SYNTHESIS AND CHARACTERIZATION OF NOVEL ANTIBACTERIAL AGENTS

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ABSTRACT:

O-phenylenediamine on condensation with substituted aromatic acids in polyphosphoric acid confer benzimidazole nucleus which over reaction with ethyl chloroacetate. Synthesis of 2-(substituted phenyl)-1H-benzimidazole 3a-d An equiable quantities of o-phenylenediamine 1 (0.05 mol) and substituted benzoic acid 2a-d (0.05 mol) were fluxed in poly phosphoric acid (15 g) and refluxed for 6-9 hrs at 180-185°C with constant exhilarating. The reaction mixtures were chilled and slowly poured into 200 g of crashed ice with constant exhilarating. 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 investigation. The final compounds were evaluated for *in vitro* antibacterial activity across both Gram-positive and Gram-negative strains of bacteria through cup-plate method. Among the different derivative, the compounds 6a-6f great inhibition of bacterial growth as analysed to standard drug ciprofloxacin.

KEYWORDS: Benzimidazole, Synthesis, Antibacterial Agents.

INTRODUCTION:

Resistance to commercially available antimicrobial agents like quinolones, β-lactam antibiotics, and macrolides is a big health problem worldwide (Ozkay Y *et al.* 2015). Due to the chemotherapeutic probable of benzimidazole compound, it is deliberated that it would-be worthwhile to synthesize novel molecules having pharmacophores in a individual chemical entity. The prevalence of drug resistant bacteria is developing at an startling rate in both developing and developed countries. From this account alone, it

should be clear that the demand for the development of novel antibacterial agents is of utmost importance. There exists a perpetual need for new antibiotics. Most drugs will be just as effective in the future as they are today, but that is not the case with antibiotics. Eventually, the inevitable rise of resistance will erode the utility of today's antibiotics (Fischbach *et al.* 2009).

`Since 1998, the FDA has approved a number of new antibiotics. However, only a limited number of these agents possess a novel mechanism of action. Having new antibiotics approved for use is a great thing, but those antibiotics which utilize the same mechanism of action as previously approved drugs always run the risk of increasing the rate of resistance.

MATERIAL AND METHODS:

O-phenylenediamine on condensation with substituted aromatic acids in polyphosphoric acid give benzimidazole nucleus which on reaction with ethyl chloroacetate.

2-(Substituted phenyl)-1H-benzimidazole 3a-d will be 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-benzimidazol1-yl] acetate 4a-d. In the next step, substituted esters reacted with hydrazine hydrate to form 2-[2-(substituted phenyl)-1H- benzimidazol1-yl] acetohydrazide 5a-d. Finally, benzimidazole-hydrazone derivatives 6a-l will be synthesized by treatment of 5a-d with substituted aldehydes in the presence of ethanol and little acetic acid.

Chemistry: The chemicals used in the synthesis were procured from Merck, SigmaAldrich, 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 F254 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 were recorded on FTIR 8400S (Shimadzu) spectrophotometer.

RESULTS:

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.

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); 1H NMR (DMSO-d6, 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, 50.19; N,14.42.

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); 1H NMR (DMSO-d6, 400 MHz) δ: 6.88-6.90 (t, 1H, 2-hydroxyphenyl), 6.956.97 (d, 1H, 2-hydroxyphenyl), 7.19-7.21 (m, 2H, benzimidazole), 7.277.29 (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 C13H10N2O: 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); 1H NMR (CDCl3, 400 MHz) δ: 7.287.37 (m, 2H, benzimidazole), 7.40-7.48 (m, 2H, 2-chlorophenyl), 7.517.54 (dd, 1H, 2-chlorophenyl), 7.71-7.73 (m, 2H, benzimidazole), 8.458.48 (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.

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); 1H NMR (DMSO-d6, 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 C13H9N2Cl: C, 68.28; H, 3.97; N, 12.25. Found: C, 68.37; H, 3.84; N, 12.24.

Synthesis of ethyl-2-[2-(substituted phenyl)-1H-benzimidazol-1yl]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.

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); 1H NMR (DMSO-d6, 400 MHz) δ: 1.18 (m, 3H, CH3), 4.3 (m, 2H, CH2), 4.7 (m, 2H, OCH2) 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 $C_{17}H_{16}N_2O2$: 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); 1H NMR (DMSO-d6, 400 MHz) δ: 1.19 (m, 3H, CH3), 4.16 (m, 2H, CH2), 4.7 (m, 2H, OCH2), 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 C17H16N2O3: 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); 1H NMR (DMSO-d6, 400 MHz) δ: 1.20 (m, 3H, CH3), 4.23 (m, 2H, CH2), 4.9 (m, 2H, OCH2), 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 C17H15N2O2Cl: 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); 1H NMR (DMSO-d6, 400 MHz) δ: 1.19 (m, 3H, CH3), 4.21 (m, 2H, CH2), 4.83 (m, 2H, OCH2), 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 C17H15N2O2Cl: 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); 1H NMR (DMSO-d6, 400 MHz) δ: 2.51 (s, 2H, NH2), 4.95 (s, 2H, CH2), 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 C15H14N4O:

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); 1H NMR (DMSO-d6, 400 MHz) δ: 2.50-2.51 (s, 2H, NH2), 4.85 (s, 2H, CH2), 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 C15H14N4O2: 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; 1H NMR (DMSO-d6, 400 MHz) δ: 2.50-2.52 (s, 2H, NH2), 4.78 (s, 2H, CH2), 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 C15H13N4OCl: 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; 1H NMR (DMSO-d6, 400 MHz) δ: 2.50-2.51 (s, 2H, NH2), 4.92 (s, 2H, CH2), 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 C15H13N4OCl: 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); 1H NMR (DMSO-d6, 400 MHz) δ: 4.62 (s, 2H, CH2), 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 C22H18N4O: 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);1H NMR (DMSO-d6, 400 MHz) δ: 4.73 (s, 2H, CH2), 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 C22H18N4O2: 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); 1H NMR (DMSO-d6, 400 MHz) δ: 4.69 (s, 2H, CH2), 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), 8.118.13 (d, 2H, benzimidazole), 8.63 (s, 1H, N=CH), 11.37 (1H, CONH); ESMS (m/z): 389 [M+1]; Anal. calcd for C22H17N4OCl: 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-d6, 400 MHz) δ: 4.76 (s, 2H, CH2), 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 C22H18N4O2: 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)-1Hbenzimidazol-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-d6, 400 MHz) δ: 4.84 (s, 2H, CH2), 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 C22H18N4O3: 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)-1Hbenzimidazol-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-d6, 400 MHz) δ: 4.62 (s, 2H, CH2), 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-d6, 400 MHz) δ: 4.32 (s, 2H, CH2), 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 C22H17N4OCl: 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)-1Hbenzimidazol-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-d6, 400 MHz) δ: 4.52 (s, 2H, CH2), 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 C22H17N4O2Cl: 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)-1Hbenzimidazole-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 (CCl); 1H NMR (CDCl3, 400 MHz) δ: 4.56 (s, 2H, CH2), 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 C22H16N4OCl2: 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 (CCl); 1H NMR (DMSO-d6, 400 MHz) δ: 4.51 (s, 2H, CH2), 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 C22H17N4OCl: 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)-1Hbenzimidazol-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 (CCl); 1H NMR (DMSO-d6, 400 MHz) δ: 4.59 (s, 2H, CH2), 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 C22H17N4O2Cl: 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)-1Hbenzimidazol-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-d6, 400 MHz) δ: 4.74 (s, 2H, CH2), 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 C22H16N4OCl2: C, 62.42; H, 3.81; N, 13.24. Found: C, 62.19; H, 3.83; N, 13.12.

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.

Physiochemical characteristics 3a-d, 4a-d, 5a-d and 6a-d

Comp	R	R1	Molecular	Molecular	M.p (°C)	Rf	Yield

ound numb er			formula	weight		valuea	(%)
3a	-H	-	C13H10N2	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 -		C15H14N4O	266.29	262-264	0.71	65.58
5b	2-OH -		C15H14N4O 2	282.29	268-280	0.76	69.23
5c	2-Cl -		C15H13N4O Cl	300.74	273-275	0.74	73.27
5d	3-Cl -		C15H13N4O Cl	300.74	267-269	0.72	68.27
6a	-H	-H	C22H18N4O	354.40	277-279	0.85	74.81
6b	-H	2-O H	C22H18N4O 2	370.40	276-278	0.83	75.61
6c	-H	2-Cl	C22H17N4O Cl	388.84	279-281	0.79	77.61
6d	2-OH	-H	C22H18N4O 2	370.40	271-273	0.77	78.56

TLC mobile phase= hexan: ethyl acetate: methanol (6:3.5:0.5)

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-benzimidazol1-yl]acetate 4a-d. In the next step, substituted esters reacted with hydrazine hydrate to form 2-[2-(substituted phenyl)-1H- benzimidazol1-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 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 1H 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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