[Egyptian Journal of Basic and Applied Sciences 5 (2018) 100–109](https://doi.org/10.1016/j.ejbas.2017.11.001)



Contents lists available at [ScienceDirect](http://www.sciencedirect.com/science/journal/2314808X)

Egyptian Journal of Basic and Applied Sciences

journal homepage: [www.elsevier.com/locate/ejbas](http://www.elsevier.com/locate/ejbas)

Full Length Article

[](http://crossmark.crossref.org/dialog/?doi=10.1016/j.ejbas.2017.11.001&domain=pdf)Synthesis and evaluation of antimicrobial, antitubercular and anticancer activities of benzimidazole derivatives

Snehlata Yadav [a](#_bookmark0), Balasubramanian Narasimhan [a](#_bookmark0),[⇑](#_bookmark6), Siong Meng Lim [b](#_bookmark1),[c](#_bookmark2), Kalavathy Ramasamy [b](#_bookmark1),[c](#_bookmark2),

Mani Vasudevan [d](#_bookmark3), Syed Adnan Ali Shah [b](#_bookmark1),[e](#_bookmark4), Abhishek Mathur [f](#_bookmark5)

a *Faculty of Pharmaceutical Sciences, Maharshi Dayanand University, Rohtak 124001, India*

b *Faculty of Pharmacy, Universiti Teknologi MARA (UiTM), 42300 Bandar Puncak Alam, Selangor Darul Ehsan, Malaysia*

c *Collaborative Drug Discovery Research (CDDR) Group, Pharmaceutical Life Sciences Community of Research, Universiti Teknologi MARA (UiTM), 40450 Shah Alam, Selangor* *Darul Ehsan, Malaysia*

d *Department of Pharmacology and Toxicology, College of Pharmacy, Qassim University, Buraidah 51452, Saudi Arabia*

e *Atta-ur-Rahman Institute for Natural Products Discovery (AuRIns), Universiti Teknologi MARA, Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor D.E., Malaysia*

f *Collaborative Research Group, National Centre of Fungal Taxonomy (in Association with Environmental Biotech & Engineering Co.), New Delhi 110012, India*

a r t i c l e i n f o

*Article history:*

Received 8 July 2017

Received in revised form 17 October 2017 Accepted 5 November 2017

Available online 14 November 2017

*Keywords:*

Microbial resistance MCF7 cell line Isocitrate lyase

Pantothenate synthetase Chorismate mutase

a b s t r a c t

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 strep- tomycin 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 (IC50 = 0.0013 mM) than 5-flourouracil against breast cancer cell line (MCF 7).

© 2017 Mansoura University. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

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 cur- rently used antimicrobial drugs led to a revitalized interest of the researchers in infectious diseases to develop new chemical entities to combat them [[1–4]](#_bookmark15).

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]](#_bookmark15). To combat the mycobacterial resistance, there is a need to identify novel tar-

\* Corresponding author.

*E-mail address:* [naru2000us@yahoo.com](mailto:naru2000us@yahoo.com) (B. Narasimhan).

gets 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]](#_bookmark15). Chorismate mutase (CM), isocitrate lyase (ICL), and pantothenate synthetase (PS) are few such unique targets for *M. tuberculosis* [[7]](#_bookmark15). 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 cat- alyzing the isomerization of chorismate to prephenate for biosyn- thesis of *L*-phenylalanine and *L*-tyrosine in the mycobacteria [[8,9]](#_bookmark15). 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]](#_bookmark16). PS catalyzes the condensation of pantothenate from D-pantoate and b-alanine for the biosynthesis of coenzyme A and acyl carrier pro- tein in mycobacterium [[11]](#_bookmark17).

<https://doi.org/10.1016/j.ejbas.2017.11.001>

2314-808X/© 2017 Mansoura University. Production and hosting by Elsevier B.V.

This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

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 industri- alized 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]](#_bookmark18). 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]](#_bookmark21). The presence of imidazole (a biologically active pharmacophore) makes it a versatile heterocycle with an extensive range of biological activities such as antihistaminic [[14]](#_bookmark22), antiulcer, antitubercular [[15]](#_bookmark25), antioxidant [[16]](#_bookmark27), anti-HIV [[17]](#_bookmark28), anti-inflammatory [[18]](#_bookmark30), anal-

gesic [[19]](#_bookmark32), antimicrobial [[20]](#_bookmark34), antiprotozoal, antitrichinellosis [[21]](#_bookmark35),

antihypertensive [[22]](#_bookmark37), anticancer [[23]](#_bookmark19) DNA binding [[24]](#_bookmark19) and

antimicrobial activities [[25]](#_bookmark19).

Prompted by the above findings and in continuation of our work on benzimidazole derivatives [[26]](#_bookmark19), we herein report the synthesis, antimicrobial, antitubercular and anticancer evaluation of a novel series of benzimidazole derivatives.

1. Experimental
   1. *Materials and method*

The chemicals of analytical grade were procured from commer- cial sources and used as such without further purification. Media for antimicrobial activity was obtained from Hi-media Laborato- ries. 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 (1HNMR) and 13CNMR spectra were recorded on Bruker Avance III 600 spectrom- eter (at 600 and 150 MHz respectively) in deuterated DMSO down- field to tetramethylsilane standard and chemical shifts were recorded as d (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).

* 1. *Synthesis*
     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 tempera- ture 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 tempera- ture at 0–10 °C. The shiny white precipitate so obtained was fil-

tered using suction and rendered free from chloride by repeated washing with water. The dried product was finally recrystallized with ethyl alcohol.

* + 1. *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.

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

A solution of ethyl-2-(1*H*-benzo[*d*]imidazol-2-ylthio)acetohy drazide (0.01 mol) was poured into a solution of appropriate aro- matic 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 chromatogra- phy. 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.

* + 1. *Spectral data of benzimidazole derivatives (1–20)*
       1. *2-(1H-Benzo[d]imidazol-2-ylthio)-N*'*-(2-methoxybenzylidene) acetohydrazide (1).* Peach coloured crystalline powder; mp 145– 147 °C; yield 94.08%; Rf 0.54 (Benzene: Chloroform 6:4); IR

(cm—1): 3425 NAH str. for 2° amide, 2838 NAH str. for imidazole,

1669 C@O str for 2° amide, 745 CAS str of thiol, 656 OCN deforma- tion of amides; 1HNMR (DMSO, d): 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); 13C NMR (DMSO, d): 39.90 CH2 aliphatic, 55.54 CH3 ali- phatic, (112.59, 119.90, 121.37, 130.18, 132.24, 158.71) C of ben-

zene, (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 C17H16N4O2S: 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.

* + - 1. *2-(1H-Benzo[d]imidazol-2-ylthio)-N’-(3-methoxybenzylidene) acetohydrazide (2).* Yellow crystalline powder; mp 158–160 °C; yield 94.08%; Rf 0.41(Benzene: Chloroform 6:4); IR (cm—1): 3431 NAH str. for 2° amide, 3047 NAH str for imidazole, 1668 C@O str for 2° amide, 1052 CAOAC str of arylalkyl ether, 750 CAS str of thiol; 1HNMR (DMSO, d): 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); 13C NMR (DMSO, d): 40.03 CH2 aliphatic, 55.18 CH3 aliphatic, (116.26, 119.97, 121.30, 129.97, 135.15, 159.48) C of ben-

zene, (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 C17H16N4O2S: 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.

* + - 1. *2-(1H-Benzo[d]imidazol-2-ylthio)-N*'*-(4-methoxybenzylidene)*

*acetohydrazide (3).* Brown powder; mp 208–212 °C; yield 94.08%; Rf 0.36 (Benzene: Chloroform 6:4); IR (cm—1): 3395 NAH str. for 2° amide, 3065 NAH str. for imidazole, 1651 C@O str for 2° amide, 1104 CAOAC str of arylalkyl ether, 728 CAS str of thiol; 1HNMR (DMSO, d): 3.81 (s, 3H of methoxy), 6.92–7.75 (m, 8H aromatic),

7.98 (s, NH of 2° amide); 13C NMR (DMSO, d): 39.90 CH2 aliphatic,

55.18 CH3 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 C17H16N4O2S: 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.

* + - 1. *2-(1H-Benzo[d]imidazol-2-ylthio)-N*'*-(2,4-dimethoxybenzyli- dene) acetohydrazide (4).* Peach coloured powder; mp 206–208

°C; yield 65.76%; Rf 0.51 (Benzene); IR (cm—1): 3439 NAH str. for

2° amide, 3019 NAH str. for imidazole, 1653 C@O str for 2° amide, 1273 CAOAC str of aralkyl ether, 888 CAH out of plane bending, 743 CAS str of thiol; 1HNMR (DMSO, d): 6.64–8.03 (m, 7H aro- matic), 8.28 (s, NH of 2° amide); 13C NMR (DMSO, d): 39.90 CH2 ali- phatic, (55.34, 55.64) CH3 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 C18H18N4O3S: 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.

* + - 1. *2-(1H-Benzo[d]imidazol-2-ylthio)-N’-(3,4,5-trimethoxyben-* *zylidene) acetohydrazide (5).* Peach coloured crystals; mp 152– 155 °C; yield 43.27%; Rf 0.24 (Benzene); IR (cm—1): 3460 NAH str

for 2° amide 3057 NAH str for imidazole, 1577 C@O str for 2°

amide, 1125 CAOAC str for asymm. ether, 761 CAS str of thiol; 1HNMR (DMSO, d): 3.82 (s, 2H of methylene), 4.61 (s, NH of benz- imidazole), 6.96–7.96 (m, 6H aromatic), 8.13 (s, NH of 2° amide); 13C NMR (DMSO, d): 34.22 CH2 aliphatic, (39.79, 39.92, 40.04)

CH3 aliphatic, (108.42, 128.16, 139.61, 139.61, 149.63) C of ben-

zene, (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 C19H20N4O4S: 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.

* + - 1. *2-(1H-Benzo[d]imidazol-2-ylthio)-N’-(4-hydroxybenzylidene) acetohydrazide (6).* Mustard yellow powder; mp 104–106 °C; yield 92.28%; Rf 0.19 (Benzene: Chloroform 6:4); IR (cm—1): 3621 OAH str for phenol, 3368 NAH str for 2° amide, 2884 NAH str. for imi- dazole, 1593 C@O str for 2° amide, 832 CAH out of plane bending, 735 CAS str of thiol; 1HNMR (DMSO, d): 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); 13C NMR (DMSO, d): 39.91 CH2 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 C17H17N4O2S: 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.

* + - 1. *2-(1H-Benzo[d]imidazol-2-ylthio)-N’-(2-chlorobenzylidene) acetohydrazide (7).* Yellow crystalline powder; mp 113–116 °C; yield 58.89%; Rf 0.26 (Benzene); IR (cm—1): 3426 NAH str for 2° amide, 3025 NAH str. for imidazole, 1609 C@O str for 2° amide, 742 CACl str of monochlorinated aromatic, 634 CAS str of thiol;

1HNMR (DMSO, d): 3.73 (s, 2H of methylene), 6.98–7.97 (m, 8H

aromatic), 8.16 (s, NH of 2° amide); 13C NMR (DMSO, d): 40.04 CH2 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 C16H13ClN4OS: 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.

* + - 1. *2-(1H-Benzo[d]imidazol-2-ylthio)-N’-(4-chlorobenzylidene) acetohydrazide (8).* Dull yellow powder; mp 210–213 °C; yield 71.63%; Rf 0.26 (Benzene: Chloroform 6:4); IR (cm—1): 3466 NAH str. for 2° amide, 3074 NAH str. for imidazole, 1622 C@O str for 2° amide, 822 CAH out of plane bending, 733 CACl str of monochlorinated aromatic, 669 CAS str of thiol; 1HNMR (DMSO, d): 6.95–7.85 (m, 8H aromatic), 8.01 (s, NH of 2° amide); 13C NMR (DMSO, d): 40.05 CH2 aliphatic, (109.42, 129.01, 129.96,

132.23) C of benzene, (119.95, 122.24, 134.18, 153.45) C of benz-

imidazole, 139.40 CH aliphatic, 168.14 C of amide; EIMS *m*/*z* 346 [M + 1]+; Anal. Calcd. for C16H13ClN4OS: 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.

* + - 1. *2-(1H-Benzo[d]imidazol-2-ylthio)-N’-(4-fluorobenzylidene) acetohydrazide (9).* Light brown crystals; mp 174–176 °C; yield 72.39%; Rf 0.23 (Benzene: Chloroform 6:4); IR (cm—1): 3542 NAH str. for 2° amide, 3258 NAH str. for imidazole, 3056 CAH aromatic str, 1638 C@O str for 2° amide, 1010 CAF str of monochlorinated compound, 799 CAS str of thiol; 1HNMR (DMSO, d): 4.14 (s, 2H of methylene), 6.91–7.78 (m, 8H aromatic), 7.96 (s, NH of 2° amide); 13C NMR (DMSO, d): 40.06 CH2 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 C16H13FN4OS: 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.

* + - 1. *2-(1H-Benzo[d]imidazol-2-ylthio)-N’-(4-bromobenzylidene) acetohydrazide (10).* Lemon yellow powder; mp 182–185 °C; yield 70.54%; Rf 0.43 (Benzene: Chloroform 6:4); IR (cm—1): 3452 NAH str. for 2° amide, 3157 NAH str. for imidazole, 1650 C@O str for 2° amide, 817 CAH out of plane bending, 738 CAS str of thiol, 604 CABr str aromatic; 1HNMR (DMSO, d): 4.17 (s, 2H of methy- lene), 6.99–7.99 (m, 8H aromatic), 8.19 (s, NH of 2° amide); 13C NMR (DMSO, d): 40.04 CH2 aliphatic, (122.25, 131.73, 131.97,

133.26) C of benzene, (120.04, 123.08, 139.61, 149.51) C of benz-

imidazole, 142.37 CH aliphatic, 169.13 C of amide; EIMS *m*/*z* 390 [M + 1]+; EIMS *m*/*z* 390 [M + 1]+; Anal. Calcd. for C16H13BrN4OS: 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.

* + - 1. *2-(1H-Benzo[d]imidazol-2-ylthio)-N’-(4-nitrobenzylidene) acetohydrazide (11).* Brick red crystalline powder; mp 208–211

°C; yield 83.57%; Rf 0.39 (Benzene: Chloroform 6:4); IR (cm—1):

3338 NAH str. for 2° amide, 2946 NAH str. for imidazole, 1641 C@O str for 2° amide, 1564 NO2 str (asym) of aromatic nitro group, 1452 CAC str of phenyl nucleus, 1330 NO2 str (sym) of aromatic

nitro group, 737 CAS str of thiol, 577 CNO bending of nitro com- pound; 1HNMR (DMSO, d): 4.18 (s, 2H of methylene), 7.01–8.05 (m, 8H aromatic), 8.16 (s, NH of 2° amide); 13C NMR (DMSO, d):

40.03 CH2 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 ali- phatic, 169.48 C of amide; EIMS *m*/*z* 356 [M + 1]+; Anal. Calcd. for C16H13N5O3S: 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.

* + - 1. *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%; Rf 0.40 (Benzene: Chloro- form 6:4); IR (cm—1): 3663 OAH str of phenol, 3374 NAH str. for 2° amide, 2880 NAH str. for imidazole, 1652 C@O str for 2° amide, 868 CAH plane bending of 1,3,5- trisubstituted benzene ring, 612 CAS str of thiol; 1HNMR (DMSO, d): 3.80 (s, 2H of methylene),

6.95–7.93 (m, 7H aromatic), 8.09 (s, NH of 2° amide); 13C NMR

(DMSO, d): 39.96 CH2 aliphatic, 56.04 C of OCH3 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 ali- phatic, 170.42 C of amide; EIMS *m*/*z* 357 [M + 1]+; Anal. Calcd. for C17H16N4O3S: 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.

* + - 1. *2-(1H-Benzo[d]imidazol-2-ylthio)-N’-(3-ethoxy-4-hydroxy- benzylidene)aceto hydrazide (13).* Dark brown crystals; mp 176– 178 °C; yield 54.08%; Rf 0.43 (Benzene); IR (cm—1): 3306 NAH str.

for 2° amide, 2942 NAH str. for imidazole, 2830 CAH str aralkyl

ether, 1669 C@O str for 2° amide, 1277 CAOAC str aralkyl asymm, 864 CAH bending of 1,3,5- trisubstituted benzene ring, 747 CAS str of thiol; 1HNMR (DMSO, d): 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); 13C NMR (DMSO, d): 14.74 C of OCH2CH3, 39.88 CH2

aliphatic, 63.80 C of OCH2CH3 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 C18H18N4O3S: 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.

* + - 1. *2-(1H-Benzo[d]imidazol-2-ylthio)-N’-(4-formylbenzylidene)* *acetohydrazide (14).* Turmeric yellow crystalline powder; mp > 30 0 °C; yield 69.64%; Rf 0.37 (Benzene); IR (cm—1): 3271 NAH str.

for 2° amide, 2948 NAH str. for imidazole, 1619 C@O str for 2°

amide, 1345 CACHO skeletal aldehydes group, 936 CAH in plane bending of aldehyde group, 748 CAS str of thiol; 1HNMR (DMSO, d): 3.99 (s, 2H of methylene), 6.98–7.85 (m, 8H aromatic), 8.05 (s, NH of 2° amide); 13C NMR (DMSO, d): 39.91 CH2 aliphatic, (109.42, 121.37, 122.26, 126.58, 126.68 127.08) C aromatic; EIMS

*m*/*z* 339 [M + 1]+; Anal. Calcd. for C17H14N4O2S: 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.

* + - 1. *2-(1H-Benzo[d]imidazol-2-ylthio)-N*'*-((E)-3-phenylallyli- dene) acetohydrazide (15).* Lemon yellow colour; mp 202–204 °C; yield 48.91%; Rf 0.47 (Benzene: Chloroform 6:4); IR (cm—1): 3415 NAH str. for 2° amide, 3032 NAH str. for imidazole, 1652 C@C str vibration of R1CH@CHR2 (*cis*), 736 CAS str of thiol; 1HNMR (DMSO, d): 6.95–7.89 (m, 9H aromatic), 7.91 (s, NH of 2° amide); 13C NMR (DMSO, d): 39.91 CH2 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 C18H16- N4OS: 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.

* + - 1. *2-(1H-Benzo[d]imidazol-2-ylthio)-N’-(2-hydroxybenzyli- dene) acetohydrazide (16).* Light yellow powder; mp 178–180 °C; yield 87.04%; Rf 0.35 (Benzene: Chloroform 6:4); IR (cm—1): 3698 OAH str of phenol, 3373 NAH str. for 2° amide, 2861 NAH str. for imidazole, 1669 C@O str for 2° amide, 749 CAH plane bending of disubstituted benzene ring, 666 CAS str of thiol; 1HNMR (DMSO, d): 4.18 (s, 2H of methylene), 6.98–7.77 (m, 8H aromatic), 8.32 (s,

NH of 2° amide); 13C NMR (DMSO, d): 39.87 CH2 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 C16H14- N4O2S: 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.

* + - 1. *2-(1H-Benzo[d]imidazol-2-ylthio)-N*'*-(4-(dimethylamino) benzylidene)acetohydrazide (17).* Bright yellow powder; mp 175– 178 °C; yield 47.16%; Rf 0.48 (Benzene); IR (cm—1): 3515 NAH

str. for 2° amide, 3089 NAH str. for imidazole, 1601 C@O str for

2° amide, 1359 CAN str of aryl tertiary amine, 766 CAS str of thiol;

1HNMR (DMSO, d): 4.11 (s, 2H of methylene), 7.11–7.89 (m, 8H

aromatic), 8.05 (s, NH of 2° amide); 13C NMR (DMSO, d): 40.04 CH2 aliphatic, (39.70, 39.71) aliphatic CH3 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 C18H19N5OS: 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.

* + - 1. *2-(1H-Benzo[d]imidazol-2-ylthio)-N’-(4-(diethylamino)ben- zylidene) acetohydrazide (18).* Peach coloured powder; mp 220– 222 °C; yield 54.46%; Rf 0.41 (Benzene: Chloroform 6:4); IR

(cm—1): 3308 NAH str. for 2° amide, 3142 NAH str. for imidazole,

1647 C@O str for 2° amide, 1345 CAN str of aryl tertiary amine, 729 CAS str of thiol; 1HNMR (DMSO, d): 3.51 (s, 2H of methylene),

6.94–7.88 (m, 8H aromatic), 8.02 (s, NH of 2° amide); 13C NMR

(DMSO, d): 39.85 CH2 aliphatic, 56.04 C of CH2CH3, 18.50 C of CH2- CH3, (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 C20H23- N5OS: 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.

* + - 1. *2-(1H-Benzo[d]imidazol-2-ylthio)-N*'*-((4-hydroxynaph- thalen-1-yl)methylene) acetohydrazide (19).* Mustard yellow crys- talline powder; mp 188–190 °C; yield 78.07%; Rf 0.35 (Benzene);

IR (cm—1): 3675 OAH aromatic, 3381 NAH str. for 2° amide,

3196 NAH str. for imidazole, 1668 C@O str for 2° amide, 841 naph- thacene, 704 CAS str of thiol; 1HNMR (DMSO, d): 4.25 (s, 2H of methylene), 6.98–7.93 (m, 10H aromatic), 8.03 (s, NH of 2° amide); 13C NMR (DMSO, d): 40.05 CH2 aliphatic, (109.83, 121.05, 121.59,

127.33, 127.74, 127.94, 128.12, 128.69, 132.78, 163.61) C of naph-

thalene, (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 C20H16N4O2S: 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.

* + - 1. *2-(1H-Benzo[d]imidazol-2-ylthio)-N’-(furan-2-ylmethylene) acetohydrazide (20).* Brown coloured crystals; mp 158–160 °C; yield 66.98%; Rf 0.49 (Benzene: Chloroform 6:4); IR (cm—1): 3350 NAH str. for 2° amide, 3103 CAH str for furan, 2973 NAH str. for imidazole, 1595 C@O str for 2° amide, 755 CAS str of thiol; 1HNMR (DMSO, d): 6.96–7.83 (m, 7H aromatic), 7.89 (s, NH of 2° amide); 13C NMR (DMSO, d): 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 benzimi-

dazole, 141.78 CH aliphatic, 172.01 C of amide. EIMS *m*/*z* 301 [M

+ 1]+; Anal. Calcd. for C14H12N4O2S: 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.

* 1. *In vitro antimicrobial evaluation*
     1. *Determination of MIC*

The *in vitro* antimicrobial potential of the synthesized benzim- idazole 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]](#_bookmark19) using Cefadroxil and fluconazole as standard antibacterial and antifungal drugs respectively. The stock solution of 100 mg/ml concentration for each test and stan-

dard 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]](#_bookmark19). The bacterial cul- tures were incubated for a period of 24 h at 37 ± 2 °C. The incuba-

tion 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 deter- mined in terms of minimum inhibitory concentration (MIC).

* + 1. *Determination of MBC/MFC*

The minimum bactericidal concentration (MBC) and fungicidal concentration (MFC) of the synthesized benzimidazole derivatives was determined by subculturing 100 mL of culture from each tube that showed no growth in MIC determination onto sterilized petri-

plates containing fresh agar medium. The petri-plates were incu- bated and analyzed for microbial growth visually [[29]](#_bookmark19).

* 1. *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), rifam- pin (R) and pyrazinamide (PZA)- H37Rv (NCFT/TB/537) was used in order to assess the antimycobacterial activity of the compounds.

* + 1. *Preparation of the drugs/compounds dilutions*

Each of the synthesized compound was dissolved in DMSO to obtain a concentration of 50 mg/ml and diluted further to concen- trations of 25 mg/ml and 12.5 mg/ml. Similarly, stock solution of 50 mg/ml concentration was prepared for standard antitubercular

drug, streptomycin and diluted to 25 mg/ml in order to check the

antitubercular activity.

* + 1. *Preparation of growth media*

It was prepared by adding dehydrated medium (19 g) to puri- fied 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 med- ium after cooling to 45 °C. No adjustment for pH was made.

* + 1. *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 108 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]](#_bookmark19).

* + 1. *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]](#_bookmark19). This methodology is nontoxic, uses a thermally-stable reagent and is suitable for random screening of the antimycobacterial activity. Briefly, 200 lL 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 lL of the Middlebrook 7H9 broth (having loopful inoculum of bacteria-108 CFU) and different dilu- tions of the respective compounds were made directly on the plate. The maximum concentration of the compounds tested was 50 mg/

ml. Plates were covered and sealed with parafilm and incubated

at 37 °C for five days. After this time, 25 lL 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.

* + 1. *Bioassay protocol for susceptibility tests of the compounds by well diffusion method*

The well diffusion method was used to determine susceptibility [[30,32]](#_bookmark19). The agar well diffusion method was modified [[33]](#_bookmark20). Middle

brook 7H10 agar medium was used for bacterial cultures. The cul- ture medium was inoculated with loopful bacteria separately sus- pended in Middle brook 7H10 broth. Wells of 8 mm diameter were punched into the agar and filled each well separately with 50 mg/

ml of test compounds and 25 mg/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.

* + 1. *Determination of minimum inhibitory concentration (MIC) by alamar blue assay*

The compounds were serially diluted to determine the mini- mum inhibitory concentration of the drug in Middle brook 7H9 medium using micro titre plate method [[30,34,35]](#_bookmark19). The compounds which were found to be satisfactory by the above two methods at a maximum concentration of 50 mg/ml were diluted further to con-

centrations viz. 25, 12.5, 6.25, 3.125 and 1.56 mg/ml respectively.

Similarly, streptomycin was further diluted to 25 mg/ml in order to check the antitubercular activity. The MIC of the potent com- pounds 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 lL 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.

* 1. *In vivo antitubercular activity evaluation*

The LD50 (lethal dose) and ED50 (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.

* 1. *Enzyme assays for antitubercular activity*

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

* + 1. *Mycobacterial isocitrate lyase assay*

Isocitrate lyase activity was assayed according to the protocol reported by Dixon and Kornberg (glyoxylate phenyl hydrazone for- mation) [[36]](#_bookmark23) at 10 mM concentration of the compounds. Isoniazid was employed as a negative control (inhibition of 0%) and strepto-

mycin sulphate (25 mg) served as a positive control [[37]](#_bookmark24).

* + 1. *Mycobacterial pantothenate synthetase assay*

About 60 mL of the PS reagent, including NADH, pantoic acid, b- alanine, ATP, phosphoenolpyruvate, MgCl2, 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 mL volumes. 39 mL 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 MgCl2, 5 mM b-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 mg/ml PS in a final volume of 100 mL. 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 col- umns, of which 12 contained the complete reaction mixture with a DMSO carrier control (full reaction) and four without PS. The fol- lowing formula was used to calculate percent inhibition % inhibi

tion = 100 × (1 — compound rate — background rate)/(full reaction rate — background rate) [[38,39]](#_bookmark26).

* + 1. *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 b-mercaptoethanol were incubated at 37 °C for 5 min. The reaction was started with the addition of 10 mL 5 pM of MtCM (i.e., 185 ng of CM equivalent to 12.5 nM final concen- tration 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 prephen- ate 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]](#_bookmark29).

* 1. *In vitro anticancer screening*

The *in vitro* cytotoxicity screening of the synthesized benzimi- dazole derivatives was assessed on MCF7 (human breast adenocar- cinoma cancer) cell line using Sulforhodamine-B (SRB) assay with minor modifications [[41]](#_bookmark31). The results of anticancer activity were expressed as IC50 (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 com- pounds. Solution of the test and standard compounds were pre- pared in DMSO and made to appropriate volume with media. Monolayer cells were then incubated at 37 °C for 72 h with differ- ent concentrations (0.01, 0.1, 1, 10, 100 mg/ml) of the test com- pounds 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.

1. Results and discussion
   1. *Chemistry*

The benzimidazole derivatives (1–20) were synthesized accord- ing to [Scheme 1](#_bookmark7) 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 SAH stretching at 2600–2550 cm—1. The appearance of C@O stretch in the range of 1680–1630 cm—1 and NAH stretch 3100–3070 cm—1 indicated the formation of secondary amide (1–

20) formed by the reaction of ester and hydrazine hydrate. The absence NAH 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 d ppm confirmed the presence of protons of benzimidazole and aryl nucleus. The compounds 1, 2 and 3 showed singlet at d 3.78 ppm corresponding to a proton of the OCH3. Further confirmation was made on the basis of mass analysis and 13CNMR data. The elemen- tal (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]](#_bookmark33) but their antitubercular/anticancer activities are not explored.

* 1. *In vitro antimicrobial activity*

The results of *in vitro* antimicrobial activity of the synthesized compounds are presented in [Table 1](#_bookmark8). Most of the synthesized derivatives were found to be highly efficient as antimicrobial agents in comparison to the standard drug cefadroxil and flu- conazole as depicted by their low MIC values compared to standard drugs. Amongst the synthesized benzimidazole derivatives, com- pound 10 was found to be the potent antibacterial agent against

*S. aureus* (MIC = 0.032 mM).

In case of *B. subtilis*, lowest MIC values of 0.021 and 0.031 mM were observed for compounds 20 and 5, respectively. Compound

10 (MIC = 0.0321 mM) showed highest inhibitory action against

*E. coli* (a Gram negative bacterium). Compounds 5, 10 and 18 exhibited most effective antifungal activity against *C. albicans*, hav- ing MIC value of 0.016 mM against each compound while com- pound 17 (respectively) possessed maximum activity against *A.*

*niger* with MIC of 0.018 mM. Compound 10 emerged as the best antibacterial agent against tested Gram positive and Gram nega- tive bacteria. All the synthesized compounds showed high antifun- gal activity than the standard drug fluconazole.

The results of minimum bactericidal concentration/minimum fungicidal concentration ([Table 2](#_bookmark9)) 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]](#_bookmark36).

* 1. *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](#_bookmark10).

* 1. *In vivo antitubercular activity*

The LD50 and ED50 for the active compounds were determined in mice models infected with *Mycobacterium tuberculosis H37Rv* ([Table 4](#_bookmark13)). It was found that the toxic dose of the compounds which proved fatal and highly toxic to mice was 5.67 mg/kg while LD50 varied from 1.82 mg/kg to 3.23 mg/kg body weight of the mice. LD50 is the dose that killed 50% of the mice population within the group. Thus, ED50 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 ani- mal was recorded.

H H O C2H5 H HNNH2

S O

N SH ClCH2COOC2H5 N

N S O NH2NH2.H2O N

## N N

1H-benzo[d]imidazole-2-thiol Ethyl 2-(1H-benzo[d]imidazol-2-

ylthio)acetate

R3

EtOH/AcOH

ArCHO

EtOH/AcOH

Ar'CH

O

H

N

N

2-(1H-Benzo[d]imidazol-2-ylthio) acetohydrazide

## H HNN

S O

R1 R2

Ar'

## N

N

R5 R4

HNN

S O

Benzimidazole derivatives

# (1-14, 16-18)

1 R1= OCH3, R2=R3=R4=R5 = H

2 R2= OCH3, R1=R3=R4=R5= H

3 R3= OCH3, R1=R2=R4=R5= H

4 R1=R3= OCH3, R2=R4=R5= H

5 R2=R3=R4= OCH3, R2=R5= H

6 R3= OH, R1=R2=R4=R5= H

7 R1= Cl, R2=R3=R4=R5 = H

8 R3= Cl, R1=R2=R4=R5 = H

9 R3= F, R1=R2=R4=R5= H

10 R3= Br, R1=R2=R =R5= H

11 R3= NO2, R1=R2=R4=R5= H

12 R2= OCH3, R3= OH, R1=R4 =R5 = H

## 13 R2= OCH2CH3, R3= OH, R1=R4=R5= H

14 R3= CHO, R1=R2=R4=R5 = H

## 16 R1= OH, R2=R3=R4=R5 = H

17 R3= N(CH3)2, R1=R2=R4=R5 = H

18 R3= N(CH2CH3)2, R1=R2=R4=R5= H

15 Ar'=

19 Ar'=

20 Ar'=

Benzimidazole derivatives

# (15, 19, 20)

## OH



O

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

Table 1

MIC of synthesized benzimidazole derivatives.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Compound No. | MIC in mM |  | | | | |
|  | *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 (mg/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.

Table 5

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

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Compound No.  1  2 | Diameter of zone of inhibition (mm)  NA  >20 | MIC in mg/ml  NA 12.5 | MLC in mg/ml  NA 25 | Potent compounds/ Percent inhibition  Positive control *M.* ICL activity *M.* PS activity *M.* CM activity  (IU/L) (IU/L) (IU/L) | | | |
| 3 | >20 | 12.5 | 25 | 2 | 52.14 | 50.13 | 48.19 |
| 4 | >20 | 12.5 | 25 | 3 | 47.23 | 38.26 | 47.45 |
| 5 | >20 | 12.5 | 25 | 4 | 58.34 | 47.78 | 40.32 |
| 6 | NA | NA | NA | 5 | 52.23 | 41.56 | 43.56 |
| 7 | NA | NA | NA | 12 | 46.56 | 48.13 | 38.45 |
| 8 | NA | NA | NA | 13 | 56.15 | 43.45 | 37.56 |
| 9 | 10 | 15 | 28 | 17 | 45.67 | 38.32 | 28.45 |
| 10 | NA | NA | NA | 18 | 57.78 | 45.90 | 32.56 |
| 11 | NA | NA | NA | 19 | 67.56 | 53.45 | 47.56 |
| 12 | >20 | 12.5 | 25 | Negative control | No reduction | No reduction | No reduction |
| 13 | >20 | 12.5 | 25 | Streptomycin sulphate | 75.12 | 77.06 | 79.56 |
| 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 |

Table 6

|  |  |  |  |
| --- | --- | --- | --- |
| 20 | NA | NA | NA |
| Streptomycin | >20 | 12.5 | 25 |
| NA – Not active. |  |  |  |

IC50 (mM) values of synthesized benzimidazole derivatives.

Table 4

|  |  |
| --- | --- |
| Compound No. | IC50 (mM) |
| 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 |

LD50 (mg/kg) of potent compounds.

|  |  |
| --- | --- |
| Potent compound (s) groups | LD50 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 |

* 1. *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



R2

N

S O

On

Replacement of phenyl with *p*- hydroxynapthol (compound **19**) increases the antitubercular acivity.

increasing chain length and

R1

Halo substution at *para*-position of phenyl ring increases anticancer activity while substitution with other groups decrease activity.

The substitution at *par*a position of the phenyl nucleus with electron withdrawing groups like

CF3 and NO2 or halogens especially Br

(compound **10**) enhanced the antimicrobial activity.

HN

N

Hydroxyl group substitution at *ortho* -position yields compound with better activity. *Di*- and *tri*-substitution on phenyl ring also decreases anticancer activity.

HC

N

conjugation, there is marked increase in activity as in case of compound synthesized using cinnamaldehyde.

H

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](#_bookmark11)). Compound 19 emerged as the best com- pound that inhibited the mycobacterial isocitrate lyase, pantothen- ate 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.

* 1. *In vitro anticancer activity*

Most of the synthesized compounds possessed more cytotoxic- ity as compared to 5-fluorouracil ([Table 6](#_bookmark12)). Compound 19 (IC50 = 0. 0013 mM) showed extremely potent cytotoxicity against MCF7 cell line as compared to 5-fluorouracil (IC50 = 0.0461 mM). Majority of the compounds were more active than standard drug carboplatin while compounds 4, 5, and 18 were as active as carboplatin (IC50 = 0.2694 mM).

* 1. *Structure activity relationship (SAR)*

From the comparison of antimicrobial, antitubercular and anti- cancer activities of synthesized benzimidazole derivatives, the fol- lowing SAR may be deduced.

1. The good antimicrobial activity (minimum MIC values) of the synthesized benzimidazole derivatives compared to the stan- dard 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 ben- zimidazole derivatives compared to the standard drug strepto- mycin 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 benzimida- zole 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 devel- oping novel anticancer agent based on synthesized benzimida- zole 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](#_bookmark14).

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 com- pounds 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 com- pounds inhibited these enzymes but to a lesser extent than strep- tomycin sulphate taken as positive control. Compound 19, the most potent one among the synthesized benzimidazole deriva- tives 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 com- pared to standard antibacterial (cefadroxil) and antifungal (flu- conazole) drugs. Compound 10 was found to be the most active antibacterial agent against Gram positive as well as Gram nega- tive 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 (IC50 = 0.0013 mM).

Conflict of interest

There is no conflict of interest among the authors.

Acknowledgement

This research work was supported by the Indian Council for Medical Research, New Delhi, India (Grant No. 45/14/2011/PHA/ BMS).

References

1. [Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0005) [1995;1:7–15](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0005).
2. Kent MM, Yin S. Controlling infectious diseases. Popul Bull 2006; 61: 1–20.
3. [Gautam N, Garg A, Bishnoi AK, Agarwal S, Gautam DC. Antioxidant and](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0015) [antimicrobial assessment of synthesized and spectrally characterized new](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0015) [nitrophenothiazines and their sulfone analogues. Phosphorus Sulfur Silicon](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0015) [Relat Elem 2015;190:528–36](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0015).
4. [Hethcote HW. The mathematics of infectious diseases. SIAM Rev](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0020) [2000;42:599–653](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0020).
5. [Camacho J, Barazarte A, Gamboa N, Rodrigues J, Rojas R, Vaisberg A, et al.](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0025) [Synthesis and biological evaluation of benzimidazole-5-carbohydrazide](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0025) [derivatives as antimalarial cytotoxic and antitubercular agents. Bioorg Med](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0025) [Chem 2011;19:2023–9](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0025).
6. [Prakash P, Aruna B, Sardesai AA, Hasnain SE. Purified recombinant hypothetical](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0030) [protein coded by open reading frame Rv1885c of *Mycobacterium tuberculosis*](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0030)[exhibits a monofunctional aroq class of periplasmic chorismate mutase](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0030) [activity. J Biol Chem 2005;280:19641–8](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0030).
7. [Agarwal H, Kumar A, Bal NC, Siddiqi MI, Arora A. Ligand based virtual](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0035) [screening and biological evaluation of inhibitors of chorismate mutase](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0035) [(Rv1885c) from *Mycobacterium tuberculosis* H37Rv. Bioorg Med Chem Lett](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0035) [2007;17:3053–8](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0035).
8. [Arora A, Chandra NR, Das A, Gopal B, Mande SC, Prakash B, et al. Structural](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0040) [biology of *Mycobacterium tuberculosis* proteins: the Indian efforts. Tuberculosis](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0040) [2011;91:456–68](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0040).
9. [Guo H, Rao N. Chorismate-mutase catalysed Claisen rearrangement. In:](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0045) [Nubbemeyer U, Hiersemann M, editors. The Claisen Rearrangement-](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0045) [Methods and applications. Weinheim: Wiley-VCH Verlag GmbH & Co. KGaA;](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0045) [2007. p. 1–23](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0045).
10. [Li JM, Li N, Zhu DY, Wan LG, He YL, Yang C. Isocitrate lyase from *Mycobacterium*](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0050)[*Tuberculosis* promotes survival of *Mycobacterium Smegmatis* within](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0050) [macrophage by suppressing cell apoptosis. Chin Med J 2008;121:1114–9](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0050).
11. [Uy VCC, Billones JB. Towards antituberculosis drugs: virtual screening for](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0055) [potential inhibitors of pantothenate synthetase of *Mycobacterium tuberculosis*.](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0055) [Philipp Sci Lett 2012;5:122–30](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0055).
12. [American Cancer Society. Breast Cancer Facts & Figures 2015–](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0060) [2016. Atlanta: American Cancer Society, Inc.; 2015. p. 1–38](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0060).
13. [Wright JB. The chemistry of the benzimidazoles. Chem Rev 1951;48:397–541](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0065).
14. [Sridevi CH, Balaji K, Naidu A, Sudhakaran R. Synthesis of some phenylpyrazolo](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0070) [benzimidazolo quinoxaline derivatives as potent antihistaminic agents. E- J](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0070) [Chem (online) 2010;7:234–8](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0070).
15. [Ranjith PK, Rajeesh P, Haridas KR, Susanta NK, Row TN, Rishikesan R, et al.](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0075) [Design and synthesis of positional isomers of 5 and 6-bromo-1-](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0075) [[(phenyl)sulfonyl]-2-[(4-nitrophenoxy)methyl]-1*H*-benzimidazoles as](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0075) [possible antimicrobial and antitubercular agents. Bioorg Med Chem Lett](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0075) [2013;23:5228–34](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0075).
16. [Arora RK, Kaur N, Bansal Y, Bansal G. Novel coumarin-benzimidazole](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0080) [derivatives as antioxidants and safer anti-inflammatory agents. Acta Pharm](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0080) [Sin B 2014;4:368–75](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0080).
17. [Pan T, He X, Chen B, Chen H, Geng G, Luo H, et al. Development of](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0085) [benzimidazole derivatives to inhibit HIV-1 replication through protecting](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0085) [APOBEC3G protein. Eur J Med Chem 2015;95:500–13](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0085).
18. [Vasantha K, Basavarajaswamy G, Rai MV, Boja P, Pai VR, Shruthi N, et al. Rapid](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0090) [‘one-pot’ synthesis of a novel benzimidazole-5-carboxylate and its hydrazone](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0090) [derivatives as potential anti-inflammatory and antimicrobial agents. Bioorg](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0090) [Med Chem Lett 2015;25:1420–6](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0090).
19. [Gaba M, Gaba P, Uppal D, Dhingra N, Bahia MS, Silakari O, et al. Benzimidazole](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0095) [derivatives: search for GI-friendly anti-inflammatory analgesic agents. Acta](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0095) [Pharm Sin B 2015;5:337–42](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0095).
20. [Wen J, Luo Y, Zhang H, Zhao H, Zhou C, Cai G. A green and convenient approach](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0100) [toward benzimidazole derivatives and their antimicrobial activity. Chin Chem](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0100) [Lett 2016;27:391–4](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0100).
21. Keri RS, Hiremathad A, Budagumpi S, Nagaraja BM. Comprehensive review in current developments of benzimidazole-based medicinal chemistry. Chem Biol Drug Des 2015;86:19–65. doi: <https://doi.org/10.1111/cbdd.12462>.
22. [Bansal Y, Silakari O. The therapeutic journey of benzimidazoles: a review.](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0110) [Bioorg Med Chem 2012;20:6208–36](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0110).
23. [Yadav S, Narasimhan B, Kaur H. Perspectives of benzimidazole derivatives as](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0115) [anticancer agents in the new era. Anticancer Agents Med Chem](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0115) [2016;16:1403–25](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0115).
24. [Haque RA, Budagumpi S, Choo SY, Choong MK, Lokesh BE, Sudesh K. Nitrile-](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0120) [functionalized Hg(II)- and Ag(I)-*N-*heterocyclic carbene complexes: synthesis,](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0120) [crystal structures, nuclease and DNA binding activities. Appl Organometal](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0120) [Chem 2012;26:689–700](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0120).
25. [Ansari KF, Lal C. Synthesis physicochemical properties and antimicrobial](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0125) [activity of some new benzimidazole derivatives. Eur J Med Chem](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0125) [2009;44:4028–33](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0125).
26. [Yadav S, Kumar P, Clercq ED, Balzarini J, Pannecouque C, Dewan SK, et al. 4-[1-](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0130) [(Substituted aryl/alkyl carbonyl)-benzoimidazol-2-yl]-benzenesulfonic acids:](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0130) [synthesis, antimicrobial activity, QSAR studies, and antiviral evaluation. Eur J](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0130) [Med Chem 2010;45:5985–97](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0130).
27. [Cappucino JG, Sherman N. Microbiology: a laboratory mannual. California:](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0135) [Addison Wesley Longman Inc; 1999. p. 263](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0135).
28. Pharmacopoeia of India, vol. II. Ministry of Health Department New Delhi: Govt. of India; 1996; p. A-88.
29. [Rodriguez-Arguelles MC, Lopez-Silva EC, Sanmartin J, Pelagatti P, Zani F.](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0145) [Copper complexes of imidazole-2-pyrrole-2- and indol-3-carbaldehyde](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0145) [thiosemicarbazones: inhibitory activity against fungi and bacteria. J Inorg](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0145) [Biochem 2005;99:2231–9](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0145).
30. Parish T, Stroker NG. Mycobacteria Protocols: Methods in molecular Biology. (Vol. 101) Totowa NJ: Humana Press Totowa; 1998, p. 395–422.
31. [Collins LA, Franzblau SG. Microplate alamar blue assay versus BACTEC 460](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0155) [system for high-throughput screening of compounds against *Mycobacterium*](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0155)[*tuberculosis* and *Mycobacterium avium*. Antimicrob Agents Chemother](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0155) [1997;41:1004–9](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0155).
32. [Kirimuhuzya C, Waako P, Joloba M, Odyek O. The antimycobacterial activity of](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0160) [*Lantana camara* a plant traditionally used to treat symptoms of tuberculosis in](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0160) [South-western Uganda. Afr Health Sci 2009;9:40–5](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0160).
33. [Perez C, Anesini C. *In vitro* antimicrobial activity of Argentine folk medicinal](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0165) [plants against *Salmonella typhii*. J Ethnopharmacol 1994;44:41–6](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0165).
34. [Sanjay MJ. Natural products: an important source for antitubercular drugs.](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0170) [CRISP 2004;5:1](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0170).
35. [Tona L, Kambu K, Ngimbi N, Cimanga K, Vlietinck AJ. Antiamoebic and](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0175) [phytochemical screening of some Congolese medicinal plants. J](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0175) [Ethnopharmacol 1998;61:57–65](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0175).
36. [Dixon GH, Kornberg HL. Assay methods for key enzymes of the glyoxylate](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0180) [cycle. Biochem J 1959;72:3P](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0180).
37. [Kratky M, Vinaova J, Rodriguez NG, Stolarikova J. Antimycobacterial activity of](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0185) [salicylanilide benzenesulfonates. Molecules 2013;17:492–503](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0185).
38. [Samala S, Devi PB, Nallangi R, Yogeeswari P, Sriram D. Development of 3-](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0190) [phenyl-4,5,6,7-tetrahydro-1??-pyrazolo[4,3-*c*]pyridine derivatives as novel](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0190) [*Mycobacterium tuberculosis* pantothenate synthetase inhibitors. Eur J Med](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0190) [Chem 2013;69:356–64](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0190).
39. [Wang S, Eisenberg D. Crystal structures of a pantothenate synthetase from *M.*](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0195)[*tuberculosis* and its complexes with substrates and a reaction intermediate.](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0195) [Protein Sci 2003;12:1097–108](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0195).
40. [Adepu R, Shiva Kumar K, Sandra S, Rambabu D, Rama Krishna G, Malla Reddy](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0200) [C, et al. C-N bond formation under Cu-catalysis: synthesis and *in vitro*](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0200)[evaluation of *N*-aryl substituted thieno[2,3-*d*]pyrimidin-4-(3*H*)-ones against](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0200) [chorismate mutase. Bioorg Med Chem 2012;20:5127–38](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0200).
41. [Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, et al. New](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0205) [colorimetric cytotoxicity assay for anticancer-drug screening. J Natl Cancer](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0205) [Inst 1990;82:1107–12](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0205).
42. [Hosamani KM, Shingalapur RV. Synthesis of 2-mercaptobenzimidazole](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0210) [derivatives as potential anti-microbial and cytotoxic agents. Arch Pharm](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0210) [(Weinheim) 2011;344:311–9](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0210).
43. [Zainab AK. Synthesis of some new 1, 2, 4-triazoles derived from 2-](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0215) [mercaptobenzimidazole. Um-Salama Sci J 2009;6:200–8](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0215).
44. Budeanu CH, Rusu G, Cojocaru Z, Nistor, C. Investigations on a series of five- membered nitrogen heterocycles. Synthesis of some new benzimidazolyl-2- mercaptoacetohydrazidehydrazones and testing of their cytostatic action on experimental tumors. Revista Medico-Chirurgicala 1976; 80: 605–9.
45. [Emami S, Falhati M, Banifafemi A, Shafiee A. Stereoselective synthesis and](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0225) [antifungal activity of (*Z*)-trans-3-azolyl-2-methylchromanone oxime ethers.](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0225) [Bioorg Med Chem 2004;12:5881–9](http://refhub.elsevier.com/S2314-808X(17)30308-1/h0225).