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Synthesis and evaluation of antimicrobial, antitubercular and anticancer activities of benzimidazole derivatives

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ABSTRACT

A series of benzimidazole derivatives (**1**–**20**) was synthesized and evaluated for its *in vitro* antimicrobial, antitubercular and anticancer activities. Compound **10** was found to be the most active antibacterial agent. The compounds active in *in vitro* evaluation against *M. tuberculosis* were further assessed for their *in vivo* activity in mice and for their capacity to inhibit the vital mycobacterial enzymes *viz*., isocitrate lyase, pantothenate synthetase and chorismate mutase. The dose of the compounds in antitubercular evaluation that proved fatal and highly toxic to mice was 5.67 mg/kg while lethal dose varied from 1.82 mg/kg to 3.23 mg/kg body weight of the mice. A dose of 1.34 mg/kg was found to be safe for each of the compounds. All compounds inhibited the mycobacterial enzymes but to a lesser extent than streptomycin sulphate used as positive control. Compound **19**, exhibiting inhibition of 67.56%, 53.45%, and 47.56% against isocitrate lyase, pantothenate synthetase and chorismate mutase, respectively is the most potent antitubercular compound among the synthesized benzimidazole derivatives. Further, compound **19** also emerged as a potent anticancer agent (IC_{50} = 0.0013 μM) than 5-flourouracil against breast cancer cell line (MCF 7).

1. Introduction

Chemotherapy has revolutionized the treatment of infectious diseases since the discovery of antibacterial dyes by Ehrlich earlier in the 20th century and paved the way to a great victory for human health and longevity. The emergence of resistance against currently used antimicrobial drugs led to a revitalized interest of the researchers in infectious diseases to develop new chemical entities to combat them [1–4].

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (*M. tuberculosis*), remains a pivotal cause of high mortality worldwide despite the handiness of highly potent antitubercular drugs due to the development of resistance by the mycobacterium as a result of gene mutation to first-line antitubercular drugs [5]. To combat the mycobacterial resistance, there is a need to identify novel targets unique to *M. tuberculosis* which are absent in humans whose blockage would either prove lethal to the bacterium or render it extremely susceptible to the host immune response [6]. Chorismate mutase (CM), isocitrate lyase (ICL), and pantothenate synthetase (PS) are few such unique targets for *M. tuberculosis* [7]. Chorismate is a precursor of important molecules such folic acid, menaquinones, mycobactins and aromatic amino acids. The shikimate pathway utilizes CM as one of the key enzymes for catalyzing the isomerization of chorismate to prephenate for biosynthesis of L-phenylalanine and L-tyrosine in the mycobacteria [8,9]. The glyoxylate metabolism shunt employs ICL as an important enzyme in the main metabolic route for the biosynthesis of cellular material i.e., fatty acids, which might be the major source of carbon for *M. tuberculosis* during growth on C2 substances [10]. PS catalyzes the condensation of pantothenate from D-pantoate and β-alanine for the biosynthesis of coenzyme A and acyl carrier protein in mycobacterium [11].

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Breast cancer is by far the most common cancer of women, comprising 23% of all female cancers, and there were an estimated 1.15 million new cases in 2002. It ranks second overall when both sexes are considered. More than half of all cases occur in industrialized countries — about 361,000 in Europe (27.3% of cancers in women) and 230,000 in North America (31.3%). Incidence rates are high in most of the developed areas of the world (except for Japan, where breast cancer is third after colorectal cancer and stomach cancer), with the highest age-standardized incidence in North America (99.4 per 100,000). The incidence is more modest in eastern Europe, South America, southern Africa, and western Asia, but breast cancer is still the most common cancer of women in these regions [12]. To curb the menace of increasing resistance and unaffordable treatment by breast cancer survivals, there is urgency for new and cost effective chemotherapeutic agents.

Benzimidazole, also known as benzoglyoxaline, is a heterocyclic moiety of choice for the researchers in modern times [13]. The presence of imidazole (a biologically active pharmacophore) makes it a versatile heterocycle with an extensive range of biological activities such as antihistaminic [14], antiulcer, antitubercular [15], antioxidant [16], anti-HIV [17], anti-inflammatory [18], analgesic [19], antimicrobial [20], antiprotozoal, antitrichinellosis [21], antihypertensive [22], anticancer [23] DNA binding [24] and antimicrobial activities [25].

Prompted by the above findings and in continuation of our work on benzimidazole derivatives [26], we herein report the synthesis, antimicrobial, antitubercular and anticancer evaluation of a novel series of benzimidazole derivatives.

2. Experimental

2.1. Materials and method

The chemicals of analytical grade were procured from commercial sources and used as such without further purification. Media for antimicrobial activity was obtained from Hi-media Laboratories. Microbial type cell cultures (MTCC) for antimicrobial activity were purchased from IMTECH, Chandigarh. Infrared (IR) spectra were recorded on Bruker 12060280, Software: OPUS 7.2.139.1294 spectrophotometer using KBr pellet method and expressed in cm^{-1}. The proton nuclear magnetic resonance (^{1}HNMR) and ^{13}CNMR spectra were recorded on Bruker Avance III 600 spectrometer (at 600 and 150 MHz respectively) in deuterated DMSO downfield to tetramethylsilane standard and chemical shifts were recorded as δ (parts per million). Melting points were determined by open glass capillary method and are uncorrected. The progress of reaction was confirmed by TLC performed on silica gel-G plates and the spots were visualized in iodine chamber. The LCMS data were recorded on Waters Q-TOF micromass (ESI-MS), at Panjab University, India. Elemental analysis for synthesized derivatives was performed on CHNN/CHNS/O analyzer (Flash EA1112N series, Thermo finnigan, Italy).

2.2. Synthesis

2.2.1. General procedure for synthesis of ethyl-2-(1H-benzo[d] imidazol-2-ylthio)acetate

A mixture containing 2-mercaptobenzimidazole (0.03 mol), potassium hydroxide (0.03 mol) and 60 ml ethanol was stirred and heated at 78–90 °C for 10–15 min. Ethyl chloroacetate (0.03 mol) was then added in one portion that led to arise in temperature of 30–40 °C due to exothermic reaction. After stirring for 24 h at room temperature, reaction mixture was added to ice (100 gm) and stirred further for half an hour, maintaining the temperature at 0–10 °C. The shiny white precipitate so obtained was filtered using suction and rendered free from chloride by repeated washing with water. The dried product was finally recrystallized with ethyl alcohol.

2.2.2. General procedure for synthesis of ethyl-2-(1H-benzo[d] imidazol-2-ylthio)acetohydrazide

Ethyl-2-(1*H*-benzo[*d*]imidazol-2-ylthio)acetohydrazide was obtained by gently refluxing a solution of ethyl-2-(1*H*-benzo[*d*] imidazol-2-ylthio)acetate (0.01 mol) and hydrazine hydrate (0.06 mol) in rectified spirit on a water bath for 3–4 h. The progress of reaction was confirmed by TLC. The solution was concentrated and kept in refrigerator overnight. The creamish white precipitate formed was filtered, dried and recrystallized from water.

2.2.3. General procedure for synthesis of benzimidazole derivatives ***(1–20)***

A solution of ethyl-2-(1*H*-benzo[*d*]imidazol-2-ylthio)acetohydrazide (0.01 mol) was poured into a solution of appropriate aromatic aldehyde (0.01 mol) in boiling ethanol and refluxed for an appropriate time using 1–2 drops of glacial acetic acid as catalyst. The progress of reaction was monitored by thin layer chromatography. Excess solvent was removed by distillation after completion of reaction and the concentrate was kept aside for precipitation. The obtained product was filtered followed by washing with dilute ethanol and recrystallization with ethanol.

2.2.4. Spectral data of benzimidazole derivatives ***(1–20)***

2.2.4.1. 2-(1H-Benzo[d]imidazol-2-ylthio)-N′-(2-methoxybenzylidene) acetohydrazide (1). Peach coloured crystalline powder; mp 145–147 °C; yield 94.08%; R_f 0.54 (Benzene: Chloroform 6:4); IR (cm^{-1}): 3425 N—H str. for 2° amide, 2838 N—H str. for imidazole, 1669 C=O str for 2° amide, 745 C—S str of thiol, 656 OCN deformation of amides; ^{1}HNMR (DMSO, δ): 3.85 (s, 3H of methoxy), 4.98 (s, NH of benzimidazole), 7.12–7.77 (m, 8H, aromatic), 8.13 (s, NH of 2° amide); ^{13}C NMR (DMSO, δ): 39.90 CH2 aliphatic, 55.54 CH3 aliphatic, (112.59, 119.90, 121.37, 130.18, 132.24, 158.71) C of benzene, (120.54, 123.01, 139.29, 149.83) C of benzimidazole, 142.52 CH aliphatic, 168.97 C of amide; EIMS *m*/*z* 341 $[M+1]^+$; Anal. Calcd. for $C_{17}H_{16}N_4O_2S$: C, 59.98; H, 4.74; N, 16.46; S, 9.42. Found: C, 59.97; H, 4.72; N, 16.47; S, 9.40.

2.2.4.2. 2-(1H-Benzo[d]imidazol-2-ylthio)-N′-(3-methoxybenzylidene) acetohydrazide (2). Yellow crystalline powder; mp 158–160 °C; yield 94.08%; R_f 0.41(Benzene: Chloroform 6:4); IR (cm^{-1}): 3431 N—H str. for 2° amide, 3047 N—H str for imidazole, 1668 C=O str for 2° amide, 1052 C—O—C str of arylalkyl ether, 750 C—S str of thiol; ^{1}HNMR (DMSO, δ): 3.79 (s, 3H of methoxy), 4.59 (s, NH of benzimidazole), 7.12–8.00 (m, 8H of benzimidazole), 8.18 (s, NH of 2° amide); ^{13}C NMR (DMSO, δ): 40.03 CH_2 aliphatic, 55.18 CH_3 aliphatic, (116.26, 119.97, 121.30, 129.97, 135.15, 159.48) C of benzene, (115.73, 122.25, 140.74, 149.81) C of benzimidazole, 143.38 CH aliphatic, 169.84 C of amide; EIMS *m*/*z* 341 $[M+1]^+$; Anal. Calcd. for $C_{17}H_{16}N_4O_2S$: C, 59.98; H, 4.74; N, 16.46; S, 9.42. Found: C, 59.96; H, 4.73; N, 16.45; S, 9.41.

2.2.4.3. 2-(1H-Benzo[d]imidazol-2-ylthio)-N′-(4-methoxybenzylidene) acetohydrazide (3). Brown powder; mp 208–212 °C; yield 94.08%; R_f 0.36 (Benzene: Chloroform 6:4); IR (cm^{-1}): 3395 N—H str. for 2° amide, 3065 N—H str. for imidazole, 1651 C=O str for 2° amide, 1104 C—O—C str of arylalkyl ether, 728 C—S str of thiol; ^{1}HNMR (DMSO, δ): 3.81 (s, 3H of methoxy), 6.92–7.75 (m, 8H aromatic), 7.98 (s, NH of 2° amide); ^{13}C NMR (DMSO, δ): 39.90 CH_2 aliphatic, 55.18 CH_3 aliphatic, (114.05, 127.76, 127.87, 153.58, 159.90) C aromatic, 140.59 CH aliphatic, 170.21 C of amide; EIMS *m*/*z* 341 $[M+1]^+$; Anal. Calcd. for $C_{17}H_{16}N_4O_2S$: C, 59.98; H, 4.74; N, 16.46; S, 9.42. Found: C, 59.97; H, 4.71; N, 16.45; S, 9.39.

2.2.4.4. 2-(1H-Benzo[d]imidazol-2-ylthio)-N′-(2,4-dimethoxybenzylidene) acetohydrazide (4). Peach coloured powder; mp 206–208 °C; yield 65.76%; R_f 0.51 (Benzene); IR (cm^{-1}): 3439 N—H str. for 2° amide, 3019 N—H str. for imidazole, 1653 C=O str for 2° amide, 1273 C—O—C str of aralkyl ether, 888 C—H out of plane bending, 743 C—S str of thiol; ^{1}HNMR (DMSO, δ): 6.64–8.03 (m, 7H aromatic), 8.28 (s, NH of 2° amide); ^{13}C NMR (DMSO, δ): 39.90 CH_2 aliphatic, (55.34, 55.64) CH_3 aliphatic, (98.02, 106.20, 126.48, 158.18, 161.49) C of benzene, (115.87, 126.48, 136.24) C of benzimidazole, 153.60 CH aliphatic, 170.15 C of amide; EIMS *m/z* 371 $[M + 1]^+$; Anal. Calcd. for $C_{18}H_{18}N_4O_3S$: C, 58.36; H, 4.90; N, 15.12; S, 8.66. Found: C, 58.34; H, 4.92; N, 15.09; S, 8.62.

2.2.4.5. 2-(1H-Benzo[d]imidazol-2-ylthio)-N′-(3,4,5-trimethoxybenzylidene) acetohydrazide (5). Peach coloured crystals; mp 152–155 °C; yield 43.27%; R_f 0.24 (Benzene); IR (cm^{-1}): 3460 N—H str for 2° amide 3057 N—H str for imidazole, 1577 C=O str for 2° amide, 1125 C—O—C str for asymm. ether, 761 C—S str of thiol; ^{1}HNMR (DMSO, δ): 3.82 (s, 2H of methylene), 4.61 (s, NH of benzimidazole), 6.96–7.96 (m, 6H aromatic), 8.13 (s, NH of 2° amide); ^{13}C NMR (DMSO, δ): 34.22 CH_2 aliphatic, (39.79, 39.92, 40.04) CH_3 aliphatic, (108.42, 128.16, 139.61, 139.61, 149.63) C of benzene, (112.35, 122.24, 136.57, 149.45) C of benzimidazole, 142.89 CH aliphatic, 169.23 C of amide; EIMS *m/z* 401 $[M + 1]^+$; Anal. Calcd. for $C_{19}H_{20}N_4O_4S$: C, 56.99; H, 5.03; N, 13.99; S, 8.01. Found: C, 56.98; H, 5.01; N, 13.96; S, 8.00.

2.2.4.6. 2-(1H-Benzo[d]imidazol-2-ylthio)-N′-(4-hydroxybenzylidene) acetohydrazide (6). Mustard yellow powder; mp 104–106 °C; yield 92.28%; R_f 0.19 (Benzene: Chloroform 6:4); IR (cm^{-1}): 3621 O—H str for phenol, 3368 N—H str for 2° amide, 2884 N—H str. for imidazole, 1593 C=O str for 2° amide, 832 C—H out of plane bending, 735 C—S str of thiol; ^{1}HNMR (DMSO, δ): 3.97 (s, 2H of methylene), 4.56 (s, NH of benzimidazole), 6.95–7.94 (m, 8H aromatic), 8.10 (s, NH of 2° amide); ^{13}C NMR (DMSO, δ): 39.91 CH_2 aliphatic, (115.59, 128.39, 131.77, 161.54) C of benzene, (115.44, 128.34, 131.79, 153.46) C of benzimidazole, 139.55 CH aliphatic, 163.17 C of amide; EIMS *m/z* 342 $[M + 1]^+$; Anal. Calcd. for $C_{17}H_{17}N_4O_2S$: C, 58.88; H, 4.32; N, 17.17; S, 9.82. Found: C, 58.86; H, 4.29; N, 17.16; S, 9.83.

2.2.4.7. 2-(1H-Benzo[d]imidazol-2-ylthio)-N′-(2-chlorobenzylidene) acetohydrazide (7). Yellow crystalline powder; mp 113–116 °C; yield 58.89%; R_f 0.26 (Benzene); IR (cm^{-1}): 3426 N—H str for 2° amide, 3025 N—H str. for imidazole, 1609 C=O str for 2° amide, 742 C—Cl str of monochlorinated aromatic, 634 C—S str of thiol; ^{1}HNMR (DMSO, δ): 3.73 (s, 2H of methylene), 6.98–7.97 (m, 8H aromatic), 8.16 (s, NH of 2° amide); ^{13}C NMR (DMSO, δ): 40.04 CH_2 aliphatic, (104.26, 105.63, 129.22, 153.14, 153.16) C aromatic, 140.25 CH aliphatic, 161.11 C of amide; EIMS *m/z* 346 $[M + 1]^+$; Anal. Calcd. for $C_{16}H_{13}ClN_4OS$: C, 55.73; H, 3.80; N, 16.25; S, 9.30. Found: C, 55.70; H, 10.27; N, 16.24; S, 9.28.

2.2.4.8. 2-(1H-Benzo[d]imidazol-2-ylthio)-N′-(4-chlorobenzylidene) acetohydrazide (8). Dull yellow powder; mp 210–213 °C; yield 71.63%; R_f 0.26 (Benzene: Chloroform 6:4); IR (cm^{-1}): 3466 N—H str. for 2° amide, 3074 N—H str. for imidazole, 1622 C=O str for 2° amide, 822 C—H out of plane bending, 733 C—Cl str of monochlorinated aromatic, 669 C—S str of thiol; ^{1}HNMR (DMSO, δ): 6.95–7.85 (m, 8H aromatic), 8.01 (s, NH of 2° amide); ^{13}C NMR (DMSO, δ): 40.05 CH_2 aliphatic, (109.42, 129.01, 129.96, 132.23) C of benzene, (119.95, 122.24, 134.18, 153.45) C of benzimidazole, 139.40 CH aliphatic, 168.14 C of amide; EIMS *m/z* 346 $[M + 1]^+$; Anal. Calcd. for $C_{16}H_{13}ClN_4OS$: C, 55.73; H, 3.80; N, 16.25; S, 9.30. Found: C, 55.72; H, 3.77; N, 16.23; S, 9.29.

2.2.4.9. 2-(1H-Benzo[d]imidazol-2-ylthio)-N′-(4-fluorobenzylidene) acetohydrazide (9). Light brown crystals; mp 174–176 °C; yield 72.39%; R_f 0.23 (Benzene: Chloroform 6:4); IR (cm^{-1}): 3542 N—H str. for 2° amide, 3258 N—H str. for imidazole, 3056 C—H aromatic str, 1638 C=O str for 2° amide, 1010 C—F str of monochlorinated compound, 799 C—S str of thiol; ^{1}HNMR (DMSO, δ): 4.14 (s, 2H of methylene), 6.91–7.78 (m, 8H aromatic), 7.96 (s, NH of 2° amide); ^{13}C NMR (DMSO, δ): 40.06 CH_2 aliphatic, (109.41, 110.59, 131.55, 153.24) C of benzene, (111.97, 122.25, 132.21, 150.22) C of benzimidazole, 143.73 CH aliphatic, 168.13 C of amide; EIMS *m/z* 329 $[M + 1]^+$; Anal. Calcd. for $C_{16}H_{13}FN_4OS$: C, 58.52; H, 3.99; N, 17.06; S, 9.77. Found: C, 58.50; H, 3.97; N, 17.03; S, 9.76.

2.2.4.10. 2-(1H-Benzo[d]imidazol-2-ylthio)-N′-(4-bromobenzylidene) acetohydrazide (10). Lemon yellow powder; mp 182–185 °C; yield 70.54%; R_f 0.43 (Benzene: Chloroform 6:4); IR (cm^{-1}): 3452 N—H str. for 2° amide, 3157 N—H str. for imidazole, 1650 C=O str for 2° amide, 817 C—H out of plane bending, 738 C—S str of thiol, 604 C—Br str aromatic; ^{1}HNMR (DMSO, δ): 4.17 (s, 2H of methylene), 6.99–7.99 (m, 8H aromatic), 8.19 (s, NH of 2° amide); ^{13}C NMR (DMSO, δ): 40.04 CH_2 aliphatic, (122.25, 131.73, 131.97, 133.26) C of benzene, (120.04, 123.08, 139.61, 149.51) C of benzimidazole, 142.37 CH aliphatic, 169.13 C of amide; EIMS *m/z* 390 $[M + 1]^+$; EIMS *m/z* 390 $[M + 1]^+$; Anal. Calcd. for $C_{16}H_{13}BrN_4OS$: C, 49.37; H, 3.37; N, 14.39; S, 8.24. Found: C, 49.36; H, 3.35; N, 14.38; S, 8.23.

2.2.4.11. 2-(1H-Benzo[d]imidazol-2-ylthio)-N′-(4-nitrobenzylidene) acetohydrazide (11). Brick red crystalline powder; mp 208–211 °C; yield 83.57%; R_f 0.39 (Benzene: Chloroform 6:4); IR (cm^{-1}): 3338 N—H str. for 2° amide, 2946 N—H str. for imidazole, 1641 C=O str for 2° amide, 1564 NO_2 str (asym) of aromatic nitro group, 1452 C—C str of phenyl nucleus, 1330 NO_2 str (sym) of aromatic nitro group, 737 C—S str of thiol, 577 CNO bending of nitro compound; ^{1}HNMR (DMSO, δ): 4.18 (s, 2H of methylene), 7.01–8.05 (m, 8H aromatic), 8.16 (s, NH of 2° amide); ^{13}C NMR (DMSO, δ): 40.03 CH_2 aliphatic, (123.96, 132.20, 140.25, 149.58) C of benzene, (122.25, 123.83, 138.67, 147.67) C of benzimidazole, 142.05 CH aliphatic, 169.48 C of amide; EIMS *m/z* 356 $[M + 1]^+$; Anal. Calcd. for $C_{16}H_{13}N_5O_3S$: C, 54.08; H, 3.69; N, 19.71; S, 9.02. Found: C, 54.06; H, 3.67; N, 19.70; S, 9.01.

2.2.4.12. 2-(1H-Benzo[d]imidazol-2-ylthio)-N′-(4-hydroxy-3-methoxybenzylidene) acetohydrazide (12). Light orange crystalline powder; mp 102–104 °C; yield 55.36%; R_f 0.40 (Benzene: Chloroform 6:4); IR (cm^{-1}): 3663 O—H str of phenol, 3374 N—H str. for 2° amide, 2880 N—H str. for imidazole, 1652 C=O str for 2° amide, 868 C—H plane bending of 1,3,5- trisubstituted benzene ring, 612 C—S str of thiol; ^{1}HNMR (DMSO, δ): 3.80 (s, 2H of methylene), 6.95–7.93 (m, 7H aromatic), 8.09 (s, NH of 2° amide); ^{13}C NMR (DMSO, δ): 39.96 CH_2 aliphatic, 56.04 C of OCH_3 aliphatic, (115.38, 119.86, 122.27, 126.52, 148.13, 149.98) C of benzene, (115.55, 123.48, 141.60, 147.60) C of benzimidazole, 144.01 CH aliphatic, 170.42 C of amide; EIMS *m/z* 357 $[M + 1]^+$; Anal. Calcd. for $C_{17}H_{16}N_4O_3S$: C, 57.29; H, 4.52; N, 15.72; S, 9.00. Found: C, 57.27; H, 4.51; N, 15.71; S, 9.01.

2.2.4.13. 2-(1H-Benzo[d]imidazol-2-ylthio)-N′-(3-ethoxy-4-hydroxybenzylidene)aceto hydrazide (13). Dark brown crystals; mp 176–178 °C; yield 54.08%; R_f 0.43 (Benzene); IR (cm^{-1}): 3306 N—H str. for 2° amide, 2942 N—H str. for imidazole, 2830 C—H str aralkyl ether, 1669 C=O str for 2° amide, 1277 C—O—C str aralkyl asymm, 864 C—H bending of 1,3,5- trisubstituted benzene ring, 747 C—S str of thiol; ^{1}HNMR (DMSO, δ): 3.98 (s, 2H of methylene), 4.56 (s, NH of benzimidazole), 7.07–7.93 (m, 7H of benzimidazole), 8.08 (s, NH

of 2° amide); ^{13}C NMR (DMSO, δ): 14.74 C of $OCH_2\underline{C}H_3$, 39.88 CH_2 aliphatic, 63.80 C of $O\underline{C}H_2CH3$ aliphatic, (111.41, 115.55, 123.25, 125.48, 150.05) C aromatic, 147.06 CH aliphatic, 160.55 C of amide; EIMS *m/z* 371 $[M + 1]^+$; Anal. Calcd. for $C_{18}H_{18}N_4O_3S$: C, 58.36; H, 4.90; N, 15.12; S, 8.66. Found: C, 58.35; H, 4.91; N, 15.10; S, 8.64.

2.2.4.14. 2-(1H-Benzo[d]imidazol-2-ylthio)-N′-(4-formylbenzylidene) acetohydrazide (14). Turmeric yellow crystalline powder; mp > 300 °C; yield 69.64%; R_f 0.37 (Benzene); IR (cm^{-1}): 3271 N—H str. for 2° amide, 2948 N—H str. for imidazole, 1619 C=O str for 2° amide, 1345 C—CHO skeletal aldehydes group, 936 C—H in plane bending of aldehyde group, 748 C—S str of thiol; ^{1}HNMR (DMSO, δ): 3.99 (s, 2H of methylene), 6.98–7.85 (m, 8H aromatic), 8.05 (s, NH of 2° amide); ^{13}C NMR (DMSO, δ): 39.91 CH_2 aliphatic, (109.42, 121.37, 122.26, 126.58, 126.68 127.08) C aromatic; EIMS *m/z* 339 $[M + 1]^+$; Anal. Calcd. for $C_{17}H_{14}N_4O_2S$: C, 60.34; H, 4.17; N, 16.56; S, 9.48. Found: C, 60.35; H, 4.15; N, 16.55; S, 9.47.

2.2.4.15. 2-(1H-Benzo[d]imidazol-2-ylthio)-N′-((E)-3-phenylallylidene) acetohydrazide (15). Lemon yellow colour; mp 202–204 °C; yield 48.91%; R_f 0.47 (Benzene: Chloroform 6:4); IR (cm^{-1}): 3415 N—H str. for 2° amide, 3032 N—H str. for imidazole, 1652 C=C str vibration of $R^1CH{=}CHR^2$ (*cis*), 736 C—S str of thiol; ^{1}HNMR (DMSO, δ): 6.95–7.89 (m, 9H aromatic), 7.91 (s, NH of 2° amide); ^{13}C NMR (DMSO, δ): 39.91 CH_2 aliphatic, (125.51, 135.32, 136.29) CH aliphatic, (126.53, 128.20, 128.81, 128.84, 143.39) C aromatic 153.09 C of amide; EIMS *m/z* 337 $[M + 1]^+$; Anal. Calcd. for $C_{18}H_{16}N_4OS$: C, 64.26; H, 4.79; N, 16.65; S, 9.53. Found: C, 64.23; H, 4.76; N, 16.64; S, 9.51.

2.2.4.16. 2-(1H-Benzo[d]imidazol-2-ylthio)-N′-(2-hydroxybenzylidene) acetohydrazide (16). Light yellow powder; mp 178–180 °C; yield 87.04%; R_f 0.35 (Benzene: Chloroform 6:4); IR (cm^{-1}): 3698 O—H str of phenol, 3373 N—H str. for 2° amide, 2861 N—H str. for imidazole, 1669 C=O str for 2° amide, 749 C—H plane bending of disubstituted benzene ring, 666 C—S str of thiol; ^{1}HNMR (DMSO, δ): 4.18 (s, 2H of methylene), 6.98–7.77 (m, 8H aromatic), 8.32 (s, NH of 2° amide); ^{13}C NMR (DMSO, δ): 39.87 CH_2 aliphatic, (116.12, 118.54, 121.36, 130.88, 132.23, 162.84) C of benzene, (115.94, 126.46, 141.15, 147.36) C of benzimidazole, 141.34 CH aliphatic, 168.71 C of amide; EIMS *m/z* 327 $[M + 1]^+$; Anal. Calcd. for $C_{16}H_{14}N_4O_2S$: C, 58.88; H, 4.32; N, 17.17; S, 9.82. Found: C, 58.85; H, 4.30; N, 17.15; S, 9.81.

2.2.4.17. 2-(1H-Benzo[d]imidazol-2-ylthio)-N′-(4-(dimethylamino) benzylidene)acetohydrazide (17). Bright yellow powder; mp 175–178 °C; yield 47.16%; R_f 0.48 (Benzene); IR (cm^{-1}): 3515 N—H str. for 2° amide, 3089 N—H str. for imidazole, 1601 C=O str for 2° amide, 1359 C—N str of aryl tertiary amine, 766 C—S str of thiol; ^{1}HNMR (DMSO, δ): 4.11 (s, 2H of methylene), 7.11–7.89 (m, 8H aromatic), 8.05 (s, NH of 2° amide); ^{13}C NMR (DMSO, δ): 40.04 CH_2 aliphatic, (39.70, 39.71) aliphatic CH_3 at N, (111.65, 119.90, 128.12, 128.42, 151.93) C of benzene, (111.72, 121.52, 129.44, 147.80) C of benzimidazole, 144.39 CH aliphatic, 168.42 C of amide; EIMS *m/z* 354 $[M + 1]^+$; Anal. Calcd. for $C_{18}H_{19}N_5OS$: C, 61.17; H, 5.42; N, 19.81; S, 9.07. Found: C, 61.17; H, 5.41; N, 19.79; S, 9.05.

2.2.4.18. 2-(1H-Benzo[d]imidazol-2-ylthio)-N′-(4-(diethylamino)benzylidene) acetohydrazide (18). Peach coloured powder; mp 220–222 °C; yield 54.46%; R_f 0.41 (Benzene: Chloroform 6:4); IR (cm^{-1}): 3308 N—H str. for 2° amide, 3142 N—H str. for imidazole, 1647 C=O str for 2° amide, 1345 C—N str of aryl tertiary amine, 729 C—S str of thiol; ^{1}HNMR (DMSO, δ): 3.51 (s, 2H of methylene), 6.94–7.88 (m, 8H aromatic), 8.02 (s, NH of 2° amide); ^{13}C NMR (DMSO, δ): 39.85 CH_2 aliphatic, 56.04 C of $\underline{C}H_2CH_3$, 18.50 C of $CH_2\text{-}\underline{C}H_3$, (115.75, 124.98, 130.05, 153.43) C of benzene, (115.48, 124.96, 141.42, 147.46) C of benzimidazole, 144.01 CH aliphatic, 172.09 C of amide; EIMS *m/z* 382 $[M + 1]^+$; Anal. Calcd. for $C_{20}H_{23}N_5OS$: C, 62.97; H, 6.08; N, 18.36; S, 8.41. Found: C, 62.95; H, 6.06; N, 18.34; S, 8.39.

2.2.4.19. 2-(1H-Benzo[d]imidazol-2-ylthio)-N′-((4-hydroxynaphthalen-1-yl)methylene) acetohydrazide (19). Mustard yellow crystalline powder; mp 188–190 °C; yield 78.07%; R_f 0.35 (Benzene); IR (cm^{-1}): 3675 O—H aromatic, 3381 N—H str. for 2° amide, 3196 N—H str. for imidazole, 1668 C=O str for 2° amide, 841 naphthacene, 704 C—S str of thiol; ^{1}HNMR (DMSO, δ): 4.25 (s, 2H of methylene), 6.98–7.93 (m, 10H aromatic), 8.03 (s, NH of 2° amide); ^{13}C NMR (DMSO, δ): 40.05 CH_2 aliphatic, (109.83, 121.05, 121.59, 127.33, 127.74, 127.94, 128.12, 128.69, 132.78, 163.61) C of naphthalene, (118.15, 123.51, 132.78, 149.39) C of benzimidazole, 143.02 CH aliphatic, 168.24 C of amide; EIMS *m/z* 377 $[M + 1]^+$; Anal. Calcd. for $C_{20}H_{16}N_4O_2S$: C, 63.81; H, 4.28; N, 14.88; S, 8.52. Found: C, 63.80; H, 4.26; N, 14.86; S, 8.50.

2.2.4.20. 2-(1H-Benzo[d]imidazol-2-ylthio)-N′-(furan-2-ylmethylene) acetohydrazide (20). Brown coloured crystals; mp 158–160 °C; yield 66.98%; R_f 0.49 (Benzene: Chloroform 6:4); IR (cm^{-1}): 3350 N—H str. for 2° amide, 3103 C—H str for furan, 2973 N—H str. for imidazole, 1595 C=O str for 2° amide, 755 C—S str of thiol; ^{1}HNMR (DMSO, δ): 6.96–7.83 (m, 7H aromatic), 7.89 (s, NH of 2° amide); ^{13}C NMR (DMSO, δ): 39.93 CH2 aliphatic, (109.42, 110.53, 127.97, 153.62) C of furan, (115.05, 122.24, 132.23, 148.02) C of benzimidazole, 141.78 CH aliphatic, 172.01 C of amide. EIMS *m/z* 301 $[M + 1]^+$; Anal. Calcd. for $C_{14}H_{12}N_4O_2S$: C, 55.99; H, 4.03; N, 18.65; S, 10.68. Found: C, 55.97; H, 4.04; N, 18.64; S, 10.65.

2.3. In vitro antimicrobial evaluation

2.3.1. Determination of MIC

The *in vitro* antimicrobial potential of the synthesized benzimidazole derivatives was assessed by tube dilution method against *Escherichia coli* MTCC 1652 (Gram-negative bacterium); *Bacillus subtilis* MTCC 2063, *Staphylococcus aureus* MTCC 2901 (Gram-positive bacteria); *Candida albicans* MTCC 227 and *Aspergillus niger* MTCC 8189 (fungal strains) [27] using Cefadroxil and fluconazole as standard antibacterial and antifungal drugs respectively. The stock solution of 100 μg/ml concentration for each test and standard drugs was prepared in dimethyl sulfoxide. These were then serially diluted in double strength nutrient broth I.P. for bacteria and Sabouraud dextrose broth I.P. for fungi [28]. The bacterial cultures were incubated for a period of 24 h at 37 ± 2 °C. The incubation time for *C. albicans* was 48 h at 37 ± 2 °C and for *A. niger* was 7 d at 25 ± 2 °C. The results of antimicrobial activity were determined in terms of minimum inhibitory concentration (MIC).

2.3.2. Determination of MBC/MFC

The minimum bactericidal concentration (MBC) and fungicidal concentration (MFC) of the synthesized benzimidazole derivatives was determined by subculturing 100 μL of culture from each tube that showed no growth in MIC determination onto sterilized petri-plates containing fresh agar medium. The petri-plates were incubated and analyzed for microbial growth visually [29].

2.4. In vitro antitubercular activity evaluation

The antimycobacterial activities of the compounds were performed in three level safety laboratories at National Centre of Fungal Taxonomy (NCFT), New Delhi in association with HIHT

University, Jolly Grant, Dehradun (U.K). Middle brook 7H10 agar (Becton Dickinson Company (DifcoTM), 7 Loveton Circle, Sparks, Maryland, USA; Lot No. 8175150) supplemented with oleic acid-albumin- catalase (OADC) (Becton Dickinson Company Lot 8136781) was used for reviving and culturing the mycobacteria for sensitivity testing. Drugs viz. Streptomycin (S) (500 mg) was obtained as gift samples from Shalina Laboratories Pvt. Ltd., Navi Mumbai, Maharashtra. Alamar blue dye (Accumed International, Westlake Ohio), microtiter plates (Falcon, 3072: Becton Dickinson, Lincoln Park NJ), sterilized glass wares, UV-cabinets with reverse pressure gas system. The preserved strains of *M. tuberculosis viz.*, mycobacterium sensitive to streptomycin (S), isoniazid (H), rifampin (R) and pyrazinamide (PZA)- H37Rv (NCFT/TB/537) was used in order to assess the antimycobacterial activity of the compounds.

2.4.1. Preparation of the drugs/compounds dilutions

Each of the synthesized compound was dissolved in DMSO to obtain a concentration of 50 μg/ml and diluted further to concentrations of 25 μg/ml and 12.5 μg/ml. Similarly, stock solution of 50 μg/ml concentration was prepared for standard antitubercular drug, streptomycin and diluted to 25 μg/ml in order to check the antitubercular activity.

2.4.2. Preparation of growth media

It was prepared by adding dehydrated medium (19 g) to purified water (900 ml) containing glycerol (15 ml). The mixture was stirred well to dissolve and autoclaved at 121 °C for 10 min. Oleic acid-albumin catalase (100 ml) was aseptically added to the medium after cooling to 45 °C. No adjustment for pH was made.

2.4.3. Preparation of inoculum for drug sensitivity testing

Preserved strains of *M. tuberculosis viz.* mycobacterium sensitive to S, H, R and PZA-H37Rv (NCFT/TB/537) were revived on Middle brook 7H10 agar, prior to antituberculosis susceptibility testing. Cells were scraped from freshly growing colonies (three weeks old) on Middle brook 7H10 plates and introduced into saline (10 ml). Bacterial suspensions of 0.5 McFarland standard turbidity equivalent to 10^8 CFU were prepared by dilution with saline. The mixture was vortexed for 30 s in a glass bottle containing glass beads and the particles were allowed to settle [30].

2.4.4. Random screening of the isolated compounds for antitubercular activity (Alamar-blue assay)

The antimycobacterial activity of compounds was assessed against mycobacterium sensitive to S, H, R and PZA-H37Rv (NCFT/TB/537) using the microplate alamar blue assay (MABA) [31]. This methodology is nontoxic, uses a thermally-stable reagent and is suitable for random screening of the antimycobacterial activity. Briefly, 200 μL of sterile deionized water was added to all outer-perimeter wells of sterile 96 well plates to minimize evaporation of the medium in the test wells during incubation. The 96 well plates received 100 μL of the Middlebrook 7H9 broth (having loopful inoculum of bacteria-10^8 CFU) and different dilutions of the respective compounds were made directly on the plate. The maximum concentration of the compounds tested was 50 μg/ml. Plates were covered and sealed with parafilm and incubated at 37 °C for five days. After this time, 25 μL of a freshly prepared 1:1 mixture of Alamar blue reagent and 10% tween 80 was added to the plate and incubated for 24 h. A blue colour in the well was interpreted as no bacterial growth (antimycobacterial activity) and a pink colour was scored as growth.

2.4.5. Bioassay protocol for susceptibility tests of the compounds by well diffusion method

The well diffusion method was used to determine susceptibility [30,32]. The agar well diffusion method was modified [33]. Middle brook 7H10 agar medium was used for bacterial cultures. The culture medium was inoculated with loopful bacteria separately suspended in Middle brook 7H10 broth. Wells of 8 mm diameter were punched into the agar and filled each well separately with 50 μg/ml of test compounds and 25 μg/ml of standard drug. The petri dishes were then left in the hood overnight to allow diffusion of the drug and then sealed with a carbon dioxide-permeable tape. These were then incubated at 37 °C in a carbon dioxide incubator for four weeks. The wells were flooded with alamar-blue dye in highly sterilized chamber and de-stained further to observe the zones of inhibition. The sensitivity of the strains to the compounds was determined by measuring the diameter of zones of inhibition surrounding the well using millimetre scale.

2.4.6. Determination of minimum inhibitory concentration (MIC) by alamar blue assay

The compounds were serially diluted to determine the minimum inhibitory concentration of the drug in Middle brook 7H9 medium using micro titre plate method [30,34,35]. The compounds which were found to be satisfactory by the above two methods at a maximum concentration of 50 μg/ml were diluted further to concentrations viz. 25, 12.5, 6.25, 3.125 and 1.56 μg/ml respectively. Similarly, streptomycin was further diluted to 25 μg/ml in order to check the antitubercular activity. The MIC of the potent compounds was performed in microtiter plates by alamar blue assay. Plates were covered and sealed with parafilm and incubated at 37 °C for five days. After this time, 25 μL of a freshly prepared 1:1 mixture of alamar blue reagent and 10% tween 80 was added to the plate and incubated for 24 h. A blue colour in the well was interpreted as no bacterial growth (antimycobacterial activity), and a pink colour was determined as growth. MIC is defined as the lowest drug concentration which prevented a colour change from blue to pink.

2.5. In vivo antitubercular activity evaluation

The LD_{50} (lethal dose) and ED_{50} (effective dose) doses were determined for the active compounds in mice models infected with *Mycobacterium* H37Rv *via* ethical permission no., NCFT/EC/16/2313 assigned to Collaborative Research Group (CRG), NCFT, New Delhi, India.

2.6. Enzyme assays for antitubercular activity

The compounds found potent in *in vivo* evaluation were assayed for inhibition of mycobacterial enzymes *viz.,* isocitrate lyase, pantothenate synthetase and chorismate mutase.

2.6.1. Mycobacterial isocitrate lyase assay

Isocitrate lyase activity was assayed according to the protocol reported by Dixon and Kornberg (glyoxylate phenyl hydrazone formation) [36] at 10 μM concentration of the compounds. Isoniazid was employed as a negative control (inhibition of 0%) and streptomycin sulphate (25 μg) served as a positive control [37].

2.6.2. Mycobacterial pantothenate synthetase assay

About 60 μL of the PS reagent, including NADH, pantoic acid, β-alanine, ATP, phosphoenolpyruvate, $MgCl_2$, myokinase, pyruvate kinase, and lactate dehydrogenase in buffer, was added to each well of a 96-well plate. The compounds were then added to plates in 1 μL volumes. 39 μL PS diluted in buffer was added to initiate the reaction. The final concentrations in the reaction contained 0.4 mM NADH, 5 mM pantoic acid, 10 mM $MgCl_2$, 5 mM β-alanine, 10 mM ATP, 1 mM potassium phosphoenolpyruvate, and 18 units/ml each of chicken muscle myokinase, rabbit muscle pyruvate kinase, and rabbit muscle lactate dehydrogenase diluted in 100 mM HEPES

buffer (pH 7.8), 1% DMSO, and 5 μg/ml PS in a final volume of 100 μL. The test plate was immediately transferred to a microplate reader and absorbance was measured at 340 nm after every 12 s for 120 s. Each plate had 16 control wells in the two outside columns, of which 12 contained the complete reaction mixture with a DMSO carrier control (full reaction) and four without PS. The following formula was used to calculate percent inhibition % inhibition = 100 × (1 − compound rate − background rate)/(full reaction rate − background rate) [38,39].

2.6.3. Mycobacterial chorismate mutase (MtCM) assay

Reaction volumes of 0.4 ml of chorismate (typically 1 mM) in 50 mM Tris HCl (pH 7.5), 0.5 mM EDTA, 0.1 mg/ml bovine serum albumin, and 10 mM β-mercaptoethanol were incubated at 37 °C for 5 min. The reaction was started with the addition of 10 μL 5 pM of MtCM (i.e., 185 ng of CM equivalent to 12.5 nM final concentration of the dimer based on the molecular mass of 36,948 Da). The reaction was allowed to proceed at 37 °C and was terminated after 1–5 min with 0.4 ml 1 M HCl. After a further incubation for 10 min at 37 °C, 0.8 ml 2.5 M NaOH was added to convert prephenate formed in the enzymatic reaction to phenyl pyruvate. The absorbance of phenylpyruvate chromophore was taken at 320 nm. A blank with no enzyme for every reaction was also set to account for the nonenzymatic conversion of chorismate to prephenate and enzyme was added after the addition of NaOH. The absorbance at 320 nm for the blank varied from 0.1 to 0.3, depending upon the concentration of chorismate and the duration of the reaction [40].

2.7. In vitro anticancer screening

The *in vitro* cytotoxicity screening of the synthesized benzimidazole derivatives was assessed on MCF7 (human breast adenocarcinoma cancer) cell line using Sulforhodamine-B (SRB) assay with minor modifications [41]. The results of anticancer activity were expressed as IC_{50} (concentration of compound required to inhibit cell viability by 50%) and compared with the standard anticancer drugs, 5-fluorouracil and carboplatin.

The cells were allowed to attach for a period of 24 h to the wells of the 96-multititre plates before treatment with the test compounds. Solution of the test and standard compounds were prepared in DMSO and made to appropriate volume with media. Monolayer cells were then incubated at 37 °C for 72 h with different concentrations (0.01, 0.1, 1, 10, 100 μg/ml) of the test compounds in an atmosphere of 5% carbon dioxide. After fixing with trichloroacetic acid for 30 min followed by washing with water, the cells were stained with 0.4% w/v solution of pink coloured aminoxanthene dye, Sulforhodamine-B, in acetic acid for 15 min. The cultures were washed with 1% acetic acid to get rid of excess stain and attached stain was recovered with Tris base solution. The colour intensity was measured using spectrophotometer. The asay was done in atleast triplicates.

3. Results and discussion

3.1. Chemistry

The benzimidazole derivatives (**1–20**) were synthesized according to Scheme 1 and characterized by physicochemical and spectral means. The spectral data of the synthesized compounds is found in agreement with the assigned molecular structures. The formation of ester from 2-mercaptobenzimidazole was confirmed by the absence of S—H stretching at 2600–2550 cm^{-1}. The appearance of C=O stretch in the range of 1680–1630 cm^{-1} and N—H stretch 3100–3070 cm^{-1} indicated the formation of secondary amide (**1–20**) formed by the reaction of ester and hydrazine hydrate. The absence N—H stretching of free primary amine at 3500 cm^{-1} in the target compounds confirmed their formation. The presence of heterocyclic furan moiety in compound **20** is demonstrated by the presence of CH stretch at 3103 cm^{-1} which is higher for furan than most aromatics. The multiplet corresponding to 6.9–7.9 δ ppm confirmed the presence of protons of benzimidazole and aryl nucleus. The compounds **1**, **2** and **3** showed singlet at δ 3.78 ppm corresponding to a proton of the OCH_3. Further confirmation was made on the basis of mass analysis and ^{13}CNMR data. The elemental (CHN) analysis results are within acceptable limits (± 0.4%). Few of the benzimidazole derivatives **3, 6, 8, 11, 15, 16, and 20** have been reported earlier [42–44] but their antitubercular/anticancer activities are not explored.

3.2. In vitro antimicrobial activity

The results of *in vitro* antimicrobial activity of the synthesized compounds are presented in Table 1. Most of the synthesized derivatives were found to be highly efficient as antimicrobial agents in comparison to the standard drug cefadroxil and fluconazole as depicted by their low MIC values compared to standard drugs. Amongst the synthesized benzimidazole derivatives, compound **10** was found to be the potent antibacterial agent against *S. aureus* (MIC = 0.032 μM).

In case of *B. subtilis*, lowest MIC values of 0.021 and 0.031 μM were observed for compounds **20** and **5**, respectively. Compound **10** (MIC = 0.0321 μM) showed highest inhibitory action against *E. coli* (a Gram negative bacterium). Compounds **5**, **10** and **18** exhibited most effective antifungal activity against *C. albicans*, having MIC value of 0.016 μM against each compound while compound **17** (respectively) possessed maximum activity against *A. niger* with MIC of 0.018 μM. Compound **10** emerged as the best antibacterial agent against tested Gram positive and Gram negative bacteria. All the synthesized compounds showed high antifungal activity than the standard drug fluconazole.

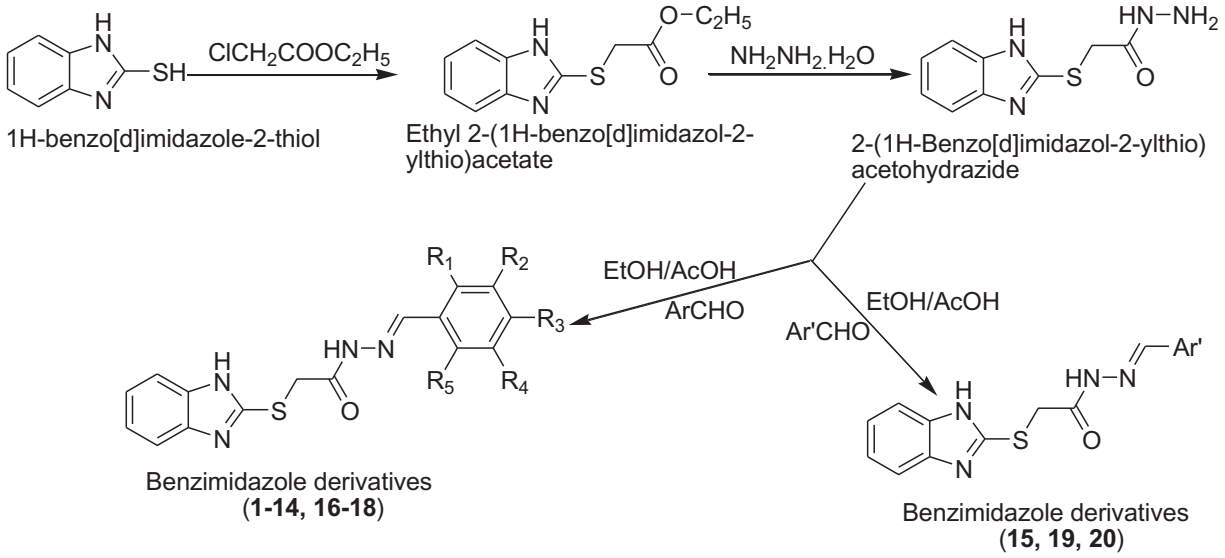
The results of minimum bactericidal concentration/minimum fungicidal concentration (Table 2) conveyed that the synthesized benzimidazole derivatives were neither bactericidal nor fungicidal except compound **3** which was fungicidal against both fungi (As a general rule, a compound is said to be bactericidal/fungicidal if its MBC/MFC is less than three times of its MIC) [45].

3.3. In vitro antitubercular activity

All the synthesized compounds were evaluated for their *in vitro* antitubercular activity against *M. tuberculosis* H37Rv (NCFT/TB/537). The zone of inhibition as well as MIC values of the test compounds was determined. The MIC and MLC (minimum lethal concentration) were determined for compounds showing zone of inhibition of >20 mm. The results of *in vitro* antitubercular activity compared with streptomycin are presented in Table 3.

3.4. In vivo antitubercular activity

The LD_{50} and ED_{50} for the active compounds were determined in mice models infected with *Mycobacterium tuberculosis H37Rv* (Table 4). It was found that the toxic dose of the compounds which proved fatal and highly toxic to mice was 5.67 mg/kg while LD_{50} varied from 1.82 mg/kg to 3.23 mg/kg body weight of the mice. LD_{50} is the dose that killed 50% of the mice population within the group. Thus, ED_{50} of 1.34 mg/kg was considered safe for each of the compounds. It was observed that this dose was effective and safe for mice in different groups before infecting the mice models with specific TB bacteria as no mortality of any single animal was recorded.



1 R_1= OCH_3, R_2=R_3=R_4=R_5 = H
2 R_2= OCH_3, R_1=R_3=R_4=R_5= H
3 R_3= OCH_3, R_1=R_2=R_4=R_5= H
4 R_1=R_3= OCH_3, R_2=R_4=R_5= H
5 R_2=R_3=R_4= OCH_3, R_2=R_5= H
6 R_3= OH, R_1=R_2=R_4=R_5= H
7 R_1= Cl, R_2=R_3=R_4=R_5 = H
8 R_3= Cl, R_1=R_2=R_4=R_5 = H
9 R_3= F, R_1=R_2=R_4=R_5= H
10 R_3= Br, R_1=R_2=R =R_5= H
11 R_3= NO_2, R_1=R_2=R_4=R_5= H
12 R_2= OCH_3, R_3= OH, R_1=R_4 =R_5 = H
13 R_2= OCH_2CH_3, R_3= OH, R_1=R_4=R_5= H
14 R_3= CHO, R_1=R_2=R_4=R_5 = H
16 R_1= OH, R_2=R_3=R4=R_5 = H
17 R_3= $N(CH_3)_2$, R_1=R_2=R_4=R_5 = H
18 R_3= $N(CH_2CH_3)_2$, R_1=R_2=R_4=R_5= H

15 Ar'=

19 Ar'=

20 Ar'=

Scheme 1. Scheme for synthesis of benzimidazole derivatives (**1**–**20**).

Table 1
MIC of synthesized benzimidazole derivatives.

Compound No.	MIC in μM				
	S. aureus	*B. subtilis*	*E. coli*	*C. albicans*	*A. niger*
1	0.073	0.073	0.073	0.037	0.073
2	0.073	0.073	0.037	0.037	0.073
3	0.037	0.037	0.037	0.018	0.037
4	0.067	0.067	0.067	0.034	0.034
5	0.062	0.031	0.062	0.016	0.062
6	0.073	0.073	0.037	0.018	0.037
7	0.036	0.073	0.036	0.073	0.036
8	0.036	0.036	0.036	0.073	0.036
9	0.038	0.038	0.038	0.019	0.038
10	0.032	0.032	0.032	0.016	0.032
11	0.035	0.035	0.035	0.018	0.035
12	0.035	0.070	0.035	0.018	0.035
13	0.034	0.034	0.067	0.017	0.034
14	0.037	0.037	0.037	0.018	0.037
15	0.037	0.037	0.037	0.019	0.037
16	0.038	0.038	0.038	0.019	0.038
17	0.035	0.035	0.035	>0.141	0.018
18	0.033	0.033	0.033	0.016	0.033
19	0.033	0.033	0.033	0.033	0.033
20	0.042	0.021	0.042	0.042	0.042
Cefadroxil	0.345	0.345	0.345	–	–
Fluconazole	–	–	–	0.40	0.82

Table 2
MBC/MFC (μg/ml) of synthesized benzimidazole derivatives.

Compound No.	*S. aureus*	*B. subtilis*	*E. coli*	*C. albicans*	*A. niger*
1	>50	>50	>50	50	>50
2	>50	>50	50	50	>50
3	>50	>50	>50	12.5	25
4	>50	>50	>50	50	50
5	>50	>50	>50	25	>50
6	>50	>50	>50	25	50
7	>50	>50	50	>50	25
8	>50	>50	>50	>50	25
9	>50	>50	>50	25	50
10	>50	>50	>50	50	50
11	>50	>50	>50	25	50
12	>50	>50	>50	25	50
13	>50	>50	>50	25	25
14	>50	>50	>50	50	50
15	>50	>50	>50	25	50
16	>50	>50	>50	25	50
17	>50	>50	>50	>50	25
18	>50	>50	>50	25	50
19	>50	>50	>50	50	50
20	>50	>50	>50	50	50

Table 3
Antimycobacterial activity, MIC and MLC of synthesized compounds against *M. tuberculosis* H37Rv.

Compound No.	Diameter of zone of inhibition (mm)	MIC in μg/ml	MLC in μg/ml
1	NA	NA	NA
2	>20	12.5	25
3	>20	12.5	25
4	>20	12.5	25
5	>20	12.5	25
6	NA	NA	NA
7	NA	NA	NA
8	NA	NA	NA
9	10	15	28
10	NA	NA	NA
11	NA	NA	NA
12	>20	12.5	25
13	>20	12.5	25
14	10	15	28
15	NA	NA	NA
16	NA	NA	NA
17	>20	12.5	25
18	>20	12.5	25
19	>20	12.5	25
20	NA	NA	NA
Streptomycin	>20	12.5	25

NA – Not active.

Table 4
LD_{50} (mg/kg) of potent compounds.

Potent compound (s) groups	LD_{50} dose (mg/kg)
2	1.82
3	1.86
4	1.89
5	1.78
12	2.89
13	2.78
17	2.56
18	3.18
19	3.23

3.5. Mycobacterial enzyme assays

The results of mycobacterial enzyme assays were expressed in terms of percent inhibition of mycobacterial enzymes *i.e.*, isocitrate lyase, chorismate mutase and pantothenate synthetase, by the

Table 5
In vitro percent inhibition of enzymes in *Mycobacterium* tuberculosis H37Rv by potent compounds.

Potent compounds/ Positive control	Percent inhibition		
	M. ICL activity (IU/L)	*M.* PS activity (IU/L)	*M.* CM activity (IU/L)
2	52.14	50.13	48.19
3	47.23	38.26	47.45
4	58.34	47.78	40.32
5	52.23	41.56	43.56
12	46.56	48.13	38.45
13	56.15	43.45	37.56
17	45.67	38.32	28.45
18	57.78	45.90	32.56
19	67.56	53.45	47.56
Negative control	No reduction	No reduction	No reduction
Streptomycin sulphate	75.12	77.06	79.56

Table 6
IC_{50} (μM) values of synthesized benzimidazole derivatives.

Compound No.	IC_{50} (μM)
1	0.0705
2	0.0764
3	0.1058
4	0.2700
5	0.2497
6	0.0410
7	0.0580
8	0.0131
9	0.0365
10	0.0193
11	0.1238
12	0.0898
13	0.2295
14	0.0975
15	0.0022
16	0.0061
17	0.1301
18	0.2621
19	0.0013
20	0.0063
5-FU	0.0461
Carboplatin	0.2694

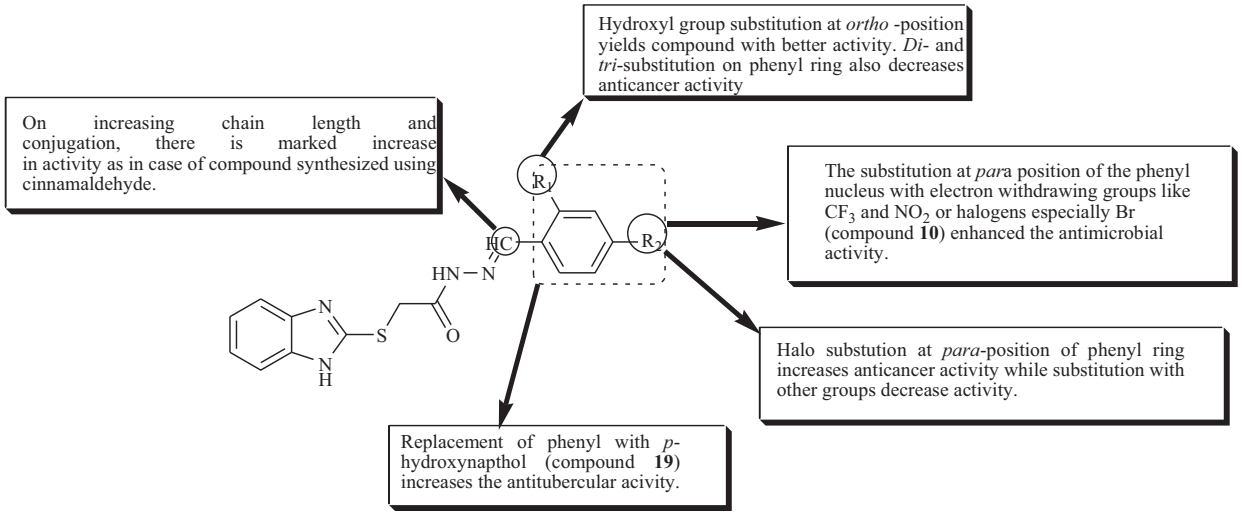


Fig. 1. Structure activity relationship of benzimidazole derivatives.

mycobacterium. The inhibition of the enzyme activity by the tested compounds was less than that of streptomycin sulphate used as positive control (Table 5). Compound **19** emerged as the best compound that inhibited the mycobacterial isocitrate lyase, pantothenate synthetase and chorismate mutase to 67.56%, 53.45% and 47.56% respectively which was comparable to inhibition of 75.12%, 77.06%, and 79.56%, respectively by streptomycin sulphate.

3.6. In vitro anticancer activity

Most of the synthesized compounds possessed more cytotoxicity as compared to 5-fluorouracil (Table 6). Compound **19** (IC_{50} = 0.0013 μM) showed extremely potent cytotoxicity against MCF7 cell line as compared to 5-fluorouracil (IC_{50} = 0.0461 μM). Majority of the compounds were more active than standard drug carboplatin while compounds **4**, **5**, and **18** were as active as carboplatin (IC_{50} = 0.2694 μM).

3.7. Structure activity relationship (SAR)

From the comparison of antimicrobial, antitubercular and anticancer activities of synthesized benzimidazole derivatives, the following SAR may be deduced.

1. The good antimicrobial activity (minimum MIC values) of the synthesized benzimidazole derivatives compared to the standard drugs cefadroxil and fluconazole may draw an attention that the synthesized benzimidazole derivatives have a very good interaction with target sites and there is a need for further *in vivo* studies to confirm the antimicrobial activity by taking the most active benzimidazole derivative (compound **10**) as a lead compound to develop novel antimicrobial agent.
2. The appreciable antitubercular activity of the synthesized benzimidazole derivatives compared to the standard drug streptomycin revealed a fact that there is a need for minor structural modifications of benzimidazole derivatives to improve the binding of molecule to tubercular target.
3. The excellent anticancer activity of the synthesized benzimidazole derivatives compared to the standard drug 5-flourouracil and carboplatin indicated a fact that there is a need for further *in vivo* studies to confirm the anticancer activity and for developing novel anticancer agent based on synthesized benzimidazole derivatives.
4. The above results also indicated a fact that different structural requirements are necessary for a compound to show different activities.

The other SAR findings are summarized in Fig.1.

4. Conclusion

A series of benzimidazole derivatives was synthesized and assessed for its *in vitro* antimicrobial and anticancer activities. The compounds were also assessed for their *in vitro* and *in vivo* antitubercular activity against *M. tuberculosis* H37Rv. The compounds found to be active in *in vivo* evaluation in mice models infected with *M. tuberculosis* were further assessed for their capacity to inhibit the vital mycobacterial enzymes *viz*., isocitrate lyase, pantothenate synthetase and chorismate mutase. All compounds inhibited these enzymes but to a lesser extent than streptomycin sulphate taken as positive control. Compound **19**, the most potent one among the synthesized benzimidazole derivatives exhibited inhibition of 67.56%, 53.45%, and 47.56% against isocitrate lyase, pantothenate synthetase and chorismate mutase, respectively which is comparable to the inhibition of these enzymes by streptomycin sulphate. Most of the synthesized derivatives emerged out as excellent antimicrobial agents as compared to standard antibacterial (cefadroxil) and antifungal (fluconazole) drugs. Compound **10** was found to be the most active antibacterial agent against Gram positive as well as Gram negative bacteria. The results of anticancer activity displayed that majority of the derivatives inhibited the viability of MCF7 cell line, especially; compound **19** was highly potent one among the series (IC_{50} = 0.0013 μM).

Conflict of interest

There is no conflict of interest among the authors.

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