_{4}: Anal calcd: C, 68.80; H, 3.70; N, 12.84; Found: C, 68.83; H, 3.72; N, 12.87 | 436.42 | 0.64<sup>b</sup> | 65 | 134–136 |
| 25    | (E)-(2-((4-Nitrobenzylidene)amino)-1H-benzo[d]imidazol-1-yl)(3-nitrophenyl)methanone | C_{21}H_{13}N_{5}O_{5}: Anal calcd: C, 60.72; H, 3.15; N, 16.86; Found: C, 60.75; H, 3.17; N, 16.89 | 415.36 | 0.66<sup>b</sup> | 74 | 119–121 |
| 26    | (E)-Naphthalen-2-yl(2-((4-nitrobenzylidene)amino)-1H-benzo[d]imidazol-1-yl)methanone | C_{25}H_{16}N_{4}O_{3}: Anal calcd: C, 71.42; H, 3.84; N, 13.33; Found: C, 71.43; H, 3.87; N, 13.37 | 420.42 | 0.52<sup>b</sup> | 62 | 176–178 |
| 27    | (E)-(2-((3,4-Dimethoxybenzylidene)amino)-1H-benzo[d]imidazol-1-yl)(3-nitro phenyl)methanone | C_{24}H_{18}N_{4}O_{5}: Anal calcd: C, 64.18; H, 4.22; N, 13.02; Found: C, 64.21; H, 4.25; N, 13.04 | 430.41 | 0.63<sup>b</sup> | 65 | 235–237 |
| 28    | (E)-1-(2-((4-Nitrobenzylidene)amino)-1H-benzo[d]imidazol-1-yl)hexadecan-1-one | C_{30}H_{40}N_{4}O_{3}: Anal calcd: C, 68.46; H, 7.99; N, 16.62; Found: C, 68.49; H, 7.97; N, 16.63 | 504.66 | 0.59<sup>b</sup> | 66 | 126–128 |
| 29    | (E)-1-(2-((4-Dimethylamino)benzylidene)amino)-1H-benzo[d]imidazol-1-yl)hexadecan-1-one | C_{34}H_{48}N_{4}O_{3}: Anal calcd: C, 71.40; H, 7.99; N, 11.10; Found: C, 71.39; H, 7.97; N, 11.12 | 528.77 | 0.64<sup>b</sup> | 68 | 131–133 |
| 30    | (E)-(2-(4-(Dimethylamino)benzylidene)amino)-1H-benzo[d]imidazol-1-yl)(naphthalen-2-yl)methanone | C_{27}H_{22}N_{4}O_{3}: Anal calcd: C, 71.40; H, 7.99; N, 11.10; Found: C, 71.39; H, 7.97; N, 11.12 | 418.49 | 0.62<sup>b</sup> | 76 | 192–195 |

TLC mobile phase: * Ethyl acetate: Methanol (7:3); ** Chloroform: Methanol (8:2)
Table 2 Anticancer screening results of synthesized compounds

| Comp. | MCF-7 cell line | Comp. | MCF-7 cell line |
|-------|----------------|-------|----------------|
|       |                |       |                |
|       |                |       |                |
|       |                |       |                |
| 1     | 31.0           | 6     | 31.0           |
| 2     | 41.8           | 17    | 41.8           |
| 3     | 170.6          | 18    | 170.6          |
| 4     | 101.1          | 19    | 101.1          |
| 5     | 67.6           | 20    | 67.6           |
| 6     | >378.3         | 21    | >378.3         |
| 7     | 112.7          | 22    | 112.7          |
| 8     | >628.1         | 23    | >628.1         |
| 9     | >3104          | 24    | >3104          |
| 10    | 157.9          | 25    | 157.9          |
| 11    | >321.2         | 26    | >321.2         |
| 12    | 7.0            | 27    | 7.0            |
| 13    | 19.1           | 28    | 19.1           |
| 14    | >394.9         | 29    | >394.9         |
| 15    | 11.7           | 30    | 11.7           |
|       | 5-Fluorouracil | 35.4  |                |
|       | S-Fluorouracil | 35.4  |                |

13C NMR spectra of the synthesized compounds were recorded on Bruker Advance-II 400 NMR spectrometer with DMSO as a solvent and the chemical shift data were expressed as delta values related to tetramethylsilane. Mass spectra were recorded using Waters, Q-TOF micro-mass spectrometer.

Procedure for the title compounds (1–11)

2-Aminobenzimidazole (0.01 mol) was refluxed with different substituted aromatic aldehyde (0.01 mol) in ethanol (20 ml) for 4–5 h (RT) in presence of glacial acetic acid (few drops). Then the reaction mixture was allowed to cool at RT and the precipitated compound was filtered and dried [12].

Synthesis of 2-(alkyl/arylideneamino)-1H-benzo[d]imidazol-1-yl-alkyl/aryl-methanones (12–30)

Compound of Schiff’s bases (1–11) (0.005 mol) were stirred at RT with different acylchlorides (0.005 mol) in dimethylformamide for 24 h with the addition of small amount of triethylamine. The resulting reaction mixture was precipitated using ice cold water and the crude product was filtered through a vacuum pump, washed with cold water, dried and recrystallized using rectified spirit [13].

Spectral data of synthesized compounds

(E)-N- ((E)-3-(4-(Dimethylamino)phenyl)allylidene)-1H-benzo[d]imidazol-2-amine (1) IR (KBr cm⁻¹): 1550 (N=CH str.), 3475 (N–H str.), 1431 (Ar, C=C str.), 1253 (C–N str.), 1300 (–N(CH3)2 str); 1H NMR (DMSO): 9.557–9.575 (d, 1H, N=CH), 6.646–6.714 (d, 1H, –CH=CH), 6.416–7.614 (m, 8H, ArH), 3.426 (s, 6H, (CH3)2); 13C NMR (DMSO): 40, 115, 119, 123, 127, 135, 138, 148, 159, 162; MS: m/z = 291.12 (M⁺ + 1).

(E)-1-(((1H-Benz[d]imidazol-2-yl)amino)methyl)naphthalen-2-ol (2) IR (KBr cm⁻¹): 3066 (N–H str., of imidazole), 3012 (C–H aromatic ring str.), 1442 (Ar, C=C str.), 1550 (N=CH str.), 1253 (C–N str.), 3518 (O–H str.); 1H NMR (DMSO): 10.295 (s, 1H, N=CH), 7.906–8.108 (m, 10H, ArH), 4.481 (s, 1H, OH), 10.809 (s, 1H, NH of imidazole); 13C NMR (DMSO): 115, 118, 123, 127, 128, 129, 132, 135, 138, 159, 162; MS: m/z = 288.39 (M⁺ + 1).

(E)-N-(3,4-Dimethoxybenzylidene)-1H-benzo[d]imidazol-2-amine (3) IR (KBr cm⁻¹): 3410 (N–H str.), 3058 (Ar, C=H str.), 1542 (C=C str.), 1610 (N=CH str.), 2827 (Ar, OCH3 str.); 1H NMR (DMSO): 9.487 (s, 1H, N=CH), 6.982–7.849 (m, 7H, ArH), 10.452 (s, 1H, NH of imidazole) 5.502 (s, 6H, (OCH3)2); 13C NMR (DMSO): 56, 115, 123, 127, 138, 152, 159, 162; MS: m/z = 282.14 (M⁺ + 1).

(E)-4-(((1H-Benz[d]imidazol-2-yl)amino)methyl)phenol (4) IR (KBr cm⁻¹): 3440 (N–H str.), 3063 (Ar, C=H str.), 1537 (C=C str.), 1613 (N=CH str.), 3452 (O–H str.); 1H NMR (DMSO): 9.582 (s, 1H, N=CH), 7.106–8.367 (m, 8H, ArH), 10.809 (s, 1H, NH of imidazole); 13C NMR (DMSO): 115, 117, 123, 126, 129, 131, 138, 159, 162; MS: m/z = 238.17 (M⁺ + 1).

(E)-N-(4-Nitrobenzylidene)-1H-benzo[d]imidazol-2-amine (5) IR (KBr cm⁻¹): 3240 (N–H str., of imidazole ring), 2974 (C–H aromatic ring str.), 1465 (Ar, C=C str.), 1550 (N=CH str.), 1548 (Ar=C–NO2 asym str.); 1H NMR (DMSO): 9.550 (s, 1H, N=CH), 7.103–8.105 (m, 4H, ArH), 8.116–8.376 (d, 4H, Ar-NO2), 12.73 (s, 1H, NH of imidazole); 13C NMR (DMSO): 115, 120, 123, 130, 138, 149, 159, 162; MS: m/z = 267.26 (M⁺ + 1).

(E)-N-(4-(Dimethylamino)benzylidene)-1H-benzo[d]imidazol-2-amine (6) IR (KBr cm⁻¹): 1550 (N=CH str.), 3374 (N–H str.), 1462 (Ar, C=C str.), 1298 (C–N str.–N(CH3)2); 1H NMR (DMSO): 9.206 (s, 1H, N=CH), 6.646–7.823 (m, 8H, ArH), 3.426 (s, 6H, (CH3)2); 13C NMR (DMSO): 40, 115, 123, 138, 159, 162; MS: m/z = 265.35 (M⁺ + 1).

(E)-N-(3-Nitrobenzylidene)-1H-benzo[d]imidazol-2-amine (7) IR (KBr cm⁻¹): 3428 (N–H str.), 3068 (Ar, C=H str.), 1531 (C=C str.), 1618 (C=N str.), 1547 (Ar=NO₂ str.); 1H NMR (DMSO): 9.515 (s, 1H, N=CH), 7.213–8.378 (m, 8H, ArH), 10.23 (s, 1H, NH of...
Table 3  Antimicrobial activity of synthesized compounds

| Comp. | Bacterial screening [MIC (μM)] | Fungal screening |
|-------|--------------------------------|-----------------|
|       | E. coli | B. subtilis | S. aureus | C. albicans | A. niger |
| 1     | 0.54    | 10.7       | 43        | 10.7        | 21.5    |
| 2     | 43.5    | 21.8       | 43.5      | 5.4         | 10.9    |
| 3     | 22.2    | 22.2       | 44.4      | 11.1        | 22.2    |
| 4     | 0.66    | 13.1       | 52.7      | 13.1        | 26.3    |
| 5     | 46.9    | 23.5       | 46.9      | 23.5        | 23.5    |
| 6     | 47.3    | 23.6       | 47.3      | 47.3        | 47.3    |
| 7     | 23.5    | 23.5       | 46.9      | 93.9        | 23.5    |
| 8     | 39.3    | 39.3       | 78.5      | 78.5        | 78.5    |
| 9     | 24.9    | 24.9       | 49.7      | 24.9        | 49.7    |
| 10    | 14.5    | 29         | 58.1      | 29          | 58.1    |
| 11    | 10.0    | 20.1       | 40.1      | 20.1        | 20.1    |
| 12    | 248.5   | 248.5      | 15.5      | 62.1        | 31.1    |
| 13    | 56.1    | 28.1       | 14        | 14          | 7       |
| 14    | 197.4   | 197.4      | 24.7      | 98.7        | 24.7    |
| 15    | 194.3   | 194.3      | 24.3      | 48.6        | 24.3    |
| 16    | 58.0    | 29         | 14.5      | 14.5        | 7.2     |
| 17    | 28.7    | 28.7       | 14.4      | 28.7        | 7.2     |
| 18    | 26.9    | 13.4       | 13.4      | 26.9        | 6.7     |
| 19    | 49.5    | 12.4       | 12.4      | 6.2         | 3.1     |
| 20    | 45.5    | 11.4       | 22.7      | 22.7        | 11.4    |
| 21    | 27.9    | 14         | 14        | 55.9        | 7       |
| 22    | 56.9    | 28.4       | 14.2      | 28.4        | 7.1     |
| 23    | 60.3    | 30.2       | 15.1      | 30.2        | 7.5     |
| 24    | 28.6    | 28.6       | 14.3      | 28.6        | 7.1     |
| 25    | 60.2    | 30.1       | 15        | 30.1        | 7.5     |
| 26    | 29.7    | 29.7       | 14.9      | 59.5        | 7.4     |
| 27    | 58.1    | 29         | 14.5      | 29          | 7.2     |
| 28    | 49.5    | 24.8       | 24.8      | 24.8        | 12.4    |
| 29    | 47.3    | 11.8       | 23.6      | 23.6        | 11.8    |
| 30    | 29.9    | 29.9       | 14.9      | 59.7        | 7.5     |
| DMSO  | NA      | NA         | NA        | NA          | NA      |
| Std. drugs | 4.7a | 4.7a | 4.7a | 5.1b | 5.1b |

NA no activity, DMSO dimethyl sulphoxide
Std. drugs: * Norfloxacin, # Fluconazole

imidazole); $^{13}$C NMR (DMSO): 115,123, 127, 135, 138, 150, 159, 162; MS: m/z=267.28 (M$^+$+1).

(E)-N-Ethylidine-1H-benzo[d]imidazol-2-yl]amino)ethanone (11) IR (KBr cm$^{-1}$): 3429 (N–H str.), 1577 (C=C str.), 1606 (N=CH str.), 2835 (Ar–CH$_3$ str.), $^{13}$C NMR (DMSO): 56, 106, 115, 123, 127, 138, 141, 152, 159, 162; MS: m/z=312.14 (M$^+$+1).

(E)-1-(2-(Ethylideneamino)-1H-benzo[d]imidazol-1-yl)ethanone (12) IR (KBr cm$^{-1}$): 1661 (C=O str.), 2919 (C–H aromatic str.), 1575 (N=CH str.), 2849 (CH str. (sym), R–CH$_3$); $^{13}$C NMR (DMSO): 7.305–7.627 (m, 4H, Ar–H), 7.233 (s, 1H, N=CH), 1.273–1.276 (d, 3H, CH$_3$), 2.856 (s, 3H, CH$_3$); $^{13}$C NMR (DMSO): 16, 24, 115, 123, 129, 138, 141, 162, 168; MS: m/z=201 (M$^+$+1).

(E)-3,5-Dinitrophenyl)-2-(4-methoxybenzylidene)amino)-1H-benzo[d]imidazol-1-yl)methanone (13) IR (KBr cm$^{-1}$): 1710 (C=O str.), 2924 (C–H aromatic str.), 1537 (N=CH str.), 1545 (Ar–NO$_2$ str.), 1110 (C=C str., O=CH$_2$); $^{13}$C NMR (DMSO): 6.785–7.943 (m, 8H, ArH), 8.632 (s, 1H, N=CH), 2.984 (s, 3H, OCH$_3$), 8.912–9.063 (m, 3H, Ar(NO$_2$)$_2$); $^{13}$C NMR (DMSO): 56, 115, 123, 125, 130, 150, 163, 168; MS: m/z=445 (M$^+$+1).

(E)-5-((1-(3,5-Dinitrobenzoyl)-1H-benzo[d]imidazol-2-yl)imino)pentanal (14) IR (KBr cm$^{-1}$): 1701 (C=O str.), 3122 (C–H aromatic str.), 1627 (N=CH str.), 1543 (Ar, NO$_2$ str.), 1727 (Aliphatic aldehyde C=O str.); $^{13}$C NMR (DMSO): 6.875–7.946 (m, 4H, ArH), 8.632 (s, 1H, N=CH), 8.912–9.063 (m, 3H, Ar(NO$_2$)$_2$), 9.254–9.678 (m, 1H, CHO); $^{13}$C NMR (DMSO): 19, 24, 44, 115, 123, 125, 130, 150, 163, 168; MS: m/z=409 (M$^+$+1).

(E)-5-((1-Acetyl-1H-benzo[d]imidazol-2-yl)imino)pentanal (15) IR (KBr cm$^{-1}$): 1695 (C=O str.), 3050 (C–H aromatic str.), 1606 (N=CH str.), 1535 (C-NO$_2$ str.), 2860...
(C–H sym. str., R-CH₃), 1728 (Aliphatic aldehyde C=O str.); ¹H NMR (DMSO): 7.875–8.246 (m, 4H, ArH), 7.632 (s, 1H, N=CH), 9.254–9.678 (m, 1H, CHO), 2.856 (s, 3H, CH₃); ¹³C NMR (DMSO): 19, 24, 28, 44, 115, 123, 138, 142, 163, 168, 202; MS: m/z = 257 (M⁺ +1).

(E)-(3,5-Dinitrophenyl)(2-((4-hydroxybenzylidene) amino)-1H-benzo[d]imidazol-1-yl)methanone (16) IR (KBr cm⁻¹): 1685 (C=O str.), 3094 (C–H aromatic str.), 1630 (N=CH str.), 1544 (C–NO₂ str.), 3465 (O–H str); ¹H NMR (DMSO): 6.885–7.632 (m, 8H, ArH), 8.654 (s, 1H, N=CH), 8.912–9.063 (m, 3H, Ar(–NO₂)₂); ¹³C NMR (DMSO): 115, 123, 125, 130, 132, 138, 150, 160, 168; MS: m/z = 431 (M⁺ +1).

(E)-(2-((2,4-Dimethoxybenzylidene)amino)-1H-benzo[d]imidazol-1-yl)(naphthalen-2-yl)methanone (17) IR (KBr cm⁻¹): 1695 (C=O str.), 2919 (C–H aromatic str.), 1634 (N=CH str.), 2850 (Ar, OCH₃ str.); 1646 (naph. ring str.); ¹H NMR (DMSO): 6.844–8.213 (m, 14H, ArH), 8.612 (s, 1H, N=CH), 2.804 (s, 6H, (OCH₃)₂); ¹³C NMR (DMSO): 56, 107, 109, 115, 123, 127, 129, 132, 138, 142, 160, 168; MS: m/z = 435 (M⁺ +1).

(E)-(E)-(3,4,5-trimethoxybenzylidene) amino)-1H-benzo[d]imidazol-1-yl)methanone (18) IR (KBr cm⁻¹): 1691 (C=O str.), 1548 (N=CH str.), 1140 (C=O–C str., OCH₃); 795 (C–H out of plane bending, naphthalene ring); ¹H NMR (DMSO): 8.590 (s, 1H, N=CH), 4.194 (s, 9H, (OCH₃)₃), 6.971–8.070 (m, 11H, Ar–H); ¹³C NMR (DMSO): 57, 107, 115, 123, 124, 127, 128, 131, 139, 142, 151, 160, 168; MS: m/z = 465 (M⁺ +1).

(E)-(3,5-Dinitrophenyl)(2-((3,4,5-trimethoxybenzylidene) amino)-1H-benzo[d]imidazol-1-yl)methanone (19) IR (KBr cm⁻¹): 1681(C=O str.), 1539 (N=CH str.), 2850 (CH₃ sym. str., R-OCH₃); 1345 (C–NO₂ str.); ¹H NMR (DMSO): 8.947 (s, 1H, N=CH), 3.955 (s, 9H, (OCH₃)₃), 9.860–9.865 (m, 3H, Ar(NO)₂); 7.948–7.951 (d, 2H, Ar–H), 7.343–7.366 (m, 2H, Ar–H); ¹³C NMR (DMSO): 57, 106, 115, 125, 128, 129, 131, 139, 142, 147, 151, 168; MS: m/z = 505 (M⁺ +1).

(E)-(3-Nitrophenyl)(2-((4-(pyridin-2-yl)benzylidene) amino)-1H-benzo[d]imidazol-1-yl)methanone (21) IR (KBr cm⁻¹): 1685 (C=O str.), 3061 (C–H aromatic str.), 1623 (N=CH str.), 2843 (Ar, O–CH₃ str.), 1266 (Palmitoyl group str.); ¹H NMR (DMSO): 7.283–7.286 (m, 6H, ArH), 1.278–2.386 (m, 28H, CH₂ of palmitoyl), 0.884–0.903 (t, 3H, CH₃), 3.264 (s, 9H, (OCH₃)₃); ¹³C NMR (DMSO): 14, 23, 26, 30, 32, 56, 106, 115, 123, 128, 130, 139, 142, 160, 170; MS: m/z = 549 (M⁺ +1).

(E)-(E)-(E)-3-(4-(Dimethylamino)phenyl)allylidene) amino)-1H-benzo[d]imidazol-1-yl)3-nitrophenyl)methanone (22) IR (KBr cm⁻¹): 1723(C=O str.), 2920 (C–H aromatic str.), 1530 (N=CH str.), 1549 (Ar(NO)₂ str.), 1349 (C=CH str., of ter. arylamine); ¹H NMR (DMSO):
8.390–8.410 (d, 1H, N=CH), 6.731–6.740 (d, 1H, –CH=CH), 6.250–8.355 (m, 11H, ArH), 8.919 (s, 1H, Ar-NO2) 3.559 (s, 6H, (CH3)2); 13C NMR (DMSO): 40, 115, 123, 127, 131, 138, 149, 164, 168; MS: m/z = 439 (M+ +1).

(E)-2-((4-(Dimethylamino)benzylidene) amino)-1H-benzo[d]imidazol-1-yl-[3-nitrophenyl]methanone (23) IR (KBr cm−1): 1719 (C=O str.), 3085 (C–H aromatic str.), 1615 (N=CH str.), 1546 (Ar-NO2 str.), 752 m/z 9.568 (s, 1H, N=CH), 2.909 (s, 6H (CH3)2); 13C NMR (DMSO): 40, 115, 123, 127, 131, 138, 149, 160, 168; MS: m/z = 413 (M+ +1).

(E)-2-(((2-hydroxynaphthalen-1-yl)-methylene) amino)-1H-benzo[d]imidazol-1-yl-[3-nitrophenyl]methanone (24) IR (KBr cm−1): 1696 (C=O str.), 2924 (C–H aromatic str.), 1553 (N=CH str.), 1546 (Ar-NO2 str.), 752 (O–H bending (out of plane)); 1H NMR (DMSO): 6.748–8.632 (m, 14H, ArH), 9.652 (s, 1H, N=CH); 13C NMR (DMSO): 115, 118, 123, 125, 127, 128, 131, 138, 149, 160, 168; MS: m/z = 436 (M+ +1).

(E)-((4-Nitrobenzylidene)amino)-1H-benzo[d]imidazol-1-yl-[3-nitrophenyl]methanone (25) IR (KBr cm−1): 1704 (C=O str.), 3107 (C–H aromatic str.), 1617 (N=CH str.), 1549 (Ar, NO2 str.); 1H NMR (DMSO): 7.206–8.987 (m, 12H, ArH), 9.672 (s, 1H, N=CH); 13C NMR (DMSO): 7.169–8.932 (m, 11H, ArH), 8.185 (s, 1H, N=CH); 13C NMR (DMSO): 40, 115, 123, 124, 125, 127, 131, 136, 139, 151, 160, 168; MS: m/z = 415 (M+ +1).

(E)-Naphthalen-2-yl-2-((4-nitrobenzylidene) amino)-1H-benzo[d]imidazol-1-yl)methanone (26) IR (KBr cm−1): 1686 (C=O str.), 3056 (C–H aromatic str.), 1600 (N=CH str.), 1545 (Ar, NO2 str.), 1592 (Naphthalene ring str.); 1H NMR (DMSO): 6.865–7.954 (m, 11H, ArH), 8.765 (s, 1H, N=CH), 8.923–9.867 (m, 4H, Ar(NO2)); 13C NMR (DMSO): 115, 123, 124, 128, 129, 131, 135, 136, 139, 142, 149, 160, 168; MS: m/z = 420 (M+ +1).

(E)-2-((3,4-Dimethoxybenzylidene)amino)-1H-benzo[d]imidazol-1-yl-[3-nitrophenyl]methanone (27) IR (KBr cm−1): 1684 (C=O str.), 1611 (N=CH str.), 2875 (Ar, O–CH3 str.); 1543 (Ar, NO2 str.); 1H NMR (DMSO): 7.463–8.932 (m, 11H, ArH), 8.185 (s, 1H, N=CH), 2.904 (s, 6H, (OCH3)2); 13C NMR (DMSO): 56, 115, 123, 125, 127, 136, 139, 142, 147, 150, 152, 160, 168; MS: m/z = 430 (M+ +1).

(E)-1-((4-Nitrobenzylidene)amino)-1H-benzo[d]imidazol-1-yl)hexadecan-1-one (28) IR (KBr cm−1): 1685 (C=O str.), 2954 (C–H aromatic str.), 1618 (N=CH str.), 1271 (Palmitoyl group str.), 1547 (Ar-NO2 str.); 1H NMR (DMSO): 7.624–8.163 (m, 8H, ArH), 8.672 (s, 1H, N=CH), 1.243–2.496 (m, 28H, CH2 of palmitoyl), 0.845–0.878 (t, 3H, CH3); 13C NMR (DMSO): 14, 23, 26, 30, 32, 56, 106, 115, 120, 123, 125, 128, 131, 135, 136, 139, 142, 149, 160, 170; MS: m/z = 504 (M+ +1).

(E)-1-((4-(Dimethylamino)benzylidene) amino)-1H-benzo[d]imidazol-1-yl)hexadecan-1-one (29) IR (KBr cm−1): 2927 (C–H, aromatic str.), 2813 (C–H str. aliphatic), 1659 (C=O str.), 1594 (N=CH str.), 1303 (C–N str.), 1278 (palmitoyl group str.); 1H NMR (DMSO): 7.878–7.901 (d, 1H, N=CH), 6.606–6.622 (d, 1H, –CH=CH), 6.661–7.519 (m, 8H, ArH), 3.773 (s, 6H, (CH3)2), 1.252–2.368 (m, 28H, CH2 of palmitoyl), 0.861–0.894 (t, 3H, CH3); 13C NMR (DMSO): 14, 23, 26, 30, 32, 56, 106, 115, 120, 123, 125, 128, 130, 139, 142, 149, 164, 170; MS: m/z = 528 (M+ +1).

Biological evaluation

In vitro antimicrobial assay

Tube dilution method [15] was used to determine the antimicrobial activity of synthesized compounds against Gram-positive bacteria: Staphylococcus aureus (MTCC-3160); Bacillus subtilis (MTCC-441), the Gram-negative bacterium Escherichia coli (MTCC-443) and fungal species: Candida albicans (MTCC-227) and Aspergillus niger (MTCC-281). Dilutions were made for test and standard compounds in appropriate double strength nutrient broth—I.P. (bacteria) or Sabouraud dextrose broth—I.P. (fungi) [16]. The test and standard compounds were incubated at 37 °C for 24 h (bacteria), at 25 °C for 7 days (A. niger) and at 37 °C for 48 h (C. albicans) and the minimum inhibitory concentration (MIC) was recorded in µg/mL.

In vitro anticancer assay

The in vitro anticancer activity of the developed compounds was performed by the Sulforhodamine B (SRB) assay as described by Skehan et al. [14]. The optimal MCF-7 cell count was seeded on flat-bottom well plates and allowed to attach overnight. The compounds (20 µL) were added in quadruplicates and incubated for 72 h.
(both drug-free control and treated cells). Cells in each well were fixed with 200 μL of 10% cold trichloroacetic acid. After incubation for 30 min, the individual wells were rinsed with water, allowed to stain in 100 μL 0.4% SRB [Sigma-Aldrich, St Louis, Missouri, USA] (w/v in 1% acetic acid) for 15 min. The air-dried plates were placed on a plate shaker and bound SRB was solubilised in 100 μL 10 mM Tris base solution. Absorbance was measured using a spectrophotometer at 570 nm and a dose–response curve was plotted from which the IC₅₀ value of each compound against each cell type was determined.

**Conclusion**

In conclusion, a series of 1,2-disubstituted benzimidazole derivatives were synthesized and assessed for in vitro antimicrobial and antiparasitic activities against five representative microbial species and cancer cell line. Antimicrobial activity results indicated that the synthesized compound 1 has promising activity towards Gram negative bacteria *E. coli*. None of the compound showed more potent activity against Gram positive bacteria *B. subtilis* and *S. aureus* when compared to reference drug norfloxacin. Moreover, compounds 2 and 19 showed interesting results against fungal strains *C. albicans* and *A. niger* and comparable to fluconazole. The results from anticancer activity indicated that compounds 12, 21, 22 and 29 showed promising activity against MCF7. These active compounds may be taken as lead compounds for discovery of novel antimicrobial and anticancer agents in future.

**Authors' contributions**

SSS, BN, NV and SK have designed, synthesized and carried out the spectral analysis, antimicrobial and SML, SAAS, KR and VM have carried out the spectral analysis, interpretation and cytotoxicity study of synthesized compounds. All authors read and approved the final manuscript.

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**Competing interests**

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