SUPPORTING INFORMATION

Studies on antimicrobial evaluation of some 1-((1-(1*H*-benzo[*d*]imidazol-2-yl)ethylidene)amino)-6-((arylidene)amino)-2-oxo-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitriles

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1. EXPERIMENTAL

1-((1-(1*H*-benzo[*d*]imidazol-2-yl)ethylidene)amino)-6-((2-hydroxybenzylidene)amino)-2-oxo-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile (4b)

Dark brown amorphous (EtOH). Yield: 75%; m.p. 228-230 °C; IR (KBr, cm⁻¹): 3444 (OH), 3332 (-NH, benzimidazole), 3052 (C-H, aromatic), 2923 (C-H, CH₃), 2212 (CN), 1685 (CO), 1642 (C=C), 1562 (C=N); ¹H NMR (400 MHz, DMSO-*d*₆, ppm): δ=1.31 (s, 3H, CH₃), 6.62-7.45 (m, 13H, Ar-H), 5.70 (s, 1H, OH), 9.27 (s, 1H, CH=N), 12.85 (s, 1H, -NH benzimidazole); ¹³C NMR (100 MHz, DMSO-*d*₆, ppm): δ=169.6, 163.7, 161.2, 160.2, 155.6, 153.1, 151.5, 134.8, 133.0, 132.5, 132.1, 128.9, 128.6, 127.9, 123.4, 121.4, 118.5, 117.8, 115.9, 115.5, 115.0, 114.8, 13.7; LCMS (ESI): m/z 497.16 [M⁺]. Anal. Calcd For (C₂₉H₁₉N₇O₂): C, 70.01; H, 3.85; N, 19.71%. Found: C, 69.78; H, 3.42; N, 19.56%.

1-((1-(1*H*-benzo[*d*]imidazol-2-yl)ethylidene)amino)-6-((3-hydroxybenzylidene)amino)-2-oxo-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile (4c)

Light brown amorphous (EtOH). Yield: 73%; m.p. 234-236 °C; IR (KBr, cm⁻¹): 3440 (OH), 3333 (-NH, benzimidazole), 3051 (C-H, aromatic), 2924 (C-H, CH₃), 2213 (CN), 1683 (CO), 1644 (C=C), 1563 (C=N); ¹H NMR (400 MHz, DMSO-*d*₆, ppm): δ=1.33 (s, 3H, CH₃), 6.63-7.42 (m, 13H, Ar-H), 5.74 (s, 1H, OH), 9.24 (s, 1H, CH=N), 12.87 (s, 1H, -NH benzimidazole); ¹³C NMR (100 MHz, DMSO-*d*₆, ppm): δ=169.5, 163.7, 160.1, 158.6, 155.5, 153.2, 151.5, 134.9, 135.0, 132.5, 130.2, 128.9, 128.6, 127.9, 123.0, 121.8, 118.3, 115.8, 115.2, 115.0, 114.8, 13.7; LCMS (ESI): m/z 497.16 [M⁺]. Anal. Calcd For (C₂₉H₁₉N₇O₂): C, 70.01; H, 3.85; N, 19.71%. Found: C, 69.23; H, 3.22; N, 19.06%.

1-((1-(1*H*-benzo[*d*]imidazol-2-yl)ethylidene)amino)-6-((4-hydroxybenzylidene)amino)-2-oxo-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile (4d)

Brown amorphous (EtOH). Yield: 79%; m.p. 228-230 °C; IR (KBr, cm⁻¹): 3444 (OH), 3335 (-NH, benzimidazole), 3053 (C-H, aromatic), 2925 (C-H, CH₃), 2215 (CN), 1684 (CO), 1642 (C=C), 1562 (C=N); ¹H NMR (400 MHz, DMSO-*d*₆, ppm): δ=1.33 (s, 3H, CH₃), 6.62-8.10 (m, 13H, Ar-H), 5.57 (s, 1H, OH), 9.25 (s, 1H, CH=N), 12.89 (s, 1H, -NH benzimidazole); ¹³C NMR (100 MHz, DMSO-*d*₆, ppm): δ=169.5, 163.7, 160.8, 160.0, 155.6, 153.2, 151.5, 134.9, 132.4, 130.6, 128.9, 128.6, 127.8, 123.0, 116.0, 115.8, 115.3, 115.2, 114.8, 13.6; LCMS (ESI): m/z: 497.16 [M⁺]. Anal. Calcd For (C₂₉H₁₉N₇O₂): C, 70.01; H, 3.85; N, 19.71%. Found: C, 69.55; H, 3.52; N, 19.46%.

1-((1-(1*H*-benzo[*d*]imidazol-2-yl)ethylidene)amino)-6-((2-nitrobenzylidene)amino)-2-oxo-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile (4e)

Reddish brown crystal (EtOH). Yield: 74%; m.p. 237-239 °C; IR (KBr, cm⁻¹): 3336 (-NH, benzimidazole), 3052 (C-H, aromatic), 2928 (C-H, CH₃), 2218 (CN), 1685 (CO), 1640 (C=C), 1561 (C=N), 1528, 1326 (N=O, Ar-NO₂); ¹H NMR (400 MHz, DMSO-*d*₆, ppm): δ=1.31 (s, 3H, CH₃), 6.60-8.28 (m, 13H, Ar-H), 9.27 (s, 1H, CH=N), 12.86 (s, 1H, -NH benzimidazole); ¹³C NMR (100 MHz, DMSO-*d*₆, ppm): δ=169.4, 163.7, 160.0, 155.6, 153.2, 151.5, 147.8, 134.9, 132.5, 131.9, 130.2, 128.9, 128.6, 128.4, 127.8, 124.0, 123.0, 115.8, 115.3, 115.2, 114.8, 13.6; LCMS (ESI): m/z 526.15 [M⁺]. Anal. Calcd For (C₂₉H₁₈N₈O₃): C, 66.16; H, 3.45; N, 21.28%. Found: C, 63.25; H, 3.20; N, 20.46%.

1-((1-(1*H*-benzo[*d*]imidazol-2-yl)ethylidene)amino)-6-((3-nitrobenzylidene)amino)-2-oxo-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile (4f)

Reddish brown crystal (EtOH). Yield: 69%; m.p. 238-240 °C; IR (KBr, cm^{-1}): 3335 (-NH, benzimidazole), 3050 (C-H, aromatic), 2930 (C-H, CH_3), 2223 (CN), 1684 (CO), 1640 (C=C), 1563 (C=N), 1524, 1325 (N=O, Ar- NO_2); ^1H NMR (400 MHz, $\text{DMSO}-d_6$, ppm): δ =1.32 (s, 3H, CH_3), 6.65-8.26 (m, 13H, Ar-H), 9.26 (s, 1H, $\text{CH}=\text{N}$), 12.80 (s, 1H, -NH benzimidazole); ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$, ppm): δ =169.6, 163.8, 160.1, 155.7, 153.2, 151.6, 148.2, 135.3, 134.9, 134.6, 132.6, 131.9, 129.6, 128.9, 128.6, 128.4, 127.8, 124.0, 123.0, 115.8, 115.3, 115.2, 114.8, 13.6; LCMS (ESI): m/z 526.15 [M^+]. Anal. Calcd For ($\text{C}_{29}\text{H}_{18}\text{N}_8\text{O}_3$): C, 66.16; H, 3.45; N, 21.28%. Found: C, 65.25; H, 3.32; N, 20.30%.

1-((1-(1*H*-benzo[*d*]imidazol-2-yl)ethylidene)amino)-6-((4-nitrobenzylidene)amino)-2-oxo-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile (4g)

Reddish brown crystal (EtOH). Yield: 72%; m.p. 230-232 °C; IR (KBr, cm^{-1}): 3338 (-NH, benzimidazole), 3053 (C-H, aromatic), 2935 (C-H, CH_3), 2228 (CN), 1684 (CO), 1640 (C=C), 1561 (C=N), 1526, 1329 (N=O, Ar- NO_2); ^1H NMR (400 MHz, $\text{DMSO}-d_6$, ppm): δ =1.31 (s, 3H, CH_3), 6.60-8.20 (m, 13H, Ar-H), 9.28 (s, 1H, $\text{CH}=\text{N}$), 12.87 (s, 1H, -NH benzimidazole); ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$, ppm): δ =169.4, 163.7, 160.0, 155.5, 153.2, 151.7, 150.2, 139.8, 134.9, 132.6, 131.9, 128.9, 128.6, 128.4, 127.9, 127.8, 124.0, 123.1, 115.8, 115.3, 115.2, 114.8, 13.7; LCMS (ESI): m/z 526.15 [M^+]. Anal. Calcd For ($\text{C}_{29}\text{H}_{18}\text{N}_8\text{O}_3$): C, 66.16; H, 3.45; N, 21.28%. Found: C, 66.02; H, 3.20; N, 21.01%.

1-((1-(1*H*-benzo[*d*]imidazol-2-yl)ethylidene)amino)-6-((2-chlorobenzylidene)amino)-2-oxo-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile (4h)

Pale yellow amorphous (EtOH). Yield: 72%; m.p. 234-236 °C; IR (KBr, cm^{-1}): 3336 (-NH, benzimidazole), 3052 (C-H, aromatic), 2936 (C-H, CH_3), 2229 (CN), 1686 (CO), 1642 (C=C),

1562 (C=N), 756 (C-Cl); ^1H NMR (400 MHz, DMSO- d_6 , ppm): δ =1.33 (s, 3H, CH₃), 6.61-7.97 (m, 13H, Ar-H), 9.29 (s, 1H, CH=N), 12.84 (s, 1H, -NH benzimidazole); ^{13}C NMR (100 MHz, DMSO- d_6 , ppm): δ =169.5, 163.7, 160.1, 155.6, 153.2, 151.6, 134.9, 133.9, 133.4, 132.6, 132.4, 131.9, 128.9, 128.6, 128.4, 127.9, 127.2, 126.2, 123.1, 115.8, 115.4, 115.2, 114.7, 13.7; LCMS (ESI): m/z 515.13 [M^+]. Anal. Calcd For (C₂₉H₁₈ClN₇O): C, 67.51; H, 3.52; N, 19.00%. Found: C, 66.25; H, 3.34; N, 18.24%.

1-((1-(1*H*-benzo[*d*]imidazol-2-yl)ethylidene)amino)-6-((3-chlorobenzylidene)amino)-2-oxo-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile (4i)

Lemon yellow (EtOH). Yield: 73%; m.p. 239-241 °C; IR (KBr, cm⁻¹): 3335 (-NH, benzimidazole), 3054 (C-H, aromatic), 2938 (C-H, CH₃), 2231 (CN), 1687 (CO), 1641 (C=C), 1560 (C=N), 759 (C-Cl); ^1H NMR (400 MHz, DMSO- d_6 , ppm): δ =1.31 (s, 3H, CH₃), 6.62-7.99 (m, 13H, Ar-H), 9.28 (s, 1H, CH=N), 12.85 (s, 1H, -NH benzimidazole); ^{13}C NMR (100 MHz, DMSO- d_6 , ppm): δ =169.4, 163.7, 160.0, 155.6, 153.2, 151.5, 135.1, 134.9, 134.4, 132.6, 131.1, 130.2, 128.9, 128.6, 128.4, 127.9, 127.3, 123.1, 115.8, 115.4, 115.2, 114.7, 13.7; LCMS (ESI) M/Z : 515.13 [M^+]. Anal. Calcd For C₂₉H₁₈ClN₇O: C, 67.51; H, 3.52; N, 19.00%. Found: C, 66.00; H, 3.21; N, 18.10%.

1-((1-(1*H*-benzo[*d*]imidazol-2-yl)ethylidene)amino)-6-((4-chlorobenzylidene)amino)-2-oxo-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile (4j)

Dark yellow crystal (EtOH). Yield: 68%; m.p. 223-225 °C; IR (KBr, cm⁻¹): 3338 (-NH, benzimidazole), 3052 (C-H, aromatic), 2936 (C-H, CH₃), 2234 (CN), 1687 (CO), 1641 (C=C), 1561 (C=N), 761 (C-Cl); ^1H NMR (400 MHz, DMSO- d_6 , ppm): δ =1.31 (s, 3H, CH₃), 6.64-7.97

(m, 13H, Ar-H), 9.26 (s, 1H, CH=N), 12.84 (s, 1H, -NH benzimidazole); ^{13}C NMR (100 MHz, DMSO- d_6 , ppm): δ =169.4, 163.7, 160.0, 155.6, 153.2, 151.5, 136.6, 134.9, 132.5, 131.8, 130.6, 128.9, 128.6, 127.9, 123.1, 115.8, 115.4, 115.2, 114.7, 13.7; LCMS (ESI): m/z 515.13 [M^+]. Anal. Calcd For ($\text{C}_{29}\text{H}_{18}\text{ClN}_7\text{O}$): C, 67.51; H, 3.52; N, 19.00%. Found: C, 67.10; H, 3.34; N, 18.75%.

1-((1-(1*H*-benzo[d]imidazol-2-yl)ethylidene)amino)-6-((2-methylbenzylidene)amino)-2-oxo-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile (4k)

Light yellow crystal (EtOH). Yield: 70%; m.p. 237-239 °C; IR (KBr, cm^{-1}): 3335 (-NH, benzimidazole), 3054 (C-H, aromatic), 2931 (C-H, CH_3), 2230 (CN), 1682 (CO), 1638 (C=C), 1569 (C=N); ^1H NMR (400 MHz, DMSO- d_6 , ppm): δ =1.32 (s, 3H, CH_3), 2.50 (s, 3H, CH_3), 6.62-7.67 (m, 13H, Ar-H), 9.24 (s, 1H, CH=N), 12.82 (s, 1H, -NH benzimidazole); ^{13}C NMR (100 MHz, DMSO- d_6 , ppm): δ =169.4, 163.6, 160.0, 155.6, 153.2, 151.5, 135.6, 134.9, 132.5, 131.1, 130.9, 129.0, 128.9, 128.6, 127.9, 126.5, 125.5, 123.1, 115.8, 115.4, 115.2, 114.7, 18.9, 13.7; LCMS (ESI): m/z 495.18 [M^+]. Anal. Calcd For ($\text{C}_{30}\text{H}_{21}\text{N}_7\text{O}$): C, 72.71; H, 4.27; N, 19.79%. Found: C, 71.12; H, 4.02; N, 18.86%.

1-((1-(1*H*-benzo[d]imidazol-2-yl)ethylidene)amino)-6-((3-methylbenzylidene)amino)-2-oxo-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile (4l)

Yellow amorphous (EtOH). Yield: 70%; m.p. 227-229 °C; IR (KBr, cm^{-1}): 3334 (-NH, benzimidazole), 3052 (C-H, aromatic), 2927 (C-H, CH_3), 2231 (CN), 1682 (CO), 1638 (C=C), 1562 (C=N); ^1H NMR (400 MHz, DMSO- d_6 , ppm): δ =1.31 (s, 3H, CH_3), 2.52 (s, 3H, CH_3), 6.67-7.69 (m, 13H, Ar-H), 9.26 (s, 1H, CH=N), 12.85 (s, 1H, -NH benzimidazole); ^{13}C NMR (100 MHz, DMSO- d_6 , ppm): δ =169.4, 163.7, 160.0, 155.6, 153.2, 151.5, 138.5, 134.9, 133.6,

132.5, 131.3, 130.9, 129.4, 128.9, 128.7, 128.6, 127.9, 126.2, 123.0, 115.8, 115.4, 115.2, 114.7, 21.3, 13.7; LCMS (ESI): m/z 495.18 [M^+]. Anal. Calcd For ($C_{30}H_{21}N_7O$): C, 72.71; H, 4.27; N, 19.79%. Found: C, 70.23; H, 3.86; N, 18.54%.

1-((1-(1*H*-benzo[*d*]imidazol-2-yl)ethylidene)amino)-6-((4-methylbenzylidene)amino)-2-oxo-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile (4m)

Light Yellowish amorphous (EtOH). Yield: 78%, m.p. 223-225 °C; IR (KBr, cm^{-1}): 3337 (-NH, benzimidazole), 3050 (C-H, aromatic), 2929 (C-H, CH_3), 2230 (CN), 1684 (CO), 1637 (C=C), 1562 (C=N); 1H NMR (400 MHz, DMSO- d_6 , ppm): δ =1.33 (s, 3H, CH_3), 2.52 (s, 3H, CH_3), 6.60-7.38 (m, 13H, Ar-H), 9.24 (s, 1H, CH=N), 12.83 (s, 1H, -NH benzimidazole); ^{13}C NMR (100 MHz, DMSO- d_6 , ppm): δ =169.5, 163.6, 160.0, 155.5, 153.2, 151.5, 140.7, 134.9, 132.5, 130.7, 129.1, 128.9, 128.6, 127.9, 123.0, 115.8, 115.4, 115.2, 114.8, 21.3, 13.7; LCMS (ESI): m/z 495.18 [M^+]. Anal. Calcd For ($C_{30}H_{21}N_7O$): C, 72.71; H, 4.27; N, 19.79%. Found: C, 71.85; H, 4.01; N, 19.57%.

1-((1-(1*H*-benzo[*d*]imidazol-2-yl)ethylidene)amino)-6-((4-methoxybenzylidene)amino)-2-oxo-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile (4n)

Light cream amorphous (EtOH). Yield: 68%; m.p. 236-238 °C; IR (KBr, cm^{-1}): 3334 (-NH, benzimidazole), 3058 (C-H, aromatic), 2929 (C-H, CH_3), 2852 (C-H, OCH_3), 2223 (CN), 1679 (CO), 1632 (C=C), 1564 (C=N); 1H NMR (400 MHz, DMSO- d_6 , ppm): δ =1.33 (s, 3H, CH_3), 3.83 (s, 3H, OCH_3), 6.65-8.28 (m, 13H, Ar-H), 9.26 (s, 1H, CH=N), 12.85 (s, 1H, -NH benzimidazole); ^{13}C NMR (100 MHz, DMSO- d_6 , ppm): δ =169.4, 163.7, 162.9, 160.0, 155.6, 153.2, 151.5, 134.9, 132.5, 130.2, 128.9, 128.6, 127.9, 126.0, 123.0, 115.8, 115.4, 115.2, 114.8,

114.4, 55.9, 13.7; LCMS (ESI): m/z 511.18 $[M^+]$. Anal. Calcd For ($C_{30}H_{21}N_7O_2$): C, 70.44; H, 4.14; N, 19.17%. Found: C, 69.55; H, 4.05; N, 18.41%.

1-((1-(1*H*-benzo[d]imidazol-2-yl)ethylidene)amino)-2-oxo-4-phenyl-6-((3,4,5-trimethoxybenzylidene)amino)-1,2-dihydropyridine-3,5-dicarbonitrile (4o)

Light orange crystal (EtOH). Yield: 70%; m.p. 219-221 °C; IR (KBr, cm^{-1}): 3336 (-NH, benzimidazole), 3055 (C-H, aromatic), 2927 (C-H, CH_3), 2857 (C-H, OCH_3), 2226 (CN), 1679 (CO), 1631 (C=C), 1568 (C=N); 1H NMR (400 MHz, $DMSO-d_6$, ppm): δ =1.33 (s, 3H, CH_3), 3.74-3.85 (s, 9H, OCH_3), 6.67-8.22 (m, 11H, Ar-H), 9.24 (s, 1H, CH=N), 12.84 (s, 1H, -NH benzimidazole); ^{13}C NMR (100 MHz, $DMSO-d_6$, ppm): δ =169.6, 163.6, 160.0, 155.6, 153.2, 151.5, 141.5, 134.9, 132.5, 128.9, 128.6, 128.0, 127.9, 123.0, 115.8, 115.4, 115.2, 114.8, 104.2, 60.9, 56.1, 13.7; LCMS (ESI): m/z 571.20 $[M^+]$. Anal. Calcd For ($C_{32}H_{25}N_7O_4$): C, 67.24; H, 4.41; N, 17.15%. Found: C, 66.87; H, 4.15; N, 16.95%.

2. Antibacterial assay

The newly synthesized compounds (**4a–o**) were screened for their antibacterial activity against Gram positive (*Staphylococcus aureus* (MTCC-96), *Streptococcus pyogenes* (MTCC-442), and Gram-negative bacteria *Escherichia coli* (MTCC-443), *Pseudomonas aeruginosa* (MTCC-1688) at different concentrations of 1000, 500, 200, 100, 50, 25, 12.5 $\mu g\ ml^{-1}$. Several of the recently synthesized compounds (**4a–o**) are found to exhibit moderate to excellent antimicrobial activity. On the basis of antibacterial screening results, All MTCC cultures were collected from the Institute of Microbial Technology, Chandigarh. Antibacterial activity was carried out by serial broth dilution method.^[33-36] The standard strains used for the antimicrobial activity was procured from Institute of Microbial Technology, Chandigarh. The drugs which were found to be active in

primary screening were similarly diluted to obtain 100, 50, 25, 12.5 $\mu\text{g ml}^{-1}$ concentrations. 10 $\mu\text{g ml}^{-1}$ suspensions were further inoculated on appropriate media and growth was noted after 24 and 48 h. The lowest concentration preventing appearance of turbidity was considered as minimum inhibitory concentration (MIC, $\mu\text{g ml}^{-1}$) i.e., the amount of growth from the control tube before incubation (which represents the original inoculum) was compared. A set of tubes containing only seeded broth and solvent controls were maintained under identical conditions so as to make sure that the solvent had no influence on strain growth. The standard drug used in this study was ‘ampicillin’ for evaluating antibacterial activity which showed (100, 100, 250, and 100 $\mu\text{g ml}^{-1}$) MIC against *E. coli*, *P. aeruginosa*, *S. aureus*, and *S. pyogene*, respectively (Table 1).

3. Antifungal assay

The newly synthesized compounds (**4a–o**) were screened for their antifungal activity against in six sets against *C. albicans*, *A. niger* and *A. clavatus* at various concentrations of 1000, 500, 250, and 100 $\mu\text{g ml}^{-1}$. The compounds found to be active in primary screening were similarly diluted to obtain 200, 125, 100, 62.5, 50, 25 and 12.5 $\mu\text{g ml}^{-1}$ concentrations for secondary screening to test in a second set of dilution against all microorganisms. Griseofulvin was used as a standard drug for antifungal activity, which shows 500, 100 and 100 $\mu\text{g ml}^{-1}$ MIC against *C. albicans*, *A. niger* and *A. clavatus*, respectively (Table 1). For fungal growth, in the present protocol, we have used Sabourauds dextrose broth at 28 °C in aerobic condition for 48 h.

4. IR, ^1H NMR and Mass Spectral files of compound

IR spectra of compound 4a

¹H NMR spectra of compound 4a

Mass spectra of compound 4a

¹H NMR spectra of compound 4b

¹H NMR spectra of compound 4d

Mass spectra of compound 4d

¹H NMR spectra of compound 4f

¹H NMR spectra of compound 4g

¹H NMR spectra of compound 4m

¹³C NMR spectra of compound 4m

Mass spectra of compound 4m

¹H NMR spectra of compound 4n

¹³C NMR spectra of compound 4n

Mass spectra of compound 4n