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Research Article

SYNTHESIS, CHARACTERIZATION AND *IN VITRO* ANTIMICROBIAL EVALUATION OF SOME NOVEL BENZIMIDAZOLE DERIVATIVES BEARING HYDRAZONE MOIETY

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ABSTRACT

Objective: The synthesis of novel benzimidazole-hydrazone derivatives has been carried out based on the previous findings that both these pharmacophores possess potent antimicrobial activities. The antibacterial properties of synthesized derivatives were screened against both Gram-positive and Gram-negative bacteria.

Methods: *O*-phenylenediamine on condensation with substituted aromatic acids in polyphosphoric acid gave benzimidazole nucleus which on reaction with ethyl chloroacetate and hydrazine hydrate in two different steps resulted in the formation of substituted acetohydrazides. The targeted compounds 6a-l were synthesized by reaction of substituted acetohydrazides with aromatic aldehydes and screened for their antibacterial potential by cup-plate method.

Results: The synthesized benzimidazole-hydrazones exhibited moderate to strong antibacterial activities against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli*, and *Pseudomonas aeruginosa.* The compounds 6a-6f were found to be most effective against *S. aureus, E. coli,* and *P. aeruginosa.* Among all the synthesized compounds, the zone of inhibition of 6f in highest concentration, i.e., 100 µg/ml were found to be >31 mm against all the stains of bacteria.

Conclusion: The antibacterial results revealed that the synthetized derivatives have significant antimicrobial properties and further structure activity relationship studies may develop more potent and less toxic molecules.

Keywords: Benzimidazole, Hydrazone, Antimicrobial, Antibacterial.

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INTRODUCTION

Resistance to commercially available antimicrobial agents such as β-lactam antibiotics, quinolones, and macrolides is a major health problem worldwide [1]. Infections caused by multidrug-resistant microorganisms are responsible for significant increase in morbidity and mortality, prolonged treatment period, and increased health-care cost [2]. Due to seriousness of this problem, the World Health Organisation in 2011 has selected "antimicrobial resistance: No action today no cure tomorrow" as the theme for World Health Day [3,4].

One way to counteract the challenge of microbial resistance is judicious use of currently available antibiotics; the other is the development of new anti-ineffective agents with enhanced activity and a novel mechanism of action [5,6]. The bacteria exhibit phenotypic resistance, i.e., tolerance, rendering them to 1000-fold more resistant to commercially available antibiotics, is due to their ability to grow in sessile or adherent state which leads to formation of biofilms [7].

Benzimidazole is an important pharmacophore and privileged structure in the field of medicinal chemistry because of its varied biological activities, *viz.,* anticancer [8,9], antihypertensive [10,11], antiviral [12,13], anti-inflammatory [14,15], antihistaminic [16,17], antiulcer [18], anticoagulant [19,20], and antimicrobial [21,22]. The antimicrobial action of benzimidazole is due to its structural similarity to purines; therefore, its derivatives inhibit nucleic acid and protein synthesis by competing with natural purines; thereby inhibit the growth as well as kill bacterial strains [23].

Hydrazones constitute another important class of pharmacologically active drug molecules which has attracted the attention of medicinal chemists due to their diverse biological activities such as analgesic, anthelmintic, anticonvulsant, antidepressant, anti-inflammatory, antimalarial, anticancer, antiviral, and antibacterial [24]. Some commercially available antibacterial drugs such as furacilin, furazolidone, ftivazide, nifuroxazide, nirofurazone, and nitrofurantoin are known to contain hydrazone group [25-27].

Due to the chemotherapeutic potential of both benzimidazole and hydrazone compounds, it was hypothesized that it would be worthwhile to synthesize novel molecules having both these pharmacophores in a single chemical entity. Furthermore, extensive literature search also suggested that only a limited efforts have been made to combine both these vital moieties in a single scaffold [1,28]. These findings boosted us to synthesis novel benzimidazole-hydrazone derivatives as antimicrobial agents.

METHODS

Chemistry

The chemicals used in the synthesis were procured from Merck, Sigma-Aldrich, and Loba Chemie, India. All the solvents were of commercial grade and distilled before use. Open capillary method was used to determine the melting points (m.p) of the synthesized derivatives using digital melting point apparatus (Popular, India). The progress of reactions was monitored by thin layer chromatography on silica gel F_{254} plates with visualization by ultraviolet or iodine vapors. The molecular structures of all the synthesized derivatives were confirmed by elemental analysis, infrared (IR), ^1H nuclear magnetic resonance (NMR) as well as mass spectra. The IR spectra (in KBr pellet) were recorded on FTIR-8400S (Shimadzu) spectrophotometer. Bruker

Avance II (400 MHz) spectrometer was used to record ^{1}H NMR and chemical shifts were given in δ (ppm) scale. Mass spectra were obtained with Waters Q-Tof micromass spectrometer, and elemental analysis was performed on a Thermo Flash 2000 analyzer.

The general strategy for synthesis of benzimidazole-hydrazone derivatives is illustrated in Scheme 1.

Experimental

Synthesis of 2-(substituted phenyl)-1H-benzimidazole 3a-d

An equimolar quantities of *o*-phenylenediamine 1 (0.05 mol) and substituted benzoic acid 2a-d (0.05 mol) were dissolved in poly phosphoric acid (15 g) and refluxed for 6-9 hrs at 180-185°C with constant stirring. The reaction mixtures were cooled and slowly poured into 200 g of crushed ice with constant stirring. The pH of the reaction mixtures were adjusted to alkaline by adding 4 N of anhydrous sodium carbonate. The precipitated solids were filtered under pressure, washed with distilled water and recrystallized from ethanol [29,30].

2-Phenyl-benzimidazole 3a

IR (KBr, cm^{-1}): 3404 (N-H sec.), 3047 (C-H str. Ar), 1664 (N=C), 1591 and 1462 (C=C), 1276 (C-N), 750 and 690 (mono subst. oop); ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.17-7.21 (t, 2H, benzimidazole), 7.40-7.50 (m, 3H, phenyl), 7.60-7.68 (d, 2H, phenyl), 8.20-8.22 (d, 2H, benzimidazole), 12.83 (s, NH); ES-MS (*m/z*): 195 [M+1]; Anal. calcd for $C_{13}H_{10}N_2$: C, 80.39; H, 5.19; N, 14.42. Found: C, 80.21; H, 5.20; N, 14.59.

2-(2-Hydroxyphenyl)-1H-benzimidazole 3b

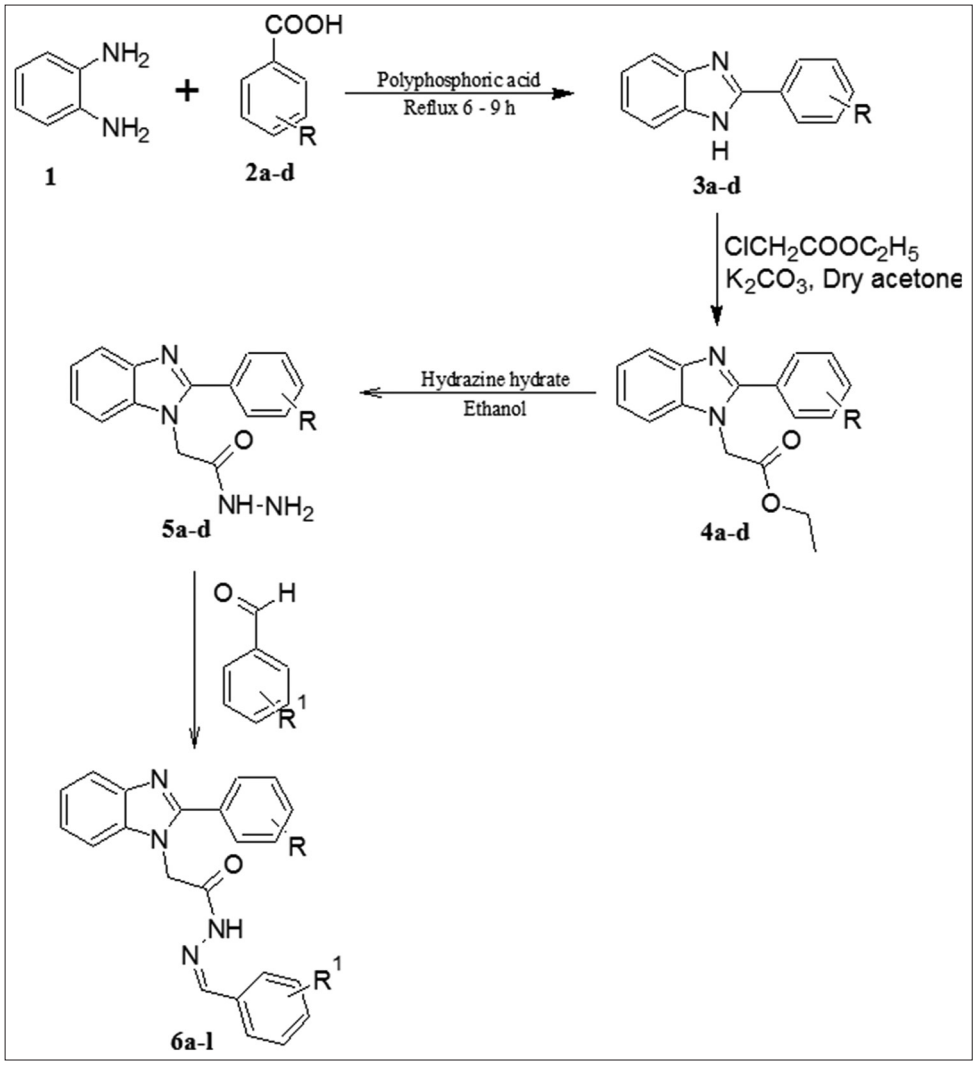
IR (KBr, cm^{-1}): 3321 (N-H sec.), 3225 (O-H), 3070 (C-H str. Ar), 1626 (N=C), 1606 and 1489 (C=C), 1246 (C-N), 750 (ortho subst. oop); ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 6.88-6.90 (t, 1H, 2-hydroxyphenyl), 6.95-6.97 (d, 1H, 2-hydroxyphenyl), 7.19-7.21 (m, 2H, benzimidazole), 7.27-7.29 (t, 1H, 2-hydroxyphenyl), 7.55-7.56 (s, 1H, OH), 7.93-7.95 (d, 2H, benzimidazole), 12.74 (s, NH); ES-MS (*m/z*): 211 [M+1]; Anal. calcd for $C_{13}H_{10}N_2O$: C, 74.27; H, 4.79; N, 13.33. Found: C, 74.21; H, 4.72; N, 13.49.

2-(2-Chlorophenyl)-1H-benzimidazole 3c

IR (KBr, cm^{-1}): 3061 (N-H sec.), 3047 (C-H str. Ar), 1589 & 1442 (C=C), 1317 (C-N), 750 (ortho subst. oop); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.28-7.37 (m, 2H, benzimidazole), 7.40-7.48 (m, 2H, 2-chlorophenyl), 7.51-7.54 (dd, 1H, 2-chlorophenyl), 7.71-7.73 (m, 2H, benzimidazole), 8.45-8.48 (dd, 1H, 2-chlorophenyl), 12.73 (s, NH); ES-MS (*m/z*): 229 [M+1]; Anal. calcd for $C_{13}H_9N_2Cl$: C, 68.28; H, 3.97; N, 12.25. Found: C, 68.34; H, 3.91; N, 12.27.

2-(3-Chlorophenyl)-1H-benzimidazole 3d

IR (KBr, cm^{-1}): 3045 (N-H sec.), 1541 and 1442 (C=C), 1317 (C-N), 895, 744 and 680 (meta subst. oop); ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.17-7.17 (dd, 2H, benzimidazole), 7.39-7.41 (m, 2H, 3-chlorophenyl), 7.52-7.53 (m, 2H, benzimidazole), 8.08-8.11 (d, 1H, 3-chlorophenyl), 8.20-8.21 (s, 1H, 3-chlorophenyl), 12.83 (s, NH); ES-MS (*m/z*): 229 [M+1]; Anal. calcd for $C_{13}H_9N_2Cl$: C, 68.28; H, 3.97; N, 12.25. Found: C, 68.37; H, 3.84; N, 12.24.



Scheme 1: Synthesis of benzimidazole-hydrazone derivatives 6a-l

Synthesis of ethyl-2-[2-(substituted phenyl)-1H-benzimidazol-1-yl]acetate 4a-d

Ethylchloroacetate (0.01 mol) was added to a solution of 2-(substituted)-*1H*-benzimidazole 3a-d (0.01 mol) in dry acetone (40 ml), followed by addition of anhydrous potassium carbonate (2 g). The reaction mixture was refluxed for 10-12 hrs. The solvent was removed under vacuum by rotary evaporator and the residue was recrystallized from ethanol to give 4a-d [31-33].

Ethyl-2-(2-phenyl-1H-benzimidazol-1-yl)acetate 4a

IR (KBr, cm^{-1}): 3047 (C-H str. ArH), 1742 (C=O), 1683 (C=N), 1622 and 1462 (C=C), 1276 (C-N), 1226 (C-O-C), 742 and 704 (mono subst. oop); ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.18 (m, 3H, CH_3), 4.3 (m, 2H, CH_2), 4.7 (m, 2H, OCH_2) 7.13-7.17 (m, 2H, benzimidazole), 7.36-7.45 (m, 3H, phenyl), 7.53-7.56 (m, 2H, phenyl), 8.12-8.14 (d, 2H, benzimidazole); ES-MS (*m/z*): 281 [M+1]; Anal. calcd for $C_{17}H_{16}N_2O_2$: C, 72.84; H, 5.75; N, 9.99. Found: C, 72.89; H, 5.84; N, 9.76.

Ethyl-2-{2-(2-hydroxyphenyl)-1H-benzimidazol-1-yl}acetate 4b

IR (KBr, cm^{-1}): 3246 (O-H), 3051 (C-H str. ArH), 1735 (C=O), 1629 (C=N), 1589 and 1417 (C=C), 1274 (C-N), 1250 (C-O-C), 746 (ortho subst. oop); ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.19 (m, 3H, CH_3), 4.16 (m, 2H, CH_2), 4.7 (m, 2H, OCH_2), 7.14-7.19 (m, 2H, benzimidazole), 7.38-7.48 (m, 4H, 2-hydroxyphenyl), 7.53-7.56 (m, 2H, benzimidazole), 7.9-8.0 (s, 1H, OH); ES-MS (*m/z*): 297 [M+1]; Anal. calcd for $C_{17}H_{16}N_2O_3$: C, 68.91; H, 5.44; N, 9.45. Found: C, 68.76; H, 5.36; N, 9.38.

Ethyl-2-{2-(2-chlorophenyl)-1H-benzimidazol-1-yl}acetate 4c

IR (KBr, cm^{-1}): 3061 (C-H str. ArH), 2966 (C-H str. Aliphatic), 1749 (C=O), 1683 (C=N), 1622 & 1489 (C=C), 1315 (C-N), 1053 (C-O-C), 742 (C-Cl); ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.20 (m, 3H, CH_3), 4.23 (m, 2H, CH_2), 4.9 (m, 2H, OCH_2), 7.16-7.20 (m, 2H, benzimidazole), 7.37-7.44 (m, 2H, 2-chlorophenyl), 7.51-7.52 (m, 1H, 2-chlorophenyl), 7.58-7.61 (m, 2H, benzimidazole), 7.88-7.92 (m, 1H, 2-chlorophenyl); ES-MS (*m/z*): 315 [M+1]; Anal. calcd for $C_{17}H_{15}N_2O_2Cl$: C, 64.87; H, 4.80; N, 8.90. Found: C, 64.64; H, 4.92; N, 8.86.

Ethyl-2-{2-(3-chlorophenyl)-1H-benzimidazol-1-yl}acetate 4d

IR (KBr, cm^{-1}): 3045 (C-H str. ArH), 2964 (C-H str. aliphatic), 1749 (C=O), 1683 (C=N), 1602 & 1471 (C=C), 1317 (C-N), 1124 (C-O-C), 744 (C-Cl); ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.19 (m, 3H, CH_3), 4.21 (m, 2H, CH_2), 4.83 (m, 2H, OCH_2), 7.14-7.19 (m, 2H, benzimidazole), 7.38-7.47 (m, 2H, 3-chlorophenyl), 7.53-7.56 (m, 2H, benzimidazole), 8.07-8.09 (d, 1H, 3-chlorophenyl), 8.19-8.20 (s, 1H, 3-chlorophenyl); ES-MS (*m/z*): 315 [M+1]; Anal. calcd for $C_{17}H_{15}N_2O_2Cl$: C, 64.87; H, 4.80; N, 8.90. Found: C, 64.69; H, 4.98; N, 8.84.

Synthesis of 2-[2-(substituted phenyl)-1H- benzimidazol-1-yl] acetohydrazide 5a-d

Hydrazine hydrate (0.01 mol) was added to ethanolic solution of ethyl [2-(substituted phenyl)-*1H*-benzimidazol-1-yl]acetate 4a-d and the reaction mixture was refluxed for 3-4 hrs. After the completion of reaction, the mixture was cooled in ice bath; the solid obtained was filtered, washed with cold water and recrystallized from methanol.

2-(2-Phenyl-1H-benzimidazol-1-yl)acetohydrazide 5a

IR (KBr, cm^{-1}): 3479 and 3416 (N-H prim.), 3047 (C-H str. ArH), 2986 (C-H str. aliphatic), 1618 (C=O), 1591 & 1475 (C=C), 1276 (C-N), 738 and 704 (mono subst. oop); ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.51 (s, 2H, NH_2), 4.95 (s, 2H, CH_2), 7.13-7.17 (m, 2H, benzimidazole), 7.38-7.47 (m, 3H, phenyl), 7.53-7.56 (m, 2H, phenyl), 8.13-8.15 (d, 2H, benzimidazole), 9.5 (1H, CONH); ES-MS (*m/z*): 267 [M+1]; Anal. calcd for $C_{15}H_{14}N_4O$: C, 67.65; H, 5.30; N, 21.04. Found: C, 67.58; H, 5.19; N, 20.92.

2-{2-(2-Hydroxyphenyl)-1H-benzimidazol-1-yl}acetohydrazide 5b

IR (KBr, cm^{-1}): 3481 and 3414 (N-H prim.), 3236 (O-H), 3057 (C-H str. ArH), 1618 (C=O), 1616 & 1489 (C=C), 1259 (C-N), 738 (ortho subst. oop); ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.50-2.51 (s, 2H, NH_2), 4.85 (s, 2H, CH_2), 6.88-6.96 (m, 2H, benzimidazole), 7.16-7.21 (m, 3H, 2-hydroxyphenyl), 7.27-7.29 (t, 1H, 2-hydroxyphenyl), 7.55 (s, 2H, benzimidazole), 7.94-7.96 (s, 1H, OH), 9.0 (1H, CONH); ES-MS (*m/z*): 283 [M+1]; Anal. calcd for $C_{15}H_{14}N_4O_2$: C, 63.82; H, 5.00; N, 19.85. Found: C, 63.89; H, 5.4; N, 19.42.

2-{2-(2-Chlorophenyl)-1H-benzimidazol-1-yl}acetohydrazide 5c

IR (KBr, cm^{-1}): 3481 and 3416 (N-H prim.), 3061 (C-H str. ArH), 2916 (C-H str. aliphatic), 1616 (C=O), 1591 & 1442 (C=C), 1232 (C-N), 742 (C-Cl), 738; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.50-2.52 (s, 2H, NH_2), 4.78 (s, 2H, CH_2), 7.16-7.20 (m, 2H, benzimidazole), 7.36-7.43 (m, 2H, 2-chlorophenyl), 7.49-7.50 (m, 1H, 2-chlorophenyl), 7.57-7.61 (m, 2H, benzimidazole), 7.88-7.90 (m, 1H, 2-chlorophenyl), 8.98 (1H, CONH); ES-MS (*m/z*): 301 [M+1]; Anal. calcd for $C_{15}H_{13}N_4OCl$: C, 59.91; H, 4.36; N, 18.63. Found: C, 59.85; H, 4.42; N, 18.75.

2-{2-(3-Chlorophenyl)-1H-benzimidazol-1-yl}acetohydrazide 5d

IR (KBr, cm^{-1}): 3481 and 3414 (N-H prim.), 3045 (C-H str. ArH), 2918 (C-H str. aliphatic), 1616 (C=O), 1570 and 1462 (C=C), 1228 (C-N), 742 (C-Cl), 744; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.50-2.51 (s, 2H, NH_2), 4.92 (s, 2H, CH_2), 7.15-7.19 (m, 2H, benzimidazole), 7.40-7.48 (m, 2H, 3-chlorophenyl), 7.54-7.56 (m, 2H, benzimidazole), 8.08-8.10 (d, 1H, 3-chlorophenyl), 8.19-8.20 (s, 1H, 3-chlorophenyl), 9.26 (1H, CONH); ES-MS (*m/z*): 301 [M+1]; Anal. calcd for $C_{15}H_{13}N_4OCl$: C, 59.91; H, 4.36; N, 18.63. Found: C, 59.82; H, 4.53; N, 18.72.

Synthesis of N-(Substituted benzylidene)-2-[(2-(substituted phenyl)-1H-benzimidazol-1-yl) acetohydrazide 6a-l

A mixture of 2-[2-(substituted phenyl)-*1H*- benzimidazol-1-yl] acetohydrazide 5a-d (0.0025 mol), substituted benzaldehyde (0.0025 mol) and glacial acetic acid (few drops) was refluxed for 5 hrs in ethanol (20 ml). After the completion of reaction, the solvent was removed by rotary evaporator and the reaction mixture was cooled and poured in ice-cold water. The precipitates obtained were filtered, dried and recrystallized from ethanol to give benzimidazole-hydrazone derivatives.

N-Benzylidene-2-[2-(phenyl)-1H-benzimidazol-1-yl] acetohydrazide 6a

IR (KBr, cm^{-1}): 3117 (N-H sec.), 3047 (C-H str. ArH), 1668 (C=O), 1622 and 1462 (C=C), 1224 (C-N), 744 and 680 (mono subst. oop); ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 4.62 (s, 2H, CH_2), 7.15-7.17 (m, 2H, benzimidazole), 7.38-7.46 (m, 6H, phenyl), 7.54-7.56 (m, 4H, phenyl), 8.11-8.14 (d, 2H, benzimidazole), 8.65 (s, 1H, N=CH), 11.07 (1H, CONH); ES-MS (*m/z*): 355 [M+1]; Anal. calcd for $C_{22}H_{18}N_4O$: C, 74.56; H, 5.12; N, 15.81. Found: C, 74.42; H, 5.19; N, 15.89.

N-(2-Hydroxybenzylidene)-2-[2-(phenyl)-1H-benzimidazol-1-yl] acetohydrazide 6b

IR (KBr, cm^{-1}): 3163 (N-H sec.), 3047 (C-H str. ArH), 1699 (C=O), 1541 and 1440 (C=C), 1274 (C-N);^{1}H NMR (DMSO-d_6, 400 MHz) δ: 4.73 (s, 2H, CH_2), 7.13-7.17 (m, 2H, benzimidazole), 7.36-7.44 (m, 4H, phenyl), 7.51-7.56 (m, 4H, phenyl), 8.11-8.13 (d, 2H, benzimidazole), 8.72 (s, 1H, N=CH), 10.97 (s, 1H, OH), 11.23 (1H, CONH); ES-MS (*m/z*): 371 [M+1]; Anal. calcd for $C_{22}H_{18}N_4O_2$: C, 71.34; H, 4.90; N, 15.13. Found: C, 71.46; H, 4.82; N, 15.02.

N-(2-Chlorobenzylidene)-2-[2-(phenyl)-1H-benzimidazol-1-yl) acetohydrazide 6c

IR (KBr, cm^{-1}): 3159 (N-H sec.), 3047 (C-H str. ArH), 1653 (C=O), 1626 and 1464 (C=C), 1276 (C-N), 742 (C-Cl); ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 4.69 (s, 2H, CH_2), 7.13-7.18 (m, 2H, benzimidazole), 7.37-7.46 (m,

3H, phenyl), 7.53-7.55 (m, 2H, phenyl), 7.84-7.85 (m, 4H, phenyl), 8.11-8.13 (d, 2H, benzimidazole), 8.63 (s, 1H, N=CH), 11.37 (1H, CONH); ES-MS (*m/z*): 389 [M+1]; Anal. calcd for $C_{22}H_{17}N_4OCl$: C, 67.95; H, 4.41; N, 14.41. Found: C, 67.86; H, 4.46; N, 14.42.

N-Benzylidene-2-{2-(2-hydroxyphenyl)-1H-benzimidazol-1-yl} acetohydrazide 6d

IR (KBr, cm^{-1}): 3234 (O-H), 3061 (C-H str. ArH), 2916 (C-H str. aliphatic), 1651 (C=O), 1629 and 1489 (C=C), 1273 (C-N), 738 (ortho subst. oop); ^1H NMR (DMSO-d_6, 400 MHz) δ: 4.76 (s, 2H, CH_2), 6.88-6.97 (m, 2H, benzimidazole), 7.19-7.23 (m, 3H, phenyl), 7.26-7.27 (d, 1H, phenyl), 7.38-7.40 (m, 3H, phenyl), 7.52-7.56 (m, 2H, benzimidazole), 7.62-7.63 (m, 2H, phenyl), 7.97-7.99 (s, 1H, OH), 8.69 (s, 1H, N=CH), 11.27 (1H, CONH); ES-MS (*m/z*): 371 [M+1]; Anal. calcd for $C_{22}H_{18}N_4O_2$: C, 71.34; H, 4.90; N, 15.13. Found: C, 71.38; H, 4.91; N, 15.01.

N-(2-Hydroxybenzylidene)-2-{2-(2-hydroxyphenyl)-1H-benzimidazol-1-yl} acetohydrazide 6e

IR (KBr, cm^{-1}): 3238 (O-H), 3059 (C-H str. ArH), 2918 (C-H str. aliphatic), 1629 (C=O), 1591 and 1489 (C=C), 1259 (C-N), 738 (ortho subst. oop); ^1H NMR (DMSO-d_6, 400 MHz) δ: 4.84 (s, 2H, CH_2), 6.89-6.96 (m, 2H, benzimidazole), 7.18-7.23 (m, 3H, phenyl), 7.26-7.27 (d, 1H, phenyl), 7.38-7.44 (m, 4H, phenyl), 7.54-7.56 (m, 2H, benzimidazole), 7.95-7.98 (s, 2H, OH), 8.54 (s, 1H, N=CH), 11.03 (1H, CONH); ES-MS (*m/z*): 387 [M+1]; Anal. calcd for $C_{22}H_{18}N_4O_3$: C, 68.38; H, 4.70; N, 14.50. Found: C, 68.42; H, 4.62; N, 14.42.

N-(2-Chlorobenzylidene)-2-{2-(2-hydroxyphenyl)-1H-benzimidazol-1-yl}acetohydrazide 6f

IR (KBr, cm^{-1}): 3238 (O-H), 3061 (C-H str. ArH), 2918 (C-H str. aliphatic), 1629 (C=O), 1591 & 1489 (C=C), 1259 (C-N), 738 (ortho subst. oop); ^1H NMR (DMSO-d_6, 400 MHz) δ: 4.62 (s, 2H, CH_2), 6.89-6.96 (m, 2H, benzimidazole), 7.19-7.22 (m, 3H, phenyl), 7.26-7.30 (d, 1H, phenyl), 7.32-7.41 (m, 4H, phenyl), 7.56-7.58 (m, 2H, benzimidazole), 7.95-7.98 (s, 1H, OH), 8.21 (s, 1H, N=CH), 10.98 (1H, CONH); ES-MS (*m/z*): 405 [M+1]; Anal. calcd for $C_{22}H_{17}N_4O_2Cl$: C, 65.27; H, 4.23; N, 13.84. Found: C, 65.22; H, 4.19; N, 13.62.

N-Benzylidene-2-{2-(2-chlorophenyl)-1H-benzimidazol-1-yl} acetohydrazide 6g

IR (KBr, cm^{-1}): 3242 (N-H sec.), 3061 (C-H str. ArH), 2916 (C-H str. aliphatic), 1651 (C=O), 1626 & 1491 (C=C), 1273 (C-N), 746 (C-Cl); ^1H NMR (DMSO-d_6, 400 MHz) δ: 4.32 (s, 2H, CH_2), 7.17-7.19 (m, 2H, benzimidazole), 7.37-7.44 (m, 6H, phenyl), 7.49-7.51 (d, 1H, phenyl), 7.58-7.60 (m, 2H, benzimidazole), 7.87-7.90 (d, 2H, phenyl), 8.12 (s, 1H, N=CH), 10.84 (1H, CONH); ES-MS (*m/z*): 389 [M+1]; Anal. calcd for $C_{22}H_{17}N_4OCl$: C, 67.95; H, 4.41; N, 14.41. Found: C, 67.79; H, 4.37; N, 14.42.

N-(2-Hydroxybenzylidene)-2-{2-(2-chlorophenyl)-1H-benzimidazol-1-yl}acetohydrazide 6h

IR (KBr, cm^{-1}): 3254 (O-H), 3061 (C-H str. ArH), 2916 (C-H str. aliphatic), 1651 (C=O), 1624 & 1489 (C=C), 1274 (C-N), 742 (C-Cl); ^1H NMR (DMSO-d_6, 400 MHz) δ: 4.52 (s, 2H, CH_2), 7.16-7.20 (m, 2H, benzimidazole), 7.36-7.43 (m, 7H, phenyl), 7.47-7.51 (d, 1H, phenyl), 7.57-7.61 (m, 2H, benzimidazole), 7.88-7.90 (s, 1H, OH), 8.32 (s, 1H, N=CH), 11.15 (1H, CONH); ES-MS (*m/z*): 405 [M+1]; Anal. calcd for $C_{22}H_{17}N_4O_2Cl$: C, 65.27; H, 4.23; N, 13.84. Found: C, 65.12; H, 4.34; N, 13.71.

N-(2-Chlorobenzylidene)-2-{2-(2-chlorophenyl)-1H-benzimidazole-1-yl}acetohydrazide 6i

IR (KBr, cm^{-1}): 3157 (N-H sec.), 3061 (C-H str. ArH), 2918 (C-H str. aliphatic), 1651 (C=O), 1622 and 1442 (C=C), 1273 (C-N), 746 (C-Cl); ^1H NMR ($CDCl_3$, 400 MHz) δ: 4.56 (s, 2H, CH_2), 7.32-7.35 (m, 2H, benzimidazole), 7.42-7.45 (m, 7H, phenyl), 7.57-7.61 (m, 2H, benzimidazole), 7.70-7.71 (d, 1H, phenyl), 8.45-8.50 (s, 1H, N=CH), 11.27 (1H, CONH); ES-MS (*m/z*): 424 [M+1]; Anal. calcd for $C_{22}H_{16}N_4OCl_2$: C, 62.42; H, 3.81; N, 13.24. Found: C, 62.38; H, 3.91; N, 13.36.

N-Benzylidene-2-{2-(3-chlorophenyl)-1H-benzimidazol-1-yl} acetohydrazide 6j

IR (KBr, cm^{-1}): 3159 (N-H sec.), 3043 (C-H str. ArH), 2918 (C-H str. aliphatic), 1651 (C=O), 1591 & 1469 (C=C), 1284 (C-N), 744 (C-Cl); ^1H NMR (DMSO-d_6, 400 MHz) δ: 4.51 (s, 2H, CH_2), 7.15-7.19 (m, 2H, benzimidazole), 7.40-7.49 (m, 8H, phenyl), 7.54-7.57 (m, 2H, benzimidazole), 8.08-8.10 (d, 1H, phenyl), 8.19-8.20 (s, 1H, N=CH), 10.98 (1H, CONH); ES-MS (*m/z*): 389 [M+1]; Anal. calcd for $C_{22}H_{17}N_4OCl$: C, 67.95; H, 4.41; N, 14.41. Found: C, 67.84; H, 4.46; N, 14.44.

N-(2-Hydroxybenzylidene-2-{2-(3-chlorophenyl)-1H-benzimidazol-1-yl}acetohydrazide 6k

IR (KBr, cm^{-1}): 3161 (O-H), 3043 (C-H str. ArH), 2918 (C-H str. aliphatic), 1622 (C=O), 1572 and 1467 (C=C), 1228 (C-N), 744 (C-Cl); ^1H NMR (DMSO-d_6, 400 MHz) δ: 4.59 (s, 2H, CH_2), 7.14-7.19 (m, 2H, benzimidazole), 7.38-7.47 (m, 7H, phenyl), 7.53-7.56 (m, 2H, benzimidazole), 8.07-8.09 (d, 1H, phenyl), 8.19-8.20 (s, 1H, N=CH), 11.02 (1H, CONH); ES-MS (*m/z*): 405 [M+1]; Anal. calcd for $C_{22}H_{17}N_4O_2Cl$: C, 65.27; H, 4.23; N, 13.84. Found: C, 65.52; H, 4.12; N, 13.76.

N-(2-Chlorobenzylidene-2-{2-(3-chlorophenyl)-1H-benzimidazol-1-yl}acetohydrazide 6l

IR (KBr, cm^{-1}): 3159 (N-H), 3043 (C-H str. ArH), 2964 (C-H str. aliphatic), 1651 (C=O), 1572 & 1440 (C=C), 1359 (C-N), 744 (C-Cl), 895 and 680 (meta oop); ^1H NMR (DMSO-d_6, 400 MHz) δ: 4.74 (s, 2H, CH_2), 7.16-7.20 (m, 2H, benzimidazole), 7.42-7.51 (m, 7H, phenyl), 7.55-7.57 (m, 2H, benzimidazole), 8.08-8.11 (d, 1H, phenyl), 8.20 (s, 1H, N=CH), 11.12 (1H, CONH); ES-MS (*m/z*): 424 [M+1]; Anal. calcd for $C_{22}H_{16}N_4OCl_2$: C, 62.42; H, 3.81; N, 13.24. Found: C, 62.19; H, 3.83; N, 13.12.

The physiochemical characterization of synthesized derivatives is presented in Table 1.

Antimicrobial activity

All the synthesized benzimidazole-hydrazone derivatives 6a-6l were screened for their *in vitro* antibacterial activity against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli,* and *Pseudomonas aeruginosa* using agar diffusion method (cup plate method). Ciprofloxacin was used as standard drugs for antibacterial activity. Three different concentrations (25, 50 and 100 µg/ml) of synthesized derivatives were used to evaluate antimicrobial potential, and the results have been summarized in Table 2.

RESULTS AND DISCUSSION

2-(Substituted phenyl)-*1H*-benzimidazole 3a-d was synthesized by refluxing *o*-phenylenediamine 1 with appropriately substituted benzoic acid 2a-d in the presence of polyphosphoric acid. The compounds 3a-d on further reaction with ethylchloroacetate in the presence of base and dry acetone gave ethyl-2-[2-(substituted phenyl)-*1H*-benzimidazol-1-yl]acetate 4a-d. In the next step, substituted esters reacted with hydrazine hydrate to form 2-[2-(substituted phenyl)-*1H*- benzimidazol-1-yl]acetohydrazide 5a-d. Finally, benzimidazole-hydrazone derivatives 6a-l were synthesized by treatment of 5a-d with substituted aldehydes in the presence of ethanol and little acetic acid.

Formulae of the synthesized compounds were confirmed by elemental analysis, and their chemical structures were elucidated by IR, ^1H NMR and electrospray mass spectrometry spectra. In the IR spectra, the vibrational bands due to N-H, C=O, C=C and C=N vibrations appeared in the expected regions. The formation of benzimidazole nucleus was confirmed by the appearance of single band of sec. N-H str. vibrations at 3045-3404 cm^{-1}. The characteristic C=O str. vibrations at 1735-1749 and 1610-1620 confirmed the formation of ester 4a-4d and amide 5a-

Table 1: Physiochemical characteristics 3a-d, 4a-d, 5a-d and 6a-d

Compound number	R	R^1	Molecular formula	Molecular weight	M.p (°C)	R_f value[a]	Yield (%)
3a	-H	-	$C_{13}H_{10}N_2$	194.23	294-296	0.73	82.63
3b	2-OH	-	$C_{13}H_{10}N_2O$	210.23	239-241	0.67	69.34
3c	2-Cl	-	$C_{13}H_9N_2Cl$	228.67	230-231	0.71	72.12
3d	3-Cl	-	$C_{13}H_9N_2Cl$	228.67	232-234	0.69	68.42
4a	-H	-	$C_{17}H_{16}N_2O_2$	280.32	253-255	0.71	58.21
4b	2-OH	-	$C_{17}H_{16}N_2O_3$	296.32	253-255	0.74	62.47
4c	2-Cl	-	$C_{17}H_{15}N_2O_2Cl$	314.76	249-251	0.76	77.26
4d	3-Cl	-	$C_{17}H_{15}N_2O_2Cl$	314.76	248-250	0.79	71.24
5a	-H	-	$C_{15}H_{14}N_4O$	266.29	262-264	0.71	65.58
5b	2-OH	-	$C_{15}H_{14}N_4O_2$	282.29	268-280	0.76	69.23
5c	2-Cl	-	$C_{15}H_{13}N_4OCl$	300.74	273-275	0.74	73.27
5d	3-Cl	-	$C_{15}H_{13}N_4OCl$	300.74	267-269	0.72	68.27
6a	-H	-H	$C_{22}H_{18}N_4O$	354.40	277-279	0.85	74.81
6b	-H	2-OH	$C_{22}H_{18}N_4O_2$	370.40	276-278	0.83	75.61
6c	-H	2-Cl	$C_{22}H_{17}N_4OCl$	388.84	279-281	0.79	77.61
6d	2-OH	-H	$C_{22}H_{18}N_4O_2$	370.40	271-273	0.77	78.56
6e	2-OH	2-OH	$C_{22}H_{18}N_4O_3$	386.40	278-280	0.75	81.06
6f	2-OH	2-Cl	$C_{22}H_{17}N_4O_2Cl$	404.84	281-283	0.79	84.06
6g	2-Cl	-H	$C_{22}H_{17}N_4OCl$	388.84	283-285	0.74	73.86
6h	2-Cl	2-OH	$C_{22}H_{17}N_4O_2Cl$	404.84	268-271	0.86	62.94
6i	2-Cl	2-Cl	$C_{22}H_{16}N_4OCl_2$	423.29	277-279	0.76	78.96
6j	3-Cl	-H	$C_{22}H_{17}N_4OCl$	388.84	272-274	0.79	57.27
6k	3-Cl	2-OH	$C_{22}H_{17}N_4O_2Cl$	404.84	277-279	0.76	59.67
6l	3-Cl	2-Cl	$C_{22}H_{16}N_4OCl_2$	423.29	279-281	0.81	64.57

[a]TLC mobile phase:hexane:ethyl acetate:methanol (6:3.5:0.5)

Table 2: Antibacterial evaluation of benzimidazole-hydrazone derivatives 6a-l

Bacterial strain	*S. aureus*			*B. subtilis*			*E. coli*			*P. aeruginosa*		
Conc. (µg/ml)	25	50	100	25	50	100	25	50	100	25	50	100
Compound number	Zone of inhibition (mm)											
6a	20	29	31	13	17	21	19	23	26	21	28	31
6b	23	26	27	20	26	28	17	26	32	17	23	27
6c	22	28	31	14	19	21	21	26	29	17	26	32
6d	20	27	32	19	28	29	22	27	31	19	24	26
6e	21	29	34	16	23	27	23	29	33	25	29	31
6f	25	32	37	17	29	31	25	32	34	26	32	33
6g	15	19	21	11	17	19	15	21	25	17	21	23
6h	16	18	22	13	18	22	12	15	19	15	19	20
6i	15	19	22	15	19	23	13	18	22	18	22	23
6j	11	16	21	16	19	21	14	17	21	16	20	22
6k	15	20	23	19	22	24	11	14	18	15	19	23
6l	15	19	23	16	18	21	10	15	17	16	20	25
Ciprofloxacin	19	21	23	23	27	29	23	25	29	23	25	27

S. aureus: Staphylococcus aureus, B. subtilis: Bacillus subtilis, E. coli: Escherichia coli, P. aeruginosa: Pseudomonas aeruginosa

5d derivatives, respectively. The out-of-plane bending vibrations that appeared in a range of 650-750 were used to assign substitution on aromatic ring. The ^{1}H NMR spectra showed amino proton of secondary amine at 12.73-12.84 ppm in compounds 3a-3d which confirmed the formation of benzimidazole nucleus. The aromatic protons of phenyl ring at 2nd position of benzimidazole nucleus appeared at 7.1-8.1 ppm in all the derivatives with variable multiplicity. The protons of ethyl chain attached to ester functional group in 4a-4d were observed at around 1.18 and 4.2 ppm respectively. The formation of hydrazides 5a-5d and benzimidazole-hydrazone derivatives 6a-6l was confirmed by appearance of NH protons in expected regions. Elemental analysis results were found to be satisfactory in all the compounds. In mass spectra, M+1 peaks in all the compounds were in agreement with their molecular formula.

The synthesized titled compounds exhibited moderate to strong antibacterial activity against both Gram-positive and Gram-negative bacteria. The compound having 2-hydroxyphenyl ring at 2nd position and 2-chloro substituent on phenyl ring at 1st position of benzimidazole nucleus 6f was found to be most active against all the stains of bacteria exhibiting zone of inhibition of 37, 31, 34, and 33 mm at concentration of 100 µg/ml against *S. aureus, B. subtilis, E. coli,* and *P. aeruginosa,* respectively. The chloro substituent at *ortho* and *meta* positions of phenyl ring attached to 2nd position of benzimidazole nucleus decreased the antibacterial potency of molecules against both Gram-positive and Gram-negative bacteria in dose-dependent manner. The compounds 6a-6f have emerged as the most effective antimicrobial agents against *S. aureus, E. coli,* and *P. aeruginosa,* whereas compounds 6b was the most active against *B. subtilis.*

CONCLUSION

A novel series of *N*-(substituted benzylidene)-2-[(2-(substituted phenyl)-*1H*-benzimidazol-1-yl) acetohydrazide derivatives 6a-l had been synthesized and characterized by IR, NMR, mass and elemental analysis. The final compounds were screened for *in vitro* antibacterial activity against both Gram-positive and Gram-negative strains of bacteria by cup-plate method. Among the various derivative, the compounds 6a-6f excellent inhibition of bacterial growth as compared to standard drug ciprofloxacin.

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