Design, synthesis and therapeutic potential of 3-(2-(1H-benzo[d]imidazol-2-ylthio)acetamido)-N-(substituted phenyl)benzamide analogues

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Abstract
Background: The emergence of bacterial resistance is a major public health problem. It is essential to develop and synthesize new therapeutic agents with better activity. The mode of actions of certain newly developed antimicrobial agents, however, exhibited very limited effect in treating life threatening systemic infections. Therefore, the advancement of multi-potent and efficient antimicrobial agents is crucial to overcome the increased multi-drug resistance of bacteria and fungi. Cancer, which remains as one of the primary causes of deaths and is commonly treated by chemotherapeutic agents, is also in need of novel and efficacious agents to treat resistant cases. As such, a sequence of novel substituted benzamides was designed, synthesized and evaluated for their antimicrobial and anticancer activities.

Methodology: All synthesized compounds were characterized by IR, NMR, Mass and elemental analysis followed by in vitro antimicrobial studies against Gram-positive (Staphylococcus aureus), Gram-negative (Salmonella typhi and Klebsiella pneumoniae) bacterial and fungal (Candida albicans and Aspergillus niger) strains by the tube dilution method. The in vitro anticancer evaluation was carried out against the human colorectal carcinoma cell line (HCT116), using the Sulforhodamine B assay.

Results, discussion and conclusion: Compound W6 (MICca, st, kp = 5.19 µM) emerged as a significant antibacterial agent against all tested bacterial strains i.e. Gram-positive (S. aureus), Gram-negative (S. typhi, K. pneumoniae) while compound W1 (MICca, an = 5.08 µM) was most potent against fungal strains (A. niger and C. albicans) and comparable to fluconazole (MIC = 8.16 µM). The anticancer screening demonstrated that compound W17 (IC50 = 4.12 µM) was most potent amongst the synthesized compounds and also more potent than the standard drug 5-FU (IC50 = 7.69 µM).

Keywords: m-Amino benzoic acid, 2-Mercaptobenzimidazole, Benzamide, Antibacterial, Antifungal, Anticancer, SAR

Background
Antibiotics with a wide spectrum of activity are considered to be most potent against Gram positive or Gram-negative microbes. Nevertheless, there is now a frightening increment of resistance against commercially accessible antimicrobials which diminishes the scope of treatment against distinctive irresistible infections. Resistance against β-lactam, macrolides, quinolones, and vancomycin is amongst the most vital medical issues. This has called for the discovery of novel and potent antimicrobials with varying chemical characteristics [1].

Colorectal tumour (CRC) is one of the most widely recognized gastrointestinal malignancies. Changes in way of
life, high-fat eating regimen, physical apathy and smoking are related to CRC pathogenesis. About 25% instances of CRC were presented with metastases at early analysis and nearly 50% of CRC patients would suffer from metastasis at some stage of life. To a large extent, the outcomes of treatment for these patients are unsatisfactory because usual regimens consider the probability of homogeneous distribution of tumor mass. It is increasingly recognized that CRC is sustained by a distinctive set of neoplastic cells named “cancer stem cells (CSCs)” which have an intrinsic potential of stemness (protection from treatment) and oncogenesis (malignant growth) [2].

Owing to their diverse potential, heterocyclic scaffolds are of major interest in the pharmaceutical industry. The heterocyclic entities which have a nitrogen group occupying a central position make them highly resemblance in structure with numerous naturally occurring molecules. In terms of improvement of biologically and therapeutically important molecules, benzimidazole nucleus has been proven as the most advantageous pharmacophore [3].

Several benzimidazole motifs with wide spectrum of diversified biological and pharmacological potentials have already been published in literature. They were reported to exhibit essential antimicrobial and antifungal [4–7], anticaner [8, 9], antiulcer [10], antihistaminic [11], antiviral [12], antihelmentic [13], analgesic [14], antihypertensive [15] and antidepressant activities [16]. Figure 1 shows a profound number of marketed medicines containing benzimidazole as a core moiety. There is now a profoundly large number of benzimidazole at several stages of evaluation in different research associations all over the globe [17].

Benzimidazole is also a vital pharmacophore, a privileged sub-structure in medicinal chemistry which contributes as a key part for different natural activities. The prominent organic applications as demonstrated by molecules related to these cores have incited wide examinations for their synthesis. Extensive biochemical and pharmacological investigations have affirmed that benzimidazole derivatives are effective against different microorganisms. Given the basic resemblance with purine, antibacterial capacity of these scaffolds showed that their opposition with purines would bring about a particular hindrance against the combination of nucleic acids and proteins inside the bacterial cell wall [18, 19]. Figure 2 reviews the biological and therapeutic profile of benzimidazoles as motivated by the structural features. As part of our continued effort in exploring new therapeutic molecules, we hereby report the synthesis, antimicrobial and anticancer evaluation as well as SAR studies of some 3-(2-(1H-benzo[d]imidazol-2-ylthio)acetamido)-N-(substituted phenyl) benzamide derivatives.

Results and discussion

Chemistry

The new target molecules (W1–W21) had been synthesized by multistep procedure as discussed in Scheme 1. Firstly, the 3-(2-chloroacetamido) benzoic acid (int-I) was synthesized by the reaction of chloroacetyl chloride with m-amino benzoic acid, then it was refluxed with 2-mercaptopbenzimidazole which yielded 3-(2-(1H-benzo[d]imidazol-2-ylthio)acetamido)benzoic acid (int-II) which on reaction with thionyl chloride in suitable alcohol resulted in formation of 3-(2-(1H-benzo[d]imidazol-2-ylthio)acetamido)benzoyl chloride (int-III). The reaction of above synthesized benzoyl chloride (int-III) with different substituted anilines in methanolic/ethanolic solvent yielded the title scaffolds (W1–W21) with appreciable yields. The physicochemical properties with Mass spectra and elemental analysis of synthesized compounds are given in experimental section. The synthesized molecules (W1–W21) were also illustrated by FT-IR, proton and carbon-NMR data which are in concurrence with the proposed molecular structures of synthesized compounds. The IR stretching vibration at 3107–3097 cm⁻¹ and 1600 cm⁻¹ indicated the occurrence of aromatic C–H and C=C groups, respectively. The IR band in the range of 653 cm⁻¹ to 651 cm⁻¹ corresponds to the C–Br stretching of Ar–Br compounds (W5 and W21). The presence of aromatic nitro group in compounds, W1, W2, W3, W4, W6 and W12 is indicated by the appearance of stretching in the range of 1554–1484 cm⁻¹. IR band appearance around 2832–2820 cm⁻¹ has established the existence of Ar-OCH₃ (an arylalkyl ether) in compounds W15, W16 and W17. Moreover, halogen group presence in compounds, W6, W12 and W13 is specified by the presence of stretching vibration at 758–742 cm⁻¹ of Ar-Cl in and compounds W18 and W19 by presence of Ar-F stretching at 1085–1084 cm⁻¹. IR stretching at 2900–2869 cm⁻¹ in the spectral data of synthesized derivatives (W9–W11) depicted the presence of Ar-CH₃. Presence of C–S group is indicated by the appearance IR stretching at 708–679 cm⁻¹ in synthesized compound's spectral data. The appearance of –CONH– group is suggested by the IR stretching at 1670–1662 cm⁻¹ spectral data of synthesized compounds. The presence of –C=N– (3° amine) and –C-NH– (2° amine) groups is suggested by the appearance of IR stretching 1372–1321 cm⁻¹ and 1338–1302 cm⁻¹ of synthesized compound's spectral data respectively. The structures of N-phenylbenzamide were further confirmed by the corresponding ¹H-NMR. The multiplet signals between 7.12 and 10.75 δ ppm in ¹H-NMR spectra indicated the aromatic proton of synthesized derivatives. The presence of –CH₂– and –CONH– between 2-mercaptopbenzimidazole and para amino benzoic acid in all the synthesized derivatives.
were indicated by appearance of singlet at 2.51–4.34 δ ppm and 7.82–8.15 δ ppm, respectively. Owing to presence of CH$_3$ of Ar-CH$_3$, the compounds W9, W10 and W11 reflected singlet at 2.51–2.53 δ ppm. As a result of presence of –OCH$_3$ of Ar-OCH$_3$, compounds W15, W16 and W17 showed singlet at range of 3.73–3.74 δ ppm. Due to presence of -NH- of benzimidazole, all synthesized compounds reflected singlet at 4.31–4.37 δ ppm.
The findings of elemental analysis of synthesized 2-mercaptobenzimidazoles were recorded within theoretical results of ±0.4%. Conclusively, the $^{13}$C-NMR spectra of synthesized benzamides were in DMSO-$d_6$ and their molecular structures were in accordance with the spectral signals. Mass spectra of the synthesized derivatives reflected the characteristic molecular ion peaks.

**In vitro antimicrobial and anticancer screening results**

The antimicrobial results of synthesized benzamides (W1–W21) are presented in Table 1, Figs. 3 and 4. The antimicrobial potential of synthesized compounds is comparable to the reference ofloxacin (antibacterial) and fluconazole (antifungal). Antimicrobial screening results revealed that among the synthesized compounds, 4-chloro-2-nitro substituted benzamide i.e. compound W6 (MIC$_{sa, st, kp}$ = 5.19 µM) exhibited significant antibacterial potency against all the tested bacterial strains i.e. Gram-positive (S. aureus), Gram-negative (S. typhi, K. pneumoniae) while the antifungal results indicated that the 2, 4-dinitro substituted derivative i.e. compound W1 (MIC$_{ca, an}$ = 5.08 µM) was found to be most potent against fungal strains (A. niger and C. albicans), even more potent than standard fluconazole (MIC= 8.16 µM). On the other hand, the in vitro anticancer activity of synthesized analogues (W1–W21) were evaluated against the human colorectal carcinoma [HCT116 (ATCC CCL-247)] cancer cell line using the SRB assay and in comparison to 5-FU, the standard drug. Table 1 shows the results in vitro anticancer activity of the synthesized analogues (W1–W21). The anticancer screening results showed that 4-methoxy substituted scaffold i.e. compound W17 (IC$_{50}$ = 4.12 µM) had the highest anticancer activity amongst the synthesized ones. It was found to be more potent than the standard drug, 5-FU (IC$_{50}$ = 7.69 µM).

![Fig. 2 Design of benzimidazole analogues for antimicrobial and anticancer activity based on biological profile](image-url)
SAR (structure activity relationship) studies

The structure activity relationship for antimicrobial and anticancer screening results of synthesized benzamides (SAR, Fig. 5): substitution of aromatic ring with 2, 4-dinitro substituent (compound W1, MIC = 5.08 µM) considerably improved the antifungal activity of benzamide derivatives against C. albicans and A. niger respectively whereas substitution with 4-chloro-2-nitro group (compound W6, MIC = 5.19 µM) resulted in improved antibacterial activity against both Gram-negative E. coli,

### Scheme 1
Synthesis of 3-(2-(1H-benzo[d]imidazol-2-ylthio)acetamido)-N-(substitutedphenyl) benzamides

| Comp. | R       | R2  | R3  | R4  | R5  | R6  | Comp. | R       | R2  | R3  | R4  | R5  | R6  |
|-------|---------|-----|-----|-----|-----|-----|-------|---------|-----|-----|-----|-----|-----|
| W1    | -       | NO2 | H   | NO2 | H   | H   | W12   | -       | Cl  | H   | NO2 | H   | H   |
| W2    | -       | NO2 | H   | H   | H   | H   | W13   | -       | H   | H   | Cl  | H   | H   |
| W3    | -       | H   | NO2 | H   | H   | H   | W14   | -       | H   | H   | H   | H   | H   |
| W4    | -       | H   | H   | NO2 | H   | H   | W15   | -       | OCH3 | H   | H   | H   | H   |
| W5    | -       | H   | H   | Br  | H   | H   | W16   | -       | H   | OCH3| H   | H   | H   |
| W6    | -       | NO2 | H   | Cl  | H   | H   | W17   | -       | H   | H   | OCH3| H   | H   |
| W7    | -       | Cl  | H   | H   | H   | H   | W18   | -       | F   | H   | H   | H   | H   |
| W8    | -       | H   | Cl  | H   | H   | H   | W19   | -       | H   | H   | F   | H   | H   |
| W9    | -       | CH3 | H   | H   | CH3 | H   | W20   | C2H5   | -   | -   | -   | -   | -   |
| W10   | -       | CH3 | H   | CH3 | H   | H   | W21   | -       | H   | Br  | H   | H   | H   |
| W11   | -       | H   | CH3 | H   | H   | H   |        |         |     |     |     |     |     |

**Step a**: Chloroacetyl chloride, Triethylamine, Ethanol, Stirred at room temperature for 1 h;
**Step b**: 2-Mercaptobenzimidazole, anhyd. Potassium carbonate, Ethanol, Reflux 5-6 h (40 °C);
**Step c**: Thionyl chloride, Methanol, Reflux 2-3 h (40 °C);
**Step d**: Substituted anilines, Methanol, Reflux 4-5 h (35-40 °C).
K. pneumoniae and Gram-positive S. aureus species, respectively. Presence of methoxy group at para position of compound W17 (IC$_{50}$ = 4.12 µM) is responsible for improved antiproliferative activity against HCT116 cancer cell line whereas methoxy group at ortho and meta position (compound W15 and W16, IC$_{50}$ = 23.12 µM) exhibited lesser activity against same cancer cell line. From the analysis of structures of most active antimicrobial compounds, it may be concluded that the introduction of nitro and halo groups to aromatic ring as an electron-withdrawing moiety may increase the antifungal as well as the antibacterial activities of the synthesized scaffolds. The above findings had demonstrated that variation in nature and position of functional groups on aromatic framework caused a considerable change in anticancer potency. The substitution of methoxy group at para position of N-phenylbenzamide enhanced the anticancer potential. These studies reveal that different functional groups are required for different activities.

### Experimental section

The reactants and reagents for syntheses were taken from commercial resource. The microbes (Microbial type cell cultures-MTCC) were acquired from Institute of Microbial Technology and Gene bank (IMTECH), Chandigarh. Reaction steps forward was checked by thin layer chromatography (TLC) using ethyl acetate as mobile phase. The scheme was drawn via ChemDraw 8.03. Melting point of synthesized derivatives was determined by open capillary tube technique. An infrared (IR) spectrum was recorded on Bruker 12060280, Software: OPUS 7.2.139.1294 spectrometer using ATR and results were in cm$^{-1}$. Bruker Avance III 600 NMR spectrometer was utilized for $^1$H/$^{13}$C-NMR (DMSO-d6, δ ppm). Waters

### Table 1 Antimicrobial and anticancer screening results of synthesized analogues

| Compounds | Minimum inhibitory concentration (MIC = µM) | IC$_{50}$ (µM) |
|-----------|---------------------------------------------|----------------|
|           | Bacterial species                          | Fungal species | Cancer cell line (HCT116) |
|           | Gram +ve                                    | Gram -ve       |                             |
|           | SA                                          | ST             | KP                          | CA  | AN  |                  |
| W1        | 10.15                                       | 10.15          | 10.15                       | 5.08 | 5.08 | > 20.31          |
| W2        | 11.17                                       | 11.17          | 11.17                       | 5.59 | 5.59 | > 22.35          |
| W3        | 11.17                                       | 11.17          | 11.17                       | 5.59 | 5.59 | > 22.35          |
| W4        | 11.17                                       | 11.17          | 11.17                       | 5.59 | 5.59 | > 22.35          |
| W5        | 10.39                                       | 10.39          | 10.39                       | 5.19 | 5.19 | > 20.77          |
| W6        | 5.19                                        | 5.19           | 5.19                        | 5.19 | 5.19 | > 20.75          |
| W7        | 11.44                                       | 11.44          | 11.44                       | 5.72 | 5.72 | > 22.89          |
| W8        | 11.44                                       | 11.44          | 11.44                       | 5.72 | 5.72 | > 22.89          |
| W9        | 11.61                                       | 11.61          | 11.61                       | 5.81 | 5.81 | > 23.23          |
| W10       | 11.61                                       | 11.61          | 11.61                       | 5.81 | 5.81 | > 23.23          |
| W11       | 12.00                                       | 12.00          | 12.00                       | 6.00 | 6.00 | > 24.01          |
| W12       | 10.38                                       | 10.38          | 10.38                       | 5.19 | 5.19 | > 20.78          |
| W13       | 11.44                                       | 11.44          | 11.44                       | 5.72 | 5.72 | > 22.89          |
| W14       | 12.42                                       | 12.42          | 12.42                       | 6.21 | 6.21 | > 24.84          |
| W15       | 11.56                                       | 11.56          | 11.56                       | 5.78 | 5.78 | > 23.12          |
| W16       | 11.56                                       | 11.56          | 11.56                       | 5.78 | 5.78 | > 23.12          |
| W17       | 11.56                                       | 11.56          | 11.56                       | 5.78 | 5.78 | > 23.12          |
| W18       | 11.89                                       | 11.89          | 11.89                       | 5.95 | 5.95 | > 23.78          |
| W19       | 11.89                                       | 11.89          | 11.89                       | 5.95 | 5.95 | > 23.78          |
| W20       | 11.61                                       | 11.61          | 11.61                       | 5.81 | 5.81 | 11.61            |
| W21       | 10.39                                       | 10.39          | 10.39                       | 5.19 | 5.19 | > 20.77          |
| DMSO      | NA                                          | NA             | NA                          | NA  | NA  | > 1.44           |
| Broth control | NG                                     | NG             | NG                          | NG  | NG  | –                 |
| Ofloxacin | 1.73                                        | 1.73           | 1.73                        | –   | –   | –                 |
| Fluconazole | –                                      | –              | –                           | 8.16| 8.16| –                 |
| S-Fluorouracil | –                                     | –              | –                           | –   | –   | 7.69              |

SA: Staphylococcus aureus (MTCC3160); ST: Salmonella typhi (MTCC3231); KP: Klebsiella pneumonia (MTCC9024); CA: Candida albicans (MTCC281) and AN: Aspergillus niger (MTCC227); DMSO: dimethyl sulfoxide; NA: no activity; NG: no growth
Micromass Q-ToF Micro instrument was used for mass spectra. Elemental analysis was performed on Perkin-Elmer 2400 C, H and N analyzer and all synthesized compounds gave C, H and N analysis within ± 0.4% of the theoretical results.

Procedure for synthesized benzamides (W1–W21)

**Step a: Synthesis of int-I**

A mixture of m-aminobenzoic acid (0.01 mol) and triethylamine (0.01 mol) in ethanol was stirred to get a clear solution. Then the solution was cooled in ice for 30 min followed by dropwise addition of chloroacetyl chloride (0.01 mol) with stirring (1 h). The resultant precipitate was strained via filtering, desiccated and recrystallized using alcohol [20].

**Step b: Synthesis of int-II**

To the reaction mixture of 3-(2-chloroacetamido) benzoic acid (int-I, 0.01 mol) and 2-mercaptobenzimidazole (0.01 mol) in alcohol potassium carbonate (0.01 mol) was added and then refluxed for 5–6 h and cooled to room temperature followed by evaporation to dryness. The resultant residue was washed with water and recrystallized from ethanol [1].

**Step c: Synthesis of int-III**

A mixture of thionyl chloride (0.3 mol) and 3-(2-(1H-benzo[d]imidazol-2-ylthio)acetamido) benzoic acid (int-II, 0.25 mol) was refluxed for 2 h. The excess of thionyl chloride was removed by distillation [21].

**Step d: Synthesis of final (W1–W21) benzamides**

The reaction mixture of 3-(2-(1H-benzo[d]imidazol-2-ylthio)acetamido)benzoyl chloride (int-III, 0.01 mol) and substituted aniline (0.01 mol) in suitable solvent was refluxed for appropriate time and thin layer chromatography was used to monitor the reaction. After completion of reaction, it was poured into ice cold water and the
resultant precipitate was filtered, desiccated and recrystallized using ethanol [22].

Spectral data elucidation of the synthesized N-substituted phenyl benzamide derivatives.

3-(2-(1H-benzo[d]imidazol-2-ylthio)acetamido)-N-(2,4-dinitrophenyl)benzamide (W1)

M.pt. °C: 200–203; Rf value: 0.70; % yield: 97.55; IR: [3099 (C–H str.), 1600 (C=C str.) of pn (phenyl nucleus)], 1520 (C–NO2 str., C6H5NO2); 1H-NMR: 7.16–10.75 (m, 11H, Ar–H), 4.34 (s, 1H, NH of imidazole), 2.53 (s, 2H, CH2), 8.15 (s, 2H, (CONH)2); 13C-NMR: 36.19, 119.67, 119.78, 121.52, 123.06, 123.26, 128.59, 129.00, 129.15, 135.06, 149.71, 149.74, 166.38, 167.02; Mol. Formula: C22H16N6O6S; Elem. Anal. Calcd: C, 53.66; H, 3.27; N, 17.07; Found: C, 53.69; H, 3.23; N, 17.03; MS: m/z 493 [M+1].

3-(2-(1H-benzo[d]imidazol-2-ylthio)acetamido)-N-(2-nitrophenyl)benzamide (W2)

M.pt. °C: 212–215; Rf value: 0.54; % yield: 67.71; IR: [3097 (C–H str.), 1598 (C–C str.) of pn], 1506 (C–NO2 str., C6H5NO2); 1H-NMR: 7.16–8.30 (m, 12H, Ar–H), 4.34 (s, 1H, NH of imidazole), 2.53 (s, 2H, CH2), 8.15 (s, 2H, (CONH)2); 13C-NMR: 36.19, 119.67, 119.78, 121.52, 123.06, 123.26, 128.59, 129.00, 129.15, 135.06, 139.015, 149.71, 149.74, 166.38, 167.02; Mol. Formula: C22H17N5O4S; Elem. Anal. Calcd: C, 59.05; H, 3.83; N, 15.65; Found: C, 53.69; H, 3.87; N, 15.69; MS: m/z 448 [M+1].

3-(2-(1H-benzo[d]imidazol-2-ylthio)acetamido)-N-(3-nitrophenyl)benzamide (W3)

M.pt. °C: 213–216; Rf value: 0.57; % yield: 71.07; IR: [3090 (C–H str.), 1598 (C–C str.) of pn], 1506 (C–NO2 str., C6H5NO2); 1H-NMR: 7.16–8.30 (m, 12H, Ar–H), 4.34 (s, 1H, NH of imidazole), 2.53 (s, 2H, CH2), 8.15 (s, 2H, (CONH)2); 13C-NMR: 36.19, 119.67, 119.78, 121.52, 123.06, 123.26, 128.59, 129.00, 129.15, 135.06, 139.015, 149.71, 149.74, 166.38, 167.02; Mol. Formula: C22H17N5O4S; Elem. Anal. Calcd: C, 59.05; H, 3.83; N, 15.65; Found: C, 59.01; H, 3.79; N, 15.61; MS: m/z 448 [M+1].

3-(2-(1H-benzo[d]imidazol-2-ylthio)acetamido)-N-(4-nitrophenyl)benzamide (W4)

M.pt. °C: 206–209; Rf value: 0.69; % yield: 78.25; IR: [3097 (C–H str.), 1595 (C–C str.) of pn], 1662 (C=O str., amide), 1331 (C=O str., N=CH), 1302 (C=N str.), 679 (C=S str., CH2-S), 2913 (C–H str., –CH2–), 1554 (C–NO2 str., C6H5NO2); 1H-NMR: 7.19–8.33 (m, 12H, Ar–H), 4.37 (s, 1H, NH of imidazole), 2.53 (s, 2H, CH2), 8.01 (s, 2H, (CONH)2); 13C-NMR: 36.21, 119.84, 119.84, 121.67, 123.17, 124.29, 129.05, 131.38, 139.03, 149.73, 166.39, 167.05; Mol. Formula: C22H17N5O4S; Elem. Anal. Calcd: C, 59.05; H, 3.83; N, 15.65; Found: C, 59.01; H, 3.79; N, 15.61; MS: m/z 448 [M+1].

**Fig. 5** Structural requirements for the antimicrobial and anticancer activities of synthesized benzimidazole analogues
3-(2-(1H-benzo[d]imidazol-2-ylthio)acetamido)-N-(4-bromophenyl)benzamide (W5)
M.p. 'C: 202–205; Rf value: 0.72; % yield: 73.96; IR: [3107 (C–H str.), 1599 (C=C str.) of pn], 1665 (–CONH str., amide), 1334 (C=N str., N=CH), 1304 (C=N str.), 681 (C=S str., CH2=S), 2915 (C–H str., –CH2–), 651 (C–Br str., C6H5Br); 1H-NMR: 7.18–8.33 (m, 12H, Ar–H), 4.36 (s, 1H, NH of imidazole), 7.85 (s, 2H, (CONH)2); 13C-NMR: 36.20, 119.84, 121.60, 123.17, 124.12, 129.05, 131.38, 149.73, 166.41, 1667.06; Mol. Formula: C22H17N4O2SBr; Elem. Anal. Calcd: C, 60.48; H, 3.92; N, 12.82; Found: C, 60.52; H, 3.88; N, 12.85; MS: m/z 437 [M+ +1].

3-(2-(1H-benzo[d]imidazol-2-ylthio)acetamido)-N-(2-nitro-4-chlorophenyl)benzamide (W6)
M.p. 'C: 215–218; Rf value: 0.76; % yield: 97.71; IR: [3100 (C–H str.), 1599 (C=C str.) of pn], 1670 (–CONH str., amide), 1367 (C=N str., N=CH), 1338 (C=N str.), 681 (C=S str., CH2=S), 2915 (C–H str., –CH2–), 758 (C=Cl str., C6H5Cl), 1503 (C–NO2 str., C6H5NO2); 1H-NMR: 7.16–8.29 (m, 11H, Ar–H), 4.33 (s, 1H, NH of imidazole), 7.96 (s, 2H, (CONH)2); 13C-NMR: 36.18, 118.30, 121.15, 123.13, 124.00, 124.26, 129.97, 131.36, 139.03, 145.03, 166.40, 167.02; Mol. Formula: C22H16N2O2SCl; Elem. Anal. Calcd: C, 60.48; H, 3.56; N, 11.64; Found: C, 54.84; H, 3.52; N, 11.60; MS: m/z 482 [M+ +1].

3-(2-(1H-benzo[d]imidazol-2-ylthio)acetamido)-N-(2-nitro-4-chlorophenyl)benzamide (W7)
M.p. 'C: 217–220; Rf value: 0.44; % yield: 89.98; IR: [3099 (C–H str.), 1598 (C=C str.) of pn], 1664 (–CONH str., amide), 1335 (C=N str., N=CH), 1304 (C=N str.), 681 (C=S str., CH2=S), 2915 (C–H str., –CH2–), 742 (C=Cl str., C6H5Cl); 1H-NMR: 7.14–8.33 (m, 12H, Ar–H), 4.33 (s, 1H, NH of imidazole), 7.84 (s, 2H, (CONH)2); 13C-NMR: 36.18, 119.80, 119.78, 121.50, 123.15, 124.28, 129.06, 131.37, 139.04, 149.71, 166.42, 167.04; Mol. Formula: C22H16N2O2SCl; Elem. Anal. Calcd: C, 60.48; H, 3.92; N, 12.82; Found: C, 60.44; H, 3.96; N, 12.86; MS: m/z 437 [M+ +1].

3-(2-(1H-benzo[d]imidazol-2-ylthio)acetamido)-N-(3-chlorophenyl)benzamide (W8)
M.p. 'C: 219–222; Rf value: 0.66; % yield: 77.47; IR: [3097 (C–H str.), 1608 (C=C str.) of pn], 1665 (–CONH str., amide), 1369 (C=N str., N=CH), 1338 (C=N str.), 688 (C=S str., CH2=S), 2945 (C–H str., –CH2–), 745 (C=Cl str., C6H5Cl); 1H-NMR: 7.15–8.31 (m, 12H, Ar–H), 4.35 (s, 1H, NH of imidazole), 7.85 (s, 2H, (CONH)2); 13C-NMR: 36.18, 119.81, 119.78, 121.51, 123.16, 124.29, 129.06, 131.38, 139.04, 149.71, 166.43, 167.05; Mol. Formula: C22H17N4O2SCl; Elem. Anal. Calcd: C, 60.48; H, 3.92; N, 12.82; Found: C, 60.52; H, 3.88; N, 12.85; MS: m/z 437 [M+ +1].
3-(2-(1H-benzo[d]imidazol-2-ylthio)acetamido)-N-(4-chlorophenyl)benzamide (W13)

M.pt. °C: 206–209; Rf value: 0.45; % yield: 79.12; IR: 3045 (C–O–C str., phenyl ether), 2842 (C–H str., –CH 2–), 743 (C–S str., CH2-S), 2914 (C–H str., –CH 2–); 1H-NMR: 7.19–8.31 (m, 12H, Ar–H), 4.37 (s, 1H, NH of imidazole), 7.99 (s, 2H, (CONH) 2); 13C-NMR: 36.18, 113.75, 119.80, 121.35, 123.17, 124.31, 128.68, 129.05, 131.38, 149.73, 166.27, 167.06; Mol. Formula: C22H18N5O4SCl; Elem. Anal. Calcd. C, 63.87; H, 4.66; N, 13.13; Found: C, 63.85; H, 4.62; N, 12.99; MS: m/z 433 [M+ +1].

3-(2-(1H-benzo[d]imidazol-2-ylthio)acetamido)-N-(phenyl)benzamide (W14)

M.pt. °C: 203–206; Rf value: 0.45; % yield: 99.18; IR: 3045 (C–O–C str., phenyl ether), 2842 (C–H str., –CH 2–), 743 (C–S str., CH2-S), 2914 (C–H str., –CH 2–); 1H-NMR: 7.13–8.29 (m, 13H, Ar–H), 4.32 (s, 1H, NH of imidazole), 7.83 (s, 2H, (CONH) 2); 13C-NMR: 36.19, 119.82, 121.52, 123.16, 124.29, 129.06, 131.38, 139.04, 149.72, 166.44, 167.06; Mol. Formula: C22H17N5O4S; Elem. Anal. Calcd. C, 62.84; H, 4.08; N, 13.33; Found: C, 62.88; H, 4.04; N, 13.29; MS: m/z 421 [M+ +1].

3-(2-(1H-benzo[d]imidazol-2-ylthio)acetamido)-N-(4-methoxyphenyl)benzamide (W18)

M.pt. °C: 216–219; Rf value: 0.42; % yield: 80.86; IR: 3045 (C–O–C str., phenyl ether), 2832 (C–H str., O-CH 3); 1H-NMR: 7.71–8.26 (m, 12H, Ar–H), 4.31 (s, 1H, NH of imidazole), 2.51 (s, 2H, CH2), 7.84 (s, 2H, (CONH) 2); 13C-NMR: 36.16, 119.77, 121.51, 123.13, 124.25, 129.07, 131.36, 139.03, 149.70, 166.39, 167.01; Mol. Formula: C22H18N4O3SF; Elem. Anal. Calcd. C, 62.84; H, 4.08; N, 13.33; Found: C, 62.88; H, 4.04; N, 13.29; MS: m/z 421 [M+ +1].

3-(2-(1H-benzo[d]imidazol-2-ylthio)acetamido)-N-(4-methoxyphenyl)benzamide (W10)

M.pt. °C: 209–212; Rf value: 0.72; % yield: 81.90; IR: 3100 (C–H str.), 1598 (C=C str. of pn), 1665 (-CONH str., amide), 1336 (C=N str., N=CH), 1304 (C–N str.), 704 (C–S str., CH2-S), 2913 (C–H str., –CH2–), 1201 (C–O–C str., phenyl ether), 2820 (C–H str., O-CH3); 1H-NMR: 7.16–8.33 (m, 12H, Ar–H), 4.36 (s, 1H, NH of imidazole), 7.86 (s, 2H, (CONH) 2); 13C-NMR: 36.18, 119.82, 121.15, 123.16, 124.29, 129.06, 131.38, 139.04, 149.72, 166.44, 167.06; Mol. Formula: C22H20N4O3S; Elem. Anal. Calcd. C, 63.87; H, 4.62; N, 12.95; Found: C, 63.85; H, 4.62; N, 12.99; MS: m/z 433 [M+ +1].
3-(2-(1H-benzo[d]imidazol-2-ylthio)acetamido)-N-ethyl-N-phenylbenzamide (W20)

M.p. °C: 216–219; Rf value: 0.42; % yield: 83.22; IR: [3099 (C–H str.), 1598 (C=C str. of pn), 1665 (–CONH str., amide), 1336 (C=N str., N=CH), 1304 (C–N str.), 704 (C=S str., CH2-S), 2915 (C–H str., –CH2–), 2932 (C–H str., CH3), 2826 (C–H str., N-CH3); 1H-NMR: 7.12–8.33 (m, 13H, Ar–H), 4.31 (s, 1H, NH of imidazole), 2.52 (s, 2H, CH2); 13C-NMR: 36.16, 119.78, 119.78, 121.49, 123.14, 124.26, 129.07, 131.37, 139.04, 149.70, 166.02; Mol. Formula C22H17N4O2S; Elem. Anal. Calcd. C, 54.89; H, 4.08; N, 13.01; Found C, 54.85; H, 3.59; N, 11.68; MS: m/z 482 [M+ +1].

3-(2-(1H-benzo[d]imidazol-2-ylthio)acetamido)-N-(3-bromophenyl)benzamide (W21)

M.p. °C: 221–223; Rf value: 0.47; % yield: 70; IR: [3099 (C–H str.), 1598 (C=C str. of pn), 1665 (–CONH str., amide), 1336 (C=N str., N=CH), 1304 (C–N str.), 704 (C=S str., CH2-S), 2915 (C–H str., –CH2–), 653 (C–Br str., Br); 1H-NMR: 7.15–8.29 (m, 12H, Ar–H), 4.31 (s, 1H, NH of imidazole), 7.82 (s, 2H, (CONH)2); 13C-NMR: 36.16, 119.78, 119.78, 121.49, 123.14, 124.26, 129.07, 131.37, 139.04, 149.70, 166.40, 167.02; Mol. Formula C23H18N4O2Br; Elem. Anal. Calcd. C, 62.84; H, 4.08; N, 13.01; Found C, 62.81; H, 4.12; N, 13.37; MS: m/z 431 [M+ +1].

Biological studies

Antimicrobial evaluation

The in vitro antimicrobial potential of the synthesized benzamides (W1–W21) in μM was determined against Gram-positive bacteria (Staphylococcus aureus- MTCC 3160), Gram-negative bacterium (Klebsiella pneumoniae- MTCC 9024, Salmonella typhi- MTCC 3231) and fungal strains (Aspergillus niger- MTCC 281 and Candida albicans MTCC 277) by tube dilution method [23] using ofloxacin (antibacterial) and fluconazole (antifungal) as standard. DMSO was used to dissolve the reference and experimental molecules (W1–W21). Dilutions were set up in nutrient broth (L.P.) for bacterial (incubated at 37 ± 1 °C for 24 h) and Sabouraud dextrose broth (L.P.) for fungal species (25 ± 1 °C for 7 days for A. niger) and (37 ± 1 °C for 48 h for C. albicans) [24].

Anticancer evaluation

The in vitro anticancer potential of the synthesized substituted benzamides was determined against the human colorectal cancer cell line using the SRB assay [25]. HCT116 cells were seeded onto the wells of 96-mL plates at 2500 cells/well for 24 h. The reference and test drug molecules were dissolved in DMSO subjected to serial dilutions. They were then incubated with monolayer cells at 37 °C for 72 h. The treated cells were then fixed with trichloroacetic acid for and then stained with 0.4% w/v of SRB in acetic acid. The unbound stain was removed by washing with 1% acetic acid. Bound SRB was solubilised in 10 mM Tris base solution. Absorbance was measured by a computer-interfaced 96-well plate spectrophotometer at 570 nm.

Conclusion

The synthetic work was conducted under appropriate experimental conditions and the expected compounds had been obtained. The biological studies were carried out to observe the effect of substituents on the antimicrobial and antitumor activities. From the outcomes of antimicrobial and antitumor studies, it is concluded that the introduction of electron-withdrawing groups such as nitro and halo substituents increased the antimicrobial potential of the synthesized scaffolds i.e. compounds W1 (3-(2-(1H-benzo[d]imidazol-2-ylthio)acetamido)-N-(2,4-dinitrophenyl)benzamide) and W6 (3-(2-(1H-benzo[d]imidazol-2-ylthio)acetamido)-N-(2-nitro-4-chlorophenyl)benzamide) while the introduction of para-methoxy substituent to phenyl ring as an electron-donating group i.e. compound W17 (3-(2-(1H-benzo[d]imidazol-2-ylthio)acetamido)-N-(4-methoxyphenyl)benzamide) exhibited the most promising antitumor activity amongst all tested compounds.

Author’s contributions

Authors BN and ST have designed, synthesized and carried out the antimicrobial and antitumor activities. From the outcomes of antimicrobial and antitumor studies, it is concluded that the introduction of electron-withdrawing groups such as nitro and halo substituents increased the antimicrobial potential of the synthesized scaffolds i.e. compounds W1 (3-(2-(1H-benzo[d]imidazol-2-ylthio)acetamido)-N-(2,4-dinitrophenyl)benzamide) and W6 (3-(2-(1H-benzo[d]imidazol-2-ylthio)acetamido)-N-(2-nitro-4-chlorophenyl)benzamide) while the introduction of para-methoxy substituent to phenyl ring as an electron-donating group i.e. compound W17 (3-(2-(1H-benzo[d]imidazol-2-ylthio)acetamido)-N-(4-methoxyphenyl)benzamide) exhibited the most promising antitumor activity amongst all tested compounds.

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References

1. Turan-Zitouni G, Kaplanicki ZA, Ozdemir A, Revial G, Guven K (2007) Synthesis and antimicrobial activity of some 2-(benzo[d]oxazol-benzox[d]imidazo[2-ylthio])-N-(9H-fluoren-9-ylacetamide derivatives. Phosphorus Sulfur Silicon 182:639–646

2. Manhas J, Bhattacharya A, Agrawal SK, Gupta B, Das P, Deo SSV, Pal S, Sen S (2016) Characterization of cancer stem cells from different grades of human colorectal cancer. Tumor Biol. https://doi.org/10.1007/s13277-016-5232-6

3. Gaba M, Mohan C (2016) Development of drugs based on imidazole and benzimidazole bioactive heterocycles: recent advances and future directions. Med Chem Res 25:173–210

4. Ozden S, Atabey D, Yildiz S, Goker H (2005) Synthesis and potent antimicrobial activity of some novel methyl or ethyl 1H-benzimidazole-5-carboxylates derivatives carrying amide or amidine groups. Biocor Med Chem 13:1587–1597

5. Moreira JB, Mann J, Neidle S, McHugh TD, Taylor PW (2013) Antibacterial activity of head-to-head bis-benzimidazoles. Int J Antimicrob Agents 42:361–366

6. Noovli M, Agrawal S, Patel H, Badiger A, Gaba M, Zamble A (2014) Synthesis, antimicrobial and cytotoxic activity of novel azetidine-2-one derivatives of 1H-benzimidazole. Arabian J Chem 7:219–226

7. Kalinowska-Lis U, Felczak A, Checinska L, Lisowska K, Ochocki J (2014) Synthesis, characterization and antimicrobial activity of silver (I) complexes of hydroxymethyl derivatives of pyridine and benzimidazole. J Organomet Chem 749:394–399

8. Wang YT, Qin YJ, Yang N, Zhang YL, Liu CH, Zhu HL (2015) Synthesis, characterization and antimicrobial activity of some 2-substituted-1H-benzimidazoles. Eur J Med Chem 22:2017–2027

9. Yang YH, Cheng MS, Wang QH, Nie H, Liao N, Wang J, Chen H (2009) Design, synthesis, and antitumor evaluation of novel symmetrical bis-benzimidazoles. Eur J Med Chem 44:1808–1812

10. Lonta M, Paglietti G, Piras S, Sparatore A, Manica D, Demontis MP, Varoni MV, Ferracce MC (1993) Synthesis and evaluation of gastroprotective and antiallergy activity of some 2-substituted-1H-benzimidazoles. Farmaco 47(3):287–303

11. Wang XJ, Xi MY, Fu JH, Zhang FR, Cheng GF, Yin DL, You QD (2012) Synthesis, biological evaluation and SAR studies of benzimidazole derivatives as H2-anthistamine agents. Chin Chem Lett 23:707–710

12. Luo Y, Yao JP, Yang L, Feng CL, Tang W, Wang GF, Zuo JP, Lu W (2010) Design and synthesis of novel benzimidazole derivatives as inhibitors of hepatitis B virus. Biocor Med Chem 18:3048–5053

13. Velazquez-Lopez JM, Hernandez-Campos A, Ypez-Mulla L, Tellez-Valencia A, Flores-Castillo P, Nieto-Meneses R, Castillo R (2015) Synthesis and trypanocidal activity of novel benzimidazole derivatives. Biocor Med Chem Lett 26(17):4377–4381

14. El-Feky SA, Thabet HK, Ubide MT (2014) Synthesis, molecular modeling and anti-inflammatory screening of novel fluorinated quinoline incorporated benzimidazole derivatives using the Pfitzinger reaction. J Fluorine Chem 161:87–94

15. Zhu W, Da Y, Wu D, Zheng H, Zhu L, Wang L, Yan Y, Chen Z (2014) Design, synthesis and biological evaluation of new 5-nitrobenzimidazole derivatives as AT1 antagonists with anti-hypertension activities. Biocor Med Chem 22:2294–2302

16. Mathew B, Jerad Suresh J, Ambazhagan S (2016) Development of novel (1H-benzimidazole bearing pyrimidine-trione based MAO-A inhibitors: synthesis, docking studies and antidepressant activity. J Saudi Chem Soc 20:5132–5139

17. Akula G, Srinivas B, Vidyasagar M, Kandikonda S (2011) Synthesis of (1H-benzimidazol-2-ylamino)-2-phenyl-1,3-thiazolidin-4-one as potential CNS depressant. Int J Pharmtech Res 3(1):360–364

18. Bandypadhyay R, Sathe M, Ponnappan S, Sharma A, Sharma P, Srisvatava AK, Kaushik MP (2011) Exploration of in vitro time point quantitative evaluation of newly synthesized benzimidazole and benzothiazole derivatives as potential antibacterial agents. Biocor Med Chem Lett 21:7306–7309

19. Spasov AA, Yahitsa IN, Bugaeva LI, Anisimova VA (1999) Benzimidazole derivatives: spectrum of pharmacological activity and toxicological properties (a review). Pharm Chem J 33(5):1–12

20. Turan-Zitouni G, Ozdemir A, Kaplanicki ZA, Cevikbas A, Gurbuz B, Gurer US (2009) Studies on some new pyrazolo[3,4-c]pyridine derivatives as antimicrobial agents. Turk J Pharm Sci 6(2):63–72

21. Kumar H, Kumar P, Narasimhan B, Ramasamy K, Mani V, Mishra RK, Majeed ABA (2013) Synthesis, in vitro antimicrobial, antiproliferative, and QSAR studies of N-(substituted phenyl)-2-(1H-indol-3-ylazo)-benzamides. Med Chem Res 22:1957–1971

22. Kumar S, Lim SM, Ramasamy K, Vasudevan M, Shah SAA Narasimhan B (2017) Bis-pyrimidine acetamides: design, synthesis and biological evaluation

23. Cappuccino JG, Sherman N (1999) In microbiology-a laboratory manual, 4th edn. Addison Wesley Longman, Inc, California, p 263

24. Pharmacopoeia of India, vol IV (2007) Controller of publication, Ministry of Health Department, Govt. of India, New Delhi, pp. 37

25. Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S, Boyd MR (1990) New colorimetric cytotoxicity assay for anticancer-drug screening. J Natl Cancer Inst 82:1107–1112