ISSN 2063-5346 *Section A-Research paper*

Synthesis of novel substituted benzimidazoles derivatives for antimicrobial activity

Monika[1], Sachdev Yadav[1], Rakesh Yadav[1, 2*]
[1]Department of Pharmacy, Banasthali Vidyapith, Banasthali-304 022, Rajasthan
[2]National Forensic Sciences University, Curti, Ponda-403401, Goa Campus
[*]Corresponding Author
Dr. Rakesh Yadav
Associate Professor (Pharmacy)
National Forensic Sciences University,
Curti, Ponda-403401, Goa Campus
E:Rakesh_pu@yahoo.co.in
Mob :(+91)9694891228

ABSTRACT

A new series of substituted benzimidazoles (6A-13B) were designed and synthesized using 1-chloro 2, 4 dinitrobenzene and substituted amine by a multi-step synthesis. By using spectroscopic methods and elemental analyses, all the produced substances were identified. All the synthesized compounds were evaluated for *in-vitro* antimicrobial activity against various human pathogenic microorganisms by employing agar cup plate process using ciprofloxacin as standard drugs. The whole strains of microorganisms were active against every chemical that was generated. Among all these 7B, 12B and 8A are the most active. Because these three compounds show valuable MIC value than other compounds .Structural activity relationship studies showed that compounds with an electron withdrawing group exhibit enhanced activity, while the other derivatives exhibit moderate activity. Based on the findings, three compounds—7B, 8A, and 12 B—were discovered to be much more active than the other compounds and standard medications in the antimicrobial experiment.

Keywords: Antimicrobial, benzimidazoles, Structural activity relationship, ciprofloxacin, agar cup plate.

INTRODUCTION

Various parasitic bacteria, including S. S. aureus S. Pyogenes E. coli and Typhimurium coli significantly affect the health of human mucosal tissues. Infection with *S. aureus*, *S. pyogenes*, *S. typhimurium* and *E. coli* may have resulted in massive host tissue degradation and potentially fatal illnesses. Millions of pgdgeople in impoverished nations suffer from food poisoning, rheumatic fever, and diarrhea as a result of these bacterial parasites.[1] Around the world, more than 50 million people are afflicted, and up to 1,10,000 of them pass away each year. The most popular antibiotics for this bacterial infection are Amoxicillin, Norfloxacin, and Ciprofloxacin, however they come with serious side effects.[2] A serious hazard that could result in treatment failures and problems is the ongoing rise of bacterial infections that are resistant to one or more antibiotic classes.[3,4] As a result, numerous research teams have put significant effort into discovering novel antimicrobial drugs.

The chemical compound benzimidazole is an aromatic heterocyclic compound. Since a few decades ago, pharmacists have been interested in the synthesis of benzimidazole-based poly heterocycles since they serve as a significant pharmacophore in medicinal chemistry and pharmacology. In essence, benzimidazole is a bicyclic molecule made by joining benzene and imidazole, which results in a favored structure. Various studies reported that benzimidazole possess a wide range of bioactivities including antimicrobial [5], antiparasitic [6]antihistaminic [7] antiallergic [8], anticancer[9-12] and antioxidant [13-15]. According to a literature review, the benzimidazole nucleus shown remarkable antitubercular[16-19] & antimicrobial[20-23] activities. Some of the compounds showed moderate activity, but some of the derivatives of benzimidazole gave considerable anti mycobacterial activity. The drugs used have serious side effects on the patients ranging from nausea, vomiting, cardiotoxicity, renal insufficiency, teratogenicity and many more. It was of concern to synthesize a new derivatives of benzimidazole in order to find more dynamic and less toxic antimicrobial agents as part of our drug research programme that focuses on the synthesis of new, safer, and more biologically active compounds.

MATERIALS AND METHODS

All used chemicals were of commercially available analytical grade and were used without further purification. On melting point equipment, melting points were calculated.

General procedure

Step 1: Mixture of 1-chloro-2,4-dinitro benzene (0.038 mol) and substituted amine (0.87mol) and heated in sealed tube in oven at 100 ºC for 10 h. Then remove excess of amine under reduced pressure.

Step 2: Reflux the mixture of substituted aniline, catalyst 0.5 gm, hydrazine monohydrate (80% , 7.4ml) and 25ml dry ethanol for 30 min under nitrogen. Filter it to remove catalyst. Dilute the filtrate with water, extract with ether and dry it with sodium sulphate and evaporate.

Procedure for Catalyst preparation: Take nickel chloride hexahydrate 23g, zinc (13gm) and dry ethanol (50ml) in a 250ml single necked round bottom flask equipped with nitrogen inlet and reflux it for 16hrs. Evaporate solvent under reduced pressure.

Step 3: Take mixture of product found in step 2, potassium hydroxide 1.29 gm, carbon disulfide 1.38ml, 230ml 95% ml ethanol in round bottom flask. Heat it under reflux for 3hrs. After refluxing add activated charcoal with heating. Filter it for removal of activated charcoal. Add 300ml warm water to filtrate and heat the mixture to 60-70 C, then add 15ml acetic acid in 30ml water with stirring. Place the mixture in refrigerator for 3hrs to complete crystallization. After that filter it and dry over-night.

Step 4: Take mixture of product found in step 3, treat it with substituted haloalkane in the presence of sodium and ethanol reflux it for 6-18 hrs.

The synthesized compounds will be characterized using various spectral methods for the chemical structure confirmation. The newly synthesized and characterized compounds were assessed for their antimicrobial potential.

General Method for antimicrobial activity

The agar layer holding the microorganisms is diffused with antibiotics using the cup plate method. The zone is formed around the cylinder. The examined samples diffuse from the cup through an agar layer in a Petri plate to the point where the development of further bacteria is solely limited to a circular area or zone in the region of the cavity containing the solution of an antibiotic agent.[24] When measured by a scale, the antibacterial activity is given as the zone diameter in millimeters.

Synthesis of 1-methyl-5-nitro 2-thiobutylbenzimidazole(6A)

1-methyl-5-nitro 2-thiobutylbenzimidazole(6A) m.pt 261-263°C % yield 79%

Spectral analysis: - IR Specturm: (KBr cm^{-1}): 2850-2960cm^{-1}(C-Hstr aliphatic), 1487cm^{-1}(C=N),788cm^{-1}(C-S), (N-O) 1550cm^{-1} NMR Specturm: ^{1}H NMR (300 MHz, $CDCl_3$): δ0.94 (t,-3H,-CH_3)δ1.49(m,-2H,-CH_2)δ1.75(m,-2H,-CH_2)δ2.44(s,-3H,-CH_3)δ3.35(t,-2H,-CH_2)δ3.62(s,-3H,-CH_3)δ6.99(d,-1H,-Ph)δ7.07(d,-1H,-Ph)δ7.45(s,-1H,Ph)

^{13}CNMR (75.45MHz,$CDCl_3$):13.54, 21.43, 21.79, 29.96, 31.36, 32.38, 107.89,117.96, 123.15,131.47,134.56,143.18,152.00 and M^{+}265.05 %found C, 54.32; H, 5.70; N, 15.84; O, 12.06

1-ethyl-5-nitro 2-thiobutylbenzimidazole (7A) $C_{11}H_{13}N_3O_2S$ **m.pt 267-269°C** % yield 76%

IR Spectrum (KBrcm^{-1}):1479cm^{-1}(C=N),800cm^{-1}(C-S)(NO2)1550cm^{-1}1350cm^{-1}

NMRSpecturm:^{1}HNMR(300MHz,$CDCl_3$):δ0.84(t,3H,CH_3) δ1.44(m,2H,CH_2) δ1.65(m,2H,CH_2) δ1.92 (t,2H,CH_2)δ3.27(t,3H,CH_3)δ4.01(q,2H,CH_2) δ7.02(d,1H,Ph)δ6.92(d,1Ph)δ7.53(s,1H,Ph)^{13}CNMR(75.45MHz,$CDCl_3$):13.49,14.36,21.26,21.55,31.32,32.33,38.83,108.01,117.87,123.19,131.51,133.27,151.11,174.04 and M^{+}251,01 % found C, 52.57; H, 5.21; N, 16.72; O, 12.73

Synthesis of 5-nitro 1-propyl-2-thiiobutylbenzimidazole (8A) $C_{14}H_{19}N_3O_2S$ **m.pt 278-281°C** % yield 79%

IR Spectrum (KBr cm^{-1}): 1475cm^{-1}(C=N),794cm^{-1}(C-S) , (NO2) 1550cm^{-}.1350cm^{-}

1HNMR(300MHz,$CDCl_3$): δ0.85(t,-3H,-CH_3)δ0.99(t,-3H,-CH_3)δ δ1.47(m,-2H,-CH_2) δ1.70(m,-2H,-CH_2)δ1.84(m,-2H,-CH_2) δ3.98(t,-2H,-CH_2) δ4.02(t,-2H,-CH_2) δ7.02(d,-1H,-Ph)δ7.44(d,-1H,-

Ph)δ7.56(s,1H,Ph)^{13}CNMR(75.45MHz,$CDCl_3$):11.52,13.44,21.72,24.35,25.72,32.57,36.44,49.83,115.84,116.40,126.82,131.33,133.76,138.43,152.12and M^+293% found C, 57.31; H, 6.53; N, 14.32; O, 10.91

Synthesis of 1-butyl 5-nitro -2-thiiobutylbenzimidazole (9A) ($C_{15}H_{21}N_3O_2S$**) m.pt 289-291°C** % yield 76%

IR Spectrum (KBr cm^{-1}): 2850-2960cm^{-1}(C-Hstr aliphatic), 1487cm^{-1}(C=N),788cm^{-1}(C-S). (NO_2) 1550 cm^{-1}, 1350 cm^{-1} NMR Specturm: ^{1}HNMR (300 MHz, $CDCl_3$): δ0.82(t,-3H,-CH_3) δ 0.86(t,-3H,-CH_3) δ 1.32(m,-2H,-CH_2) δ 1.45(m,-2H,-CH_2)δ 1.68(m,-2H,-CH_2)δ 1.73(m,-2H,-CH_2)δ δ 3.34(t,-2H,-CH_2) δ 3.98(t,-2H,-CH_2)δ δ 6.96(d,-1H,-Ph)δ 7.06(d,-1H,-Ph)δ δ 7.43(s,-1H,-Ph)^{13}CNMR (75.45 MHz, $CDCl_3$): 13.53, 13.61, 20.0, 21.40, 21.76, 31.27, 31.30, 32.67, 44.14, 108.41, 117.55, 123.59,132.10,133.42,141.81,151.50 and M^+307

%found C, 58.61; H, 6.89; N, 13.67; O, 10.41

1-methyl 5-nitro2-thiobphenylbenzimidazole (10A) $C_{14}H_{11}N_3O_2S$ **m.pt 305-307°C** % yield 72%

IR Spectrum**:** (KBr cm^{-1}): 1450cm^{-1}(C=N), 790cm^{-1}(C-S).

NMR Spectrum: ^{1}H NMR (300 MHz, $CDCl_3$): δ3.5 4(s,-3H,-CH_3) , δ7.05(q,-1H,-Ph) δ7.33(m,-5H,-Ph) δ7.38(dd,-1H,-Ph), δ7.50(s,-1H,-Ph) ^{13}CNMR(75.45MHz,$CDCl_3$) :24.34, 31.26, 38.51, 112.56, 115.46, 125.82 ,128.82,131.32,132.71,138.83,139.77,152.61 m/z: 285 (100.0%), 286 (17.3%), 287 (6.3%)% found C, 58.93; H, 3.89; N, 14.73; O, 11.22

1-ethyl 5-nitro -2-thiophenylbenzimidazole (11A)$C_{15}H_{13}N_3O_2S$ **m.pt 316-319°C** % yield 70%

IR Spectrum **:** (KBr cm^{-1})1479cm^{-1}(C=N), 800cm^{-1}(C-S), (NO_2) 1550cm^{-1}1350cm^{-1}. NMR Spectrum: ^{1}HNMR (300MHz, $CDCl_3$): δ1.41(t,-3H,-CH_3) δ4.33(q,-2H,-CH_2) δ 7.01(d,-1H,-Ph) δ 7.02 (d,-1H,-Ph) δ7.52(s,-1H,-Ph) δ 11.33(s,-1H,-SH) δ 7.57 (d,-1H,-Ph), δ7.58(d,-1H,-Ph), δ 7.27(T,-1H,-Ph), δ 7.27(d,-1H,-Ph)
^{13}CNMR(75.45MHz,$CDCl_3$):13.18,21.50,39.0,108.48,110.55,123.63,130.54,131.17,133.11,167.49 and M^+299.07% found: C, 60.18; H, 4.38; N, 14.04; O, 10.69

5-nitro 1-propyl -2-thiiophenylbenzimidazole(12A) $C_{16}H_{15}N_3O_2S$ **m.pt 327-329°C** % yield 74%

IR Spectrum**:** (KBr cm^{-1}): 1475cm^{-1}(C=N), 794cm^{-1}(C-S), (NO2) 1550cm^{-},1350cm^{-}

^{1}HNMR(300MHz,$CDCl_3$): δ1.01(t,-3H,-CH_3) δ1.87(m,-2H,-CH_2) δ, δ4.23(t,-2H,-CH_2)δ δ6.99(d,-1H,-Ph)δ δ7.26(d,-1H,-Ph)δ δ7.56(s,-1H,-Ph)δ δ7.56(d,-1H,-Ph) δ1_H7.56(d,-1H,-Ph) δ 7.27(t,-1H,-Ph) δ, 7.27(t,-1H,-Ph) δ7.07(t,-1H,-Ph) δ1.76(s,-1H,SH)

^{13}CNMR(75.45MHz,$CDCl_3$):11.52,24.84,26.15,49.56,115.27,116.48,125.89,131.33,132.76,138.86,168.75 and M^+313.94 % found: C, 61.32; H, 4.82; N, 13.41; O, 10.21

1-butyl 5-nitro2-thiophenylbenzimidazole (13A) ($C_{17}H_{17}N_3O_2S$) **m.pt 339-341°C** % yield 78%

IR Spectrum**:** (KBr cm^{-1}) 1477cm^{-1}(C=N), 790cm^{-1}(C-S), (NO_2) 1550 cm^{-1}, 1350 cm^{-1}

NMR Spectrum: ^{1}HNMR (300 MHz, $CDCl_3$) :δ δ0.98(t,-3H,-CH_3)δ 1.46(m,-2H,-CH_2) δ 1.83(m,-2H,CH_2)δ 2.41(s,3H,CH_3) δ 4.26(t,2H,CH_2)δ δ 6.997.09(m,3H,Ph)δ 11.39(s,1H,SH)^{13}CNMR(75.45MHz,$CDCl_3$):13.77,20.13,21.32, 30.06,44.05,108.81,110.41,123.69, 130.64, 130.85, 133.32, 167.33 and M^+327 % found C, 62.36; H, 5.23; N, 12.83; O, 9.77

1-Phenyl-5-nitro 2-thiobutylbenzimidazole (6B) ($C_{18}H_{20}N_3O_2S$) **m.pt 338-341°C** % yield 65%

IR Spectrum (KBr cm^{-1}):2850-2960cm^{-1} (C-Hstr aliphatic)1487cm^{-1}(C=N),790cm^{-1}(C-S),1313cm^{-1}(C-N)NMR Spectrum: ^{1}HNMR(300MHz,$CDCl_3$): δ0.83(t,-3H,-CH_3)δ 1.35(m,-2H,-CH_2) δ 1.61(m,-2H,-CH_2) δ 2.37(s,-3H,-CH_3)δ δ 3.25(t,-2H,-CH_2) δ δ 6.82-7.40(m,-8H,-Ph)

^{13}CNMR(75.45MHz,$CDCl_3$):13.46,21.78,24.34,32.58,36.48,48.32,115.76,115.95,124.82,126.25,127.83,129.86,131.32,134.73,145.67, 154.44,165.62 and M^+342 %found C, 63.13; H, 5.89; N, 12.27; O, 9.34

5-nitro 1-phenyl -2-thiiophenybenzimidazole (7B) ($C_{19}H_{13}N_3O_2S$) **m.pt 387-389°C** % yield 75%

IR Spectrum (KBr cm^{-1}) 1494cm^{-1}(C=N), 796cm^{-1}(C-S).

NMR Spectrum:^{1}HNMR(300MHz,$CDCl_3$):δ 7.43-8.42(m,-13H,-Ph)

^{13}CNMR(75.45MHz,$CDCl_3$):24.89,36.83,50.67,113.57,115.25,121.65,123.78,127.23,127.46,127.85,128.23,128.83,129.62, 137.73,139.74,144.42, 144.83,163.0 and M^+347

%found C, 65.69; H, 3.77; N, 12.10; O, 9.21

1-cyclohexyl 5-nitro2-thiiobutytlbenzimidazole (8B) ($C_{17}H_{24}N_3O_2S$) **m.pt 319-321°C** % yield 82%

Spectral analysis: IR Spectrum**:** νmax (KBr): 2850-2960cm^{-1} (C-Hstr aliphatic), 1425cm^{-1} (C=N),784cm^{-1}(C-S), 1314cm^{-1} (C-N)

NMR Spectrum: ^{1}H NMR (300 MHz, $CDCl_3$): δ0.88(t,-2H,-CH_2) δ 1.26(m,-2H,-CH_2) δ 1.43(m,-2H,-CH_2) δ 1.51(m,-2H,-CH_2) δ 1.65(m,-2H,-CH_2)δ 1.72(m,-2H,-CH_2) δ 1.92(m,-2H,CH_2) δ 2.14(m,-2H, CH_2) δ 3.45(t,-2H,-CH_2) δ 4.22(m,1H,CH) δ 6.96(d,1HPh) δ 7.20(d,1H,Ph), δ 7.52 (s,1H,Ph)[13]

CNMR(75.45MHz,$CDCl_3$):13.45,21.72,23.25,24.38,28.04,32.51,33.40,36.41,53.24,112.06,115.33,125.84,131.39,132.73,138.85,152.65 and M^+334 %found: C, 61.05; H, 7.23; N, 12.56; O, 9.57

1-Hexyl 5-nitro -2-thiiopropylbenzimidazole (9B) ($C_{16}H_{19}N_3O_2S$)**m.pt 307-309°C** % yield 74%

IR Spectrum (KBr cm^{-1})2850-2960cm^{-1}(C-Hstr aliphatic), 1473cm^{1}(C=N),781cm^{1}(CS).

^{1}HNMR(300MHz,$CDCl_3$): δ1.35 (t,-2H,-CH_2) δ1.53(m,-2H,-CH_2) δ1.92 (d,-2H,-CH_2) δ 2.18 (q,-2H,-CH_2) δ 5.15 (t,-1H,-CH) δ 6.98 (d,-1H,-Ph) δ7.08 (s,-1H,-Ph)δ7.34 (d,-1H,-Ph) δ 1.92 (m,-2H,CH_2) δ 2.14 (m,-2H,CH_2) δ 3.45 (t,-2H,-CH_2)

^{13}CNMR (75.45MHz,$CDCl_3$) :23.23,24.36, 28.04, 33.47, 52.93, 115.25, 115.47, 125.85, 131.34, 132.70, 133.84,163.73 and M^+317 %found C, 60.54; H, 6.03; N, 13.24; O, 10.08

1-cycloHexyl 5-nitro -2-thioethyllbenzimidazole (10B) ($C_{15}H_{20}N_3O_2S$)**m.pt 316-319°C** % yield 70%

Spectral analysis:- IR Spectrum **:**νmax (KBr): 2850-2960cm^{-1}(C- Hstr aliphatic), 1473cm^{1}(C=N),781cm^{1}(CS).^{1}HNMR (300MHz,$CDCl_3$): δ1.35 (t,-2H,-CH_2) δ 1.53 (m,-2H,-CH_2) δ1.92(d,-2H,-CH_2) δ2.18(q,-2H,-CH_2) δ5.15(t,-1H,-CH) δ 6.98(d,-1H,-Ph) δ7.08 (s,-1H,-Ph) δ7.34 (d,-1H,-Ph) δ1.92 (m,-2H,CH_2) δ 3.45 (t,-2H,-CH_2)

^{13}CNMR (75.45MHz,$CDCl_3$): 23.23, 24.36, 28.04, 33.47, 52.93,115.25, 115.47, 125.85, 131.34, 132.70, 133.84, 163.73 and M^+ 306 %found C, 58.80; H, 6.58; N, 13.71; O, 10.4

1-cyclohexyl 5-nitro -2-thiophenylbenzimidazole (11B) ($C_{19}H_{20}N_3O_2S$) **m.pt 367-369°C** % yield 74%

Spectral analysis: IR Spectrum**:**νmax (KBr): 2850-2960cm^{-1}(C-Hstr aliphatic), 1423cm^{-1}(C=N), 779cm^{-1}(C-S)

NMR Spectrum: ^{1}H NMR (300 MHz, $CDCl_3$):δ 1.26(m,-2H,-CH_2) δ 1.39(m,-2H,-CH_2) δ 1.71(m,-2H,-CH_2) δ1.74 (m,-2H,-CH_2) δ 2.10 (q,-2H,-CH_2) δ δ4.24(t,-1H,-CH) δ 7.48(d,-1H,-Ph) δ 7.56(m,-3H,-Ph) δ 7.507.63 (m,3H,Ph) δ7.72(s,1H,Ph)

^{13}CNMR(75.45MHz,$CDCl_3$):21.28,24.80,25.61,30.69,39.59,59.33,112.03,115.38,125.82,126.82,128.42,128.92,129.35,132.75,134.50,136.46,147.91and M^+354 %found C, 64.38; H, 5.69; N, 11.86; O, 9.03

1-Hexyl 5-nitro -2-thiomethyllbenzimidazole (12B) $C_{14}H_{18}N_3O_2S$**m.pt 285-288°C** % yield 71%

IR Spectrum**:** (KBr):2850-2960cm^{-1}(C-Hstr aliphatic), 1473cm^{1}(C=N), 781cm^{1}(CS).

^{1}HNMR (300MHz,$CDCl_3$) :δ1.35 (t,-2H,-CH_2) δ1.53 (m,-2H,CH_2) δ1.92 (d,-2H,-CH_2) δ2.18 (q,2H,CH_2),δ δ5.15 (t,1H,CH) δ 6.98 (d,1H,Ph) δ 7.08 (s,1H,Ph) δ7.34 (d,1H,Ph) δ11.03 (s,-1H,SH)

^{13}CNMR(75.45MHz,$CDCl_3$):23.23,24.36,28.04,33.47,52.93,115.25,115.47,125.85,131.34,132.70,133.84,163.73 and M^+292 % found C, 57.51; H, 6.21; N, 14.37; O, 10.94; S, 10.97

1-Hexyl 5-nitro -2-thiiohexylbenzimidazole (13B) $C_{19}H_{27}N_3O_2S$**m.pt 351-354°C** % yield 71%

IR Spectrum (KBr cm^{-1}) 2850-2960cm^{-1}(C-Hstraliphatic), 1473cm^{1}(C=N),781cm^{1}(CS).

^{1}HNMR (300MHz,$CDCl_3$):δ1.35(t,-2H,-CH_2) δ1.53 (m,-2H,CH_2)δ1.92 (d,-2H,-CH_2) δ2.18(q,-2H,-CH_2), δ 5.15(t,-1H,-CH) δ6.98(d,-1H,-Ph) δ7.08(s,-1H,-Ph) δ7.34 (d,-1H,-Ph) δ11.03δ1.35 (t,2H,CH_2) δ1.53(m,2H,CH_2) δ1.92(d,2H,CH_2) δ2.18 (q,2H,CH_2), δ5.15(t,1H,CH)

^{13}CNMR(75.45MHz,$CDCl_3$):23.23,24.36,28.04,33.47,52.93,115.25,115.47,125.85,131.34,132.70,133.84,163.73 and M^+361 %found C, 63.13; H, 7.53; N, 11.62; O, 8.85

RESULTS AND DISCUSSION

Chemistry

In the present work, a series of new substituted benzimidazole and characterized. In the first step1-chloro 2, 4 dinitrobenzene was reacted with substituted amine which was further heated with catalyst and carbon disulfide ,substituted benzimidazole (3A,3B) was formed. Further treatment of compound 3A, 3B with different halogenated aryl or alkyl groups in the presence of DMF. Finally, the title compounds 6A-13A and6B-13B were synthesized . Synthetic Schemes 1 and 2 provide an overview of the chemical processes implicated in the production of the title compounds. As shown by their spectrum analyses and TLC, the procedure utilised for the production and isolation of the compounds produced materials of good purity. The produced compounds' IR, 1H-NMR, mass spectra, and elemental analyses match the prescribed structures. The IR spectra of all the synthesized compounds showed some characteristic peaks, representing the presence of particular groups.. The occurrence of an absorption band in IR at 1450 cm- 1 corresponding to C=O and 750 cm- 1 C-S stretching confirms the formation of compound. Formation of benzimidazole 2 was confirmed by the appearance of a singlet at δ 3.54 ppm for one proton of benzimidazole NCH3 This was further confirmed by the appearance of a

singlet at δ 11.53 ppm for SH protons and a singlet at δ 7.26 ppm for two protons of phenyl. Mass spectroscopy was also performed with all synthesized compounds. with reference to literature study that Electron withdrawing group substituted derivative exhibited better activity than electron donating group substituted analogs. [25] We take electron withdrawing group substitution at position 5 and 2 Recently, there has been reported work done on utilizing benzimidazole derivatives to counter antimicrobial activity with relatively good results

Antimicrobial screening

The cup plate method was used to test all of the title compounds for their in vitro antibacterial properties. In parallel trials, the MICs of Ciprofloxacin were established in order to regulate the sensitivity of the test organisms. The lowest concentration that completely prevented the bacteria' ability to develop visibly was determined to be the MIC value. The MICs of the standard drugs and test compounds 6A-13B was competently presented in Table 1. From the results, it was observed that compound 7A,9A,11A and 7B,10B and 12B and displayed comparable activity like Ciprofloxacin against *S. aureus*. whereas the rest of the sequ ence displayed lesser activity (MIC: 15.62–62.5 μg/mL).. Compound 9A,7B and 11B displayed potent activity (MIC: 7.81 μg/mL) than the standard, while the rest of the series exhibited lower activity against *E. coli* (MIC:31.25–62.5 μg/mL) some compounds like 7B,11Band 12B displayed an equivalent activity (MIC: 7.81 μg/mL)as standard against *P. aeruginosa.)*. compounds 7B and 12B exhibited higher activity against *S.typhi.* (MIC: 3.9 μg/mL)

Structure Activity Relationship

The antibacterial research revealed that substances 7A,9A,11A and 7B,9B,10B, 12B showed strong antimicrobial activity, which might be due to

1. Electron withdrawing group at position 5 increases activity.
2. Alkyl group at position 1 also increases antimicrobial activity.
3. Even aryl group at position 1 also increases antimicrobial activity
4. Substitution at position 2thio with cyclohexyl decreases antimicrobial activity
5. Substitution at position 2thio with phenyl increases antimicrobial activity.

Scheme 1 for the synthesis benzimidazole (aliphatic substitutions at position 1)

Antimicrobial activity

The cup plate method was used in this work to test all the produced compounds for antibacterial activity. Four microorganisms were used to test the compounds' antibacterial properties i.e. *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhii*. For antibacterial tests, yeast was cultivated overnight at 30°C in agar and bacterial strains were done so in broth at 37°C.

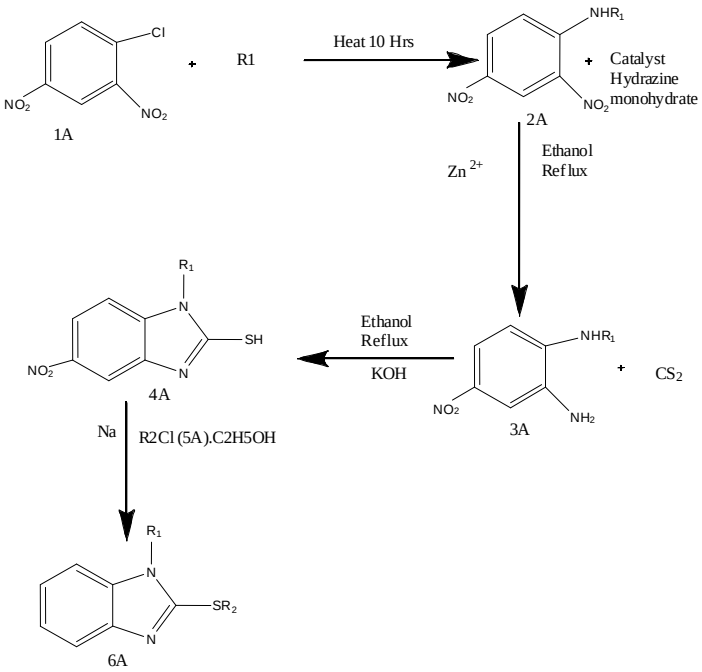
Minimum inhibitory concentration (MIC)

The cup plate dilution method was used to determine the compound's MIC.[22] Graded amounts of the test compounds were added to a prescribed amount of molten sterile agar in a stock solution of the produced chemical in dimethyl formamide. The MIC was regarded as the lowest amount of the test material demonstrating no discernible microbial or fungal growth on the plate.

CONCLUSION

A number of novel benzimidazoles were created from 1-chloro 2,4 dinitrobenzene using a multistep reaction synthesis with the goal of creating powerful antibacterial agents. These compounds were then examined using FT-IR, 1H-NMR, mass spectroscopy, and elemental analysis. The agar plate dilution method was used to test all of the title compounds for their in vitro antibacterial activity, and its MIC was calculated against various types of microbes. According to the findings, substances with an electron-withdrawing group at the phenyl group attached 1 position exhibited stronger antibacterial properties than substances with an electron-releasing group. Moreover, the propyl butyl derivatives also displayed reasonable activity. 5 nito1-phenyl 2-thiophenyl benzimidazole outperformed other evaluated substances in terms of activity

S. NO	R1	R2
6A	CH_3	nC_4H_9Cl
7A	C_2H_5	nC_4H_9Cl
8A	C_3H_7	nC_4H_9Cl
9A	C_4H_9	nC_4H_9Cl
10A	CH_3	C_6H_5Cl
11A	C_2H_5	C_6H_5Cl
12A	C_3H_7	C_6H_5Cl
13A	C_4H_9	C_6H_5Cl



Scheme 2 for the synthesis benzimidazole (aromatic substitutions at position 2

S.NO	R3	R^4
6B	C_6H_5	n-C_4H_9Cl
7B	C_6H_5	C_6H_5Cl
8B	C_6H_{12}	n-C_4H_9Cl
9B	C_6H_{12}	C_3H_7Cl
10B	C_6H_5	C_2H_5Br
11B	C_6H_{12}	C_6H_5Cl
12B	C_6H_{12}	CH_3Br
13B	C_6H_{12}	$C_6H_{12}Cl$

Table 1 Minimum inhibitory concentration in µg/mL of the synthesized compounds(6A-13B)

Compounds	***S. aureus***	***E.coli***	***P. aeruginosa***	***S. typhi***
6A	125	31.25	125	62.5
7A	31.25	62.5	31.25	15.62
8A	**15.62**	**15.62**	**7.81**	15.62
9A	**15.62**	**15.62**	15.62	31.25
10A	31.25	31.25	62.5	15.62
11A	125	31.25	31.25	31.25
12A	62.5	62.5	125	62.5
13A	62.5	125	62.5	31.25
6B	31.25	**15.62**	31.62	15.62
7B	**15.62**	**15.62**	**7.81**	**3.9**
8B	31.25	15.62	15.62	7.81
9B	62.5	31.62	15.62	7.81

10B	**15.62**	31.62	15.62	15.62
11B	31.62	**15.62**	7.81	7.81
12B	**15.62**	**31.62**	**7.81**	**3.9**
13B	125	62.5	62.5	7.81
Ciprofloxacin	15.62	15.62	7.81	3.9

REFERENCES

1. Puerto AS, Fernández JG, Del Castillo JD, Pino MJ, Angulo GP. In vitro activity of β-lactam and non–β-lactam antibiotics in extended-spectrum β-lactamase–producing clinical isolates of Escherichia coli. Diagnostic microbiology and infectious disease. 2006 Feb 1;54 (2):135-9.
2. Nolan CM, Chalhub EG, Nash DG, Yamauchi T. Treatment of bacterial meningitis with intravenous amoxicillin. Antimicrobial agents and chemotherapy. 1979 Aug; 16 (2):171-5.
3. Beekmann SE, Heilmann KP, Richter SS, García-de-Lomas J, Doern GV, GRASP Study Group. Antimicrobial resistance in Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis and group A β-haemolytic streptococci in 2002–2003: Results of the multinational GRASP Surveillance Program. International journal of antimicrobial agents. 2005 Feb 1; 25(2):148-56.
4. Spellberg B, Guidos R, Gilbert D, Bradley J, Boucher HW, Scheld WM, Bartlett JG, Edwards Jr J, Infectious Diseases Society of America. The epidemic of antibiotic-resistant infections: a call to action for the medical community from the Infectious Diseases Society of America. Clinical infectious diseases. 2008 Jan 15;46(2):155-64.
5. Chen JP, Battini N, Ansari MF, Zhou CH. Membrane active 7-thiazoxime quinolones as novel DNA binding agents to decrease the genes expression and exert potent anti-methicillin-resistant Staphylococcus aureus activity. European Journal of Medicinal Chemistry. 2021 May 5; 217:113340.
6. Alp M, Göker H, Brun R, Yıldız S. Synthesis and antiparasitic and antifungal evaluation of 2′-arylsubstituted-1H, 1′ H-[2, 5′] bisbenzimidazolyl-5-carboxamidines. European journal of medicinal chemistry. 2009 May 1; 44 (5):2002-8.
7. GÖKER A, Ayhan-Kilcigil G, Tuncbilek M, KUŞ C, Ertan R, Kendi E, Oezbey S, Fort M, Garcia C, Farré A. Synthesis and antihistaminic H1 activity of 1, 2, 5 (6)-trisubstituted benzimidazoles Heterocycles. 1999;51(11).
8. Nakano H, Inoue T, Kawasaki N, Miyataka H, Matsumoto H, Taguchi T, Inagaki N, Nagai H, Satoh T. Synthesis and biological activities of novel antiallergic agents with 5-lipoxygenase inhibiting action. Bioorganic & medicinal chemistry. 2000 Feb 1;8(2):373-80.
9. Hu Z., Ou L., Li S., Yang. *Medicinal C* L.*Chemistry Research.* 2014,**23**; 3029.
10. Akhtar MJ, Siddiqui AA, Khan AA, Ali Z, Dewangan RP, Pasha S, Yar MS. Design, synthesis, docking and QSAR study of substituted benzimidazole linked oxadiazole as cytotoxic agents, EGFR and erbB2 receptor inhibitors. European Journal of Medicinal Chemistry. 2017 Jan 27;126:853-69.
11. Demirel S, Kilcigil GA, Zümra KA, Güven B, Beşikci AO. Synthesis and pharmacologic evaluation of some benzimidazole acetohydrazide derivatives as EGFR inhibitors. Turkish Journal of Pharmaceutical Sciences. 2017 Dec;14(3):285.:
12. Cheong JE, Zaffagni M, Chung I, Xu Y, Wang Y, Jernigan FE, Zetter BR, Sun L. Synthesis and anticancer activity of novel water soluble benzimidazole carbamates. European journal of medicinal chemistry. 2018 Jan 20;144:372-85.

13. Kuş C, Ayhan-Kılcıgil G, Özbey S, Kaynak FB, Kaya M, Çoban T, Can-Eke B. Synthesis and antioxidant properties of novel N-methyl-1, 3, 4-thiadiazol-2-amine and 4-methyl-2H-1, 2, 4-triazole-3 (4H)-thione derivatives of benzimidazole class. Bioorganic & medicinal chemistry. 2008 Apr 15;16(8):4294-303.
14. Ayhan- Kılcıgil G, Gürkan S, Çoban T, Özdamar ED, Can- Eke B. Synthesis and Evaluation of Antioxidant Properties of Novel 2- [2- (4- chlorophenyl) benzimidazole- 1- yl]- N- (2- arylmethylene amino) acetamides and 2- [2- (4- chlorophenyl) benzimidazole- 1- yl]- N- (4- oxo- 2- aryl- thiazolidine- 3- yl) acetamides- I. Chemical biology & drug design. 2012 May;79(5):869-77.:
15. Ayhan- Kilcigil G, Kuş C, Çoban T, Özdamar ED, Can- Eke B. Identification of a Novel Series of N- P henyl- 5- [(2- phenylbenzimidazol- 1- yl) methyl]- 1, 3, 4- oxadiazol- 2- amines as Potent Antioxidants and Radical Scavengers. Archiv der Pharmazie. 2014 Apr;347(4):276-82.
16. Munuswamy H, Thirunavukkarasu T, Rajamani S, Elumalai EK, Ernest D. A review on antimicrobial efficacy of some traditional medicinal plants in Tamilnadu. Journal of Acute Disease. 2013 Jan 1;2(2):99-105.
17. Birajdar SS, Hatnapure GD, Keche AP, Kamble VM. Synthesis and biological evaluation of amino alcohol derivatives of 2-methylbenzimidazole as antitubercular and antibacterial agents. J. Chem. Pharm. Res. 2013;5(11):583-9.
18. Birajdar SS, Hatnapure GD, Keche AP, Kamble VM. Synthesis and biological evaluation of amino alcohol derivatives of 2-methylbenzimidazole as antitubercular and antibacterial agents. J. Chem. Pharm. Res. 2013;5(11):583-9..
19. Maste MM, Jeyarani P, Kalekar MC, Bhat AR. Synthesis and Evaluation of Benzimidazole Derivatives for Anti-tubercular and Antimicrobial Activities. Asian Journal of Research in Chemistry. 2011;4(7):1055-8.
20. Maste MM, Jeyarani P, Kalekar MC, Bhat AR. Synthesis and Evaluation of Benzimidazole Derivatives for Anti-tubercular and Antimicrobial Activities. Asian Journal of Research in Chemistry. 2011;4(7):1055-8.
21. Mukesh C.S.. *Drug Designing*. 2015,**4**; 1.
22. Mehta MR, Satish B. Design, synthesis and biological activity of some benzimidazole-benzthiazole carbohydrazide derivatives. Journal of Current Chemical and Pharmaceutical Sciences. 2015;5(2):75-86.
23. Gupta SK, Kumar N, Pathak D. Synthesis and biological evaluation of 2-substituted phenyl-1-(substituted piperazin-1-yl) methyl)-1H-benzo [d] imidazoles. Indian drugs. 2013 Jan;50(1):50-8.
24. Sen A, Batra A. Evaluation of antimicrobial activity of different solvent extracts of medicinal plant: Melia azedarach L. Int J Curr Pharm Res. 2012 Mar;4(2):67-73.
25. Laib DE, Benzehra A, Rahmani Y, Boulaouad BA, Akkal S. L'emploi de l'extrait du champignon endophyte Aspergillus niger isolé à partir des feuilles du ricin commun Ricinus communis L.(Euphorbiaceae, Malpighiales) comme agent de lutte biologique contre le criquet migrateur Locusta migratoria. Journal of Applied Biosciences. 2022 Feb 28;170(1):17720-38.