

Research Article

Synthesis of novel 2-substituted benzimidazole derivatives as potential anti microbial agents

Panneer Selvam, T. ^{1*}, P. P. Radhika¹, S. Janagaraj¹, A. Siva Kumar²

¹Department of Pharmaceutical Chemistry, Srinivas College of Pharmacy, Valachil, Mangalore -574143, Karnataka, India

²School of Bio Science and Technology, VIT University, Vellore-632014 Tamil Nadu, India

*Corresponding author email: t.panneerselvam@gmail.com

In the present study, a novel series of 2-substituted benzimidazole derivatives were synthesized and characterized by means of IR, ¹H-NMR, ¹³C-NMR, mass spectral and elemental analysis. The compounds were screened for antibacterial (*Staphylococcus aureus* ATCC9144, *Staphylococcus epidermidis* ATCC 155, *klebsiella pneumoniae* ATCC 29665 and *Esherichia coli* ATCC 25922) and antifungal (*Candida albicans* ATCC 2091 and *Aspergillus niger* ATCC 9029) activities. The Minimum Inhibitory Concentrations was determined by agar streak dilution method. 1-(4-(1H-benzo[d]imidazol-2-yl)phenyl)-3-chloro-4-(4-nitro phenyl)azetidin-2-one (3a) was found to exhibit the most potent *in vitro* antimicrobial activity with MIC of 15, 17, 19, 9, 11 and 15 µg/mL against *E.coli*, *K.pneumoniae*, *S.aureus*, *S.epidermidis*, *C.albicans* and *A.niger* respectively. All the other compounds exhibited moderate activity against the bacterial and fungal organism tested.

Keywords: Benzimidazoles; Antibacterial; Antifungal.

Benzimidazoles were reported to poses anticancer (Nare et al., 1994), antitubercular (Khyati et al., 2000, Jitendar et al., 2002), angiotensin-II receptor antagonists (Kohara et al., 1996) and antimicrobial properties (Davidet al., 2004). The azetidinone ring bearing compounds showed varied biological activities like antibacterial, antifungal and antitubercular activities and the thiazolidinone ring bearing compounds exhibited anticancer, antimicrobial, anti inflammatory & analgesic activities (Shiva et al 1981). The wide range of therapeutic value of these nucleuses promoted us to synthesize compounds comprised of the benzimidazole schiff base, thiazolidine and azetidinone ring system with substitution at 2nd position and

also substitution with different electron withdrawing and electron donating groups which would poses potential antimicrobial properties. In the present study, a novel series of 2-substituted benzimidazole and its derivates were synthesized and characterized by IR, ¹H-NMR, ¹³C-NMR, mass spectral and elemental analysis. The compounds were screened for antibacterial and antifungal activities. The minimum inhibitory concentrations (MIC) were also determined by agar streak dilution method.

Materials and Methods

Materials

The melting points were taken in open capillary tube and are uncorrected. The IR

spectra of the compounds were recorded on ABB Bomem FTIR spectrometer MB 104 with KBr Pellets. ¹H-NMR spectra were recorded on 300 MHz – Bruker DPX 200. The chemical shifts are reported as parts per million down field from tetramethylsilane. Mass spectra were recorded on Finnigan MAT 8230. Micro analyses for C, H, N were performed in Heraeus CHN repaid analyzer. All the compounds gave satisfactory chemical analyses (± 0.4%). The purity of the compounds were checked by TLC on precoated SiO₂ gel (HF₂₅₄ 200 mesh) aluminium plates (E Merck).

General Procedures. The synthetic strategy leading to the target compounds are illustrated in **scheme 1**. The thiazolidinone derivatives synthesized by equimolar quantities (0.01mol) of *o*-phenylenediamine, *p*-amino benzoic acid (0.01mol) in 4N HCl (20mL) was refluxed for 30 min. The mixture is cooled and filtered off. The residue is the 4-(1*H*-benzo[*d*]imidazol-2-yl) benzenamine **1**. The product is recrystallized from absolute alcohol. This compound was obtained as a pale yellow solid; Yield 89%; mp 209°C - 211°C.

General method of synthesis of schiff bases (2a-2e)

A mixture of equimolar quantities (0.01mol) of aromatic aldehyde and 4-(1*H*-benzo[*d*]imidazol-2-yl) benzenamine **1** was refluxed for 20 min in 20 mL of ethanol. The reaction mixture was cooled and kept for 24 h. The crystals found was filtered and dried. The schiff base N-(4-substituted benzylidene)-4-(1*H*-benzo[*d*]imidazol-2-yl) benzenamine (**2a-2e**) recrystallized from ethanol.

General method of synthesis of azetidiones (3a-3e)

A mixture of schiff base (0.001mol) and triethylamine (0.003mol) was dissolved in 1,4 - Dioxan (25mL), to this well stirred cooled solution of chloro acetyl chloride (0.0012mol)

was added drop wise at 10°C. The reaction mixture was stirred for 6 hours. Half of the solvent separated and yield 1-(4-(1*H*-benzo[*d*]imidazol-2-yl) phenyl)-3-chloro-4-(4-substituted phenyl) azetidion-2-one (**3a-3e**) recrystallized from chloroform.

General method of synthesis of thiazolidinones (4a-4e)

A mixture of schiff base (0.001mol) and thioglycollic acid (0.001mol) dissolved in 1,4 dioxane (20mL), anhydrous zinc chloride (0.5mg) was added and refluxed for 8 h. The reaction was then cooled and the resulting solid was washed with sodium bicarbonate solution and final compound 3-(4-(1*H*-benzo[*d*]imidazol-2-yl)phenyl)-2-(4-substituted phenyl)thiazolidin-4-one (**4a-4e**) recrystallized from absolute ethanol.

N-(4-nitro benzylidene)-4-(1*H*-benzo[*d*]imidazol-2-yl) benzenamine (2a)

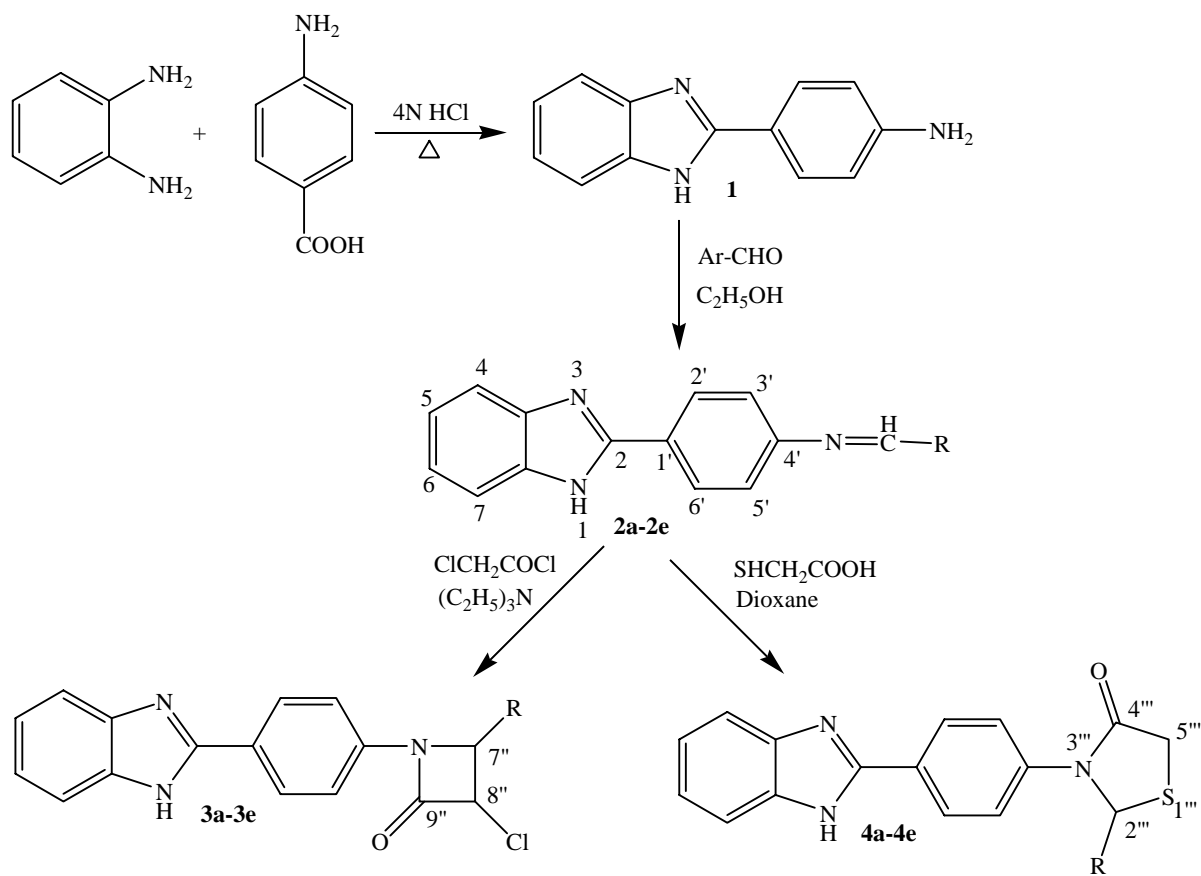
This compound was obtained as a yellow solid; Yield 91%; mp 265°C-267°C; IR (KBr) cm⁻¹; 3048 (Ar-H), 3338 (N-H), 1423 (C-N), 1601 (C=N), 1518, 1344 (Ar-NO₂), ¹H-NMR (CDCl₃) δ: 7.2-8.2 (m, 12H Ar-H); 10.1 (1H, NH), 8.39 (s, 1H, N-CH); ¹³C- NMR (CDCl₃) δ: 160.1 (N-CH), 152.9 (C-2), 150.7 (C-4"), 139.7 (C-1"), 138.4 (C-8 & C-9) 130.1 (C-2"), 129.0 (C-1'), 128.5 (C-2'&C-6'), 123.2 (C-5&C-6), 122.7 (C-3'), 121.0 (C-3" & C-5"), 120.8 (C-6"), 115.1 (C-4 & C-7) 153.2 (C-4'); EI-MS m/z (M⁺): 342 (calcd for C₂₀H₁₄N₄O₂: 342.35). Anal calcd for C₂₀H₁₄N₄O₂: C, 70.17; H, 4.12; N, 16.37, Found: C, 69.97; H, 4.13; N, 16.28.

N-(4-chloro benzylidene)-4-(1*H*-benzo[*d*]imidazol-2-yl) benzenamine (2b)

This compound was obtained as a pale yellow solid; Yield 78%; mp 215°C-217°C; IR (KBr) cm⁻¹; 2998 (Ar-H), 3358 (N-H), 1382 (C-N), 1601 (C=N), 778 (Ar-Cl), ¹H-NMR(CDCl₃) δ : 7.0-8.1 (m, 12H, Ar-H), 9.9 (1H, NH), 8.35 (s, 1H, N-CH); ¹³C- NMR (CDCl₃) δ : 160.1 (N-CH), 153.1 (C-4'), 152.6 (C-2), 138.7 (C-8 & C-9), 136.7 (C-4"), 131.7 (C-1"), 130.5 (C-2" & C-

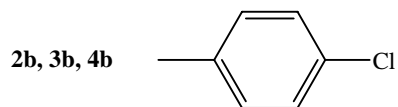
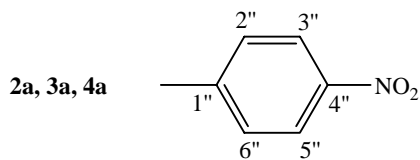
6''), 129.0 (C-3''&C-5''), 128.6 (C-2'&C-6'), 129.7 (C-1''), 123.1 (C-5&C-6), 115.3 (C-4& C-7), 122.7(C-3'); EI-MS m/z (M⁺): 332 (calcd for C₂₀H₁₄ClN₃ : 331.79). Anal calcd for C₂₀H₁₄ClN₃: C, 72.40; H, 4.25; N, 12.66, Found: C, 72.51; H, 4.22; N, 12.68.

Scheme 1



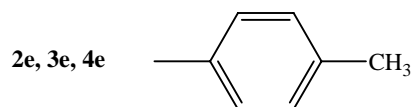
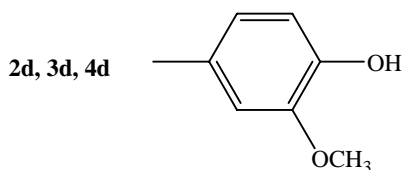
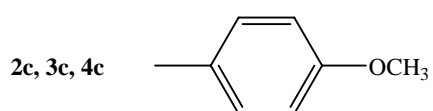
COMPOUND CODES

R



COMPOUND CODES

R



N-(4-methoxybenzylidene)-4-(1H-benzo[d]imidazol-2-yl) benzenamine (2c)

This compound was obtained as a cream solid; Yield 78%; mp 210°C-212°C; IR (KBr) cm^{-1} : 3048 (Ar-H), 3366 (N-H), 1410 (C-N), 1597 (C=N), 1202 (Ar-OCH₃); ¹H-NMR (CDCl₃) δ : 7.0-7.8 (m, 12H, Ar-H), 9.8 (1H, NH), 3.9 (3H, OCH₃); ¹³C-NMR (CDCl₃) δ : 163.0 (C-4'), 160.1 (N-CH), 153.1 (C-4'), 152.7 (C-2), 138.7 (C-8 & C-9), 130.2 (C-2'' & C-6''), 129.2 (C-1''), 128.8 (C-2' & C-6'), 126.1 (C-1'), 123.0 (C-5 & C-6), 122.8 (C-3'), 115.3 (C-4 & C-7), 114.4 (C-3'' & C-5''), 55.9 (C-7''); EI-MS m/z (M⁺): 327 (calcd for C₂₁H₁₇ClN₃O: 327.37). Anal calcd for C₂₁H₁₇ClN₃O: C, 77.04; H, 5.23; N, 12.84, Found: C, 77.19; H, 5.21; N, 12.81.

4-(4-(1H-benzo[d]imidazol-2-yl) phenylimino) methyl-2-methoxy phenol (2d)

This compound was obtained as a brown crystals; Yield 72%; mp 260°C-262°C; IR (KBr) cm^{-1} : 3124 (Ar-H), 1408 (N-H), 1603 (C-N), 1221 (C=N), 1201 (Ar-OCH₃), 3319 (Ar-OH); ¹H-NMR (CDCl₃) δ : 6.6-7.8 (m, 12H, Ar-H), 9.8 (1H, NH), 8.1 (s, 1H, N-CH), 3.9 (3H, Ar-OCH₃), 11.9 (1H Ar-OH); ¹³C-NMR (CDCl₃) δ : 160.1 (N-CH), 153.2 (C-4'), 152.9 (C-2), 151.5 (C-3''), 148.0 (C-4''), 138.9 (C-8 & C-9), 129.2 (C-1'), 128.8 (C-2' & C-6'), 127.4 (C-1''), 123.0 (C-5 & C-6), 122.8 (C-3' & C-5'), 117.0 (C-5''), 115.3 (C-4 & C-7), 56.2 (C-3''); EI-MS m/z (M⁺): 342 (calcd for C₂₁H₁₇N₃O₂: 343.37). Anal calcd for C₂₁H₁₇N₃O₂: C, 73.45; H, 4.99; N, 12.24, Found: C, 73.29; H, 4.96; N, 12.17.

N-(4-methyl benzylidene)-4-(1H-benzo[d]imidazol-2-yl) benzenamine (2e)

This compound was obtained as a white solid; Yield 60%; mp 230°C-232°C; IR (KBr) cm^{-1} : 3028 (Ar-H), 3376 (N-H), 1422 (C-N), 1594 (C=N), 2918, 2859 (Ar-CH₃), ¹H-NMR (CDCl₃) δ : 7.1-7.9 (m, 12H, Ar-H), 9.9 (1H, NH), CH₃ (3H, CH₃), 8.4 (s, 1H, N-CH); ¹³C-NMR (CDCl₃) δ : 160.1 (N-CH), 153.2 (C-4'), 152.1 (C-2), 140.7 (C-4''), 138.9 (C-8 & C-9),

130.8 (C-1''), 129.2 (C-1', C-5' & C-3''), 128.8 (C-2' & C-6'), 129.2 (C-2'' & C-5''), 123.0 (C-5 & C-6), 122.8 (C-3'), 115.3 (C-4 & C-7), 24.3 (C-7''); EI-MS m/z (M⁺): 311 (calcd for C₂₁H₁₇N₃: 311.37). Anal calcd for C₂₁H₁₇N₃: C, 81.00; H, 5.50; N, 13.49, Found: C, 81.09; H, 5.57; N, 13.38.

1-(4-(1H-benzo[d]imidazol-2-yl) phenyl)-3-chloro-4-(4-nitrophenyl)azetid-2-one (3a)

This compound was obtained as a pale brown solid; Yield 76%; mp 234°C-236°C; IR (KBr) cm^{-1} : 2975 (Ar-H), 3200 (N-H), 1398 (C-N), 1601 (C=N), 1518, 1344 (Ar-NO₂), 812 (CH-Cl) 1686 (β -Lactam, C=O); ¹H-NMR (CDCl₃) δ : 7.3-8.7 (m, 12H, Ar-H), 10.1 (s, 1H, NH), 3.1 (d, 1H, aze.CH); ¹³C-NMR (CDCl₃) δ : 162.1 (C-9''), 152.1 (C-2), 149.8 (C-1''), 148.4 (C-4''), 141.7 (C-4'), 138.9 (C-8 & C-9), 127.9 (C-2'' & C-6''), 127.7 (C-2' & C-6'), 123.0 (C-5 & C-6), 122.1 (C-3' & C-5'), 120.9 (C-3'' & C-5''), 115.3 (C-4 & C-7), 126.3 (C-1'), 63.1 (C-7''), 62.0 (C-8''); EI-MS m/z (M⁺): 418 (calcd for C₂₂H₁₅ClN₄O₃: 418). Anal calcd for C₂₂H₁₅ClN₄O₃: C, 60.09; H, 3.61; N, 13.38, Found: C, 63.01; H, 3.67; N, 13.41.

1-(4-(1H-benzo[d]imidazol-2-yl)phenyl)-3-chloro-4-(4-chlorophenyl)azetid-2-one (3b)

This compound was obtained as a pale yellow solid; Yield 48%; mp 228°C-230°C; IR (KBr) cm^{-1} : 3073 (Ar-H), 3264 (NH), 1406 (C-N), 1599 (C=N), 826 (Ar-Cl), 772 (CH-Cl) 1681 (β -Lactam C=O); ¹H-NMR (CDCl₃) δ : 7.0-8.4 (m, 12H, Ar-H), 9.9 (s, 1H, NH), 3.3 (d, 1H, aze.CH); ¹³C-NMR (CDCl₃) δ : 162.2 (C-9''), 152.9 (C-2), 141.7 (C-4'), 141.6 (C-1''), 138.9 (C-8 & C-9), 132.3 (C-4''), 128.7 (C-3'' & C-5''), 128.4 (C-2'' & C-6''), 127.7 (C-2' & C-6'), 126.3 (C-1'), 123.0 (C-5 & C-6), 122.1 (C-3' & C-5'), 115.3 (C-4 & C-7), 63.1 (C-7''), 62.0 (C-8''); EI-MS m/z (M⁺): 408 (calcd for C₂₂H₁₅Cl₂N₃O: 408.28). Anal calcd for C₂₂H₁₅Cl₂N₃O: C, 64.72; H, 3.70; N, 10.29, Found: C, 64.77; H, 3.67; N, 10.26.

1-(4-(1H-benzo[d]imidazol-2-yl)phenyl)-3-chloro-4-(4-methoxyphenyl)azetid-2-one (3c)

This compound was obtained as a yellow solid; Yield 72%; mp 210°C-212°C; IR (KBr) cm^{-1} : 3022 (Ar-H), 3337 (NH), 1407 (C-N), 1605 (C=N), 1247 (Ar- OCH₃), 799 (CH-Cl) 1681 (β -Lactam C=O); ¹H- NMR (CDCl₃) δ : 7.0-7.8 (m, 12H, Ar-H), 9.8 (s, 1H, NH), 3.9 (d, 1H, aze.CH); ¹³C- NMR (CDCl₃) δ : 162.2 (C-9''), 158.7 (C-4''), 152.1 (C-2), 141.7 (C-4'), 138.9 (C-8&C-9), 135.8 (C-1''), 128.0 (C-2''&C-6''), 127.7 (C-2'&C-6'), 123.0 (C-5&C-6), 126.3 (C-1'), 122.1 (C-3'&C-5'), 115.3 (C-4&C-7), 114.4 (C-5''), 63.1 (C-7''), 62.0 (C-8''), 55.9 (C-10''); EI-MS m/z (M⁺): 403 (calcd for C₂₃H₁₈ClN₃O₂: 403.86). Anal calcd for C₂₃H₁₈ClN₃O₂: C, 68.40; H, 4.49; N, 10.40, Found: C, 68.37; H, 4.51; N, 10.36.

1-(4-(1H-benzo[d]imidazol-2-yl)phenyl)-3-chloro-4-(4-hydroxy-3-methoxyphenyl)azetid-2-one (3d)

This compound was obtained as a yellow solid; Yield 76%; mp 220°C-222°C; IR (KBr) cm^{-1} : 3001 (Ar-H), 3227 (NH), 1406 (C-N), 1593 (C=N), 1246 (Ar-OCH₃), 795 (CHCl) 1675 (β -Lactam C=O); ¹H-NMR (CDCl₃) δ : 6.6-7.8 (m, 12H, Ar-H), 9.8(s, 1H, NH), 3.1 (d, 1H, aze.CH); ¹³C- NMR (CDCl₃) δ : 162.2 (C-9''), 152.9 (C-2), 151.2 (C-3''), 143.7 (C-4''), 141.7 (C-4'), 138.9 (C-8&C-9), 137.1 (C-1''), 127.7 (C-2'&C-6'), 126.3 (C-1'), 123.0 (C-5&C-6), 122.1 (C-3'&C-5'), 120.7 (C-6''), 116.7 (C-5''), 115.3 (C-7&C-4), 112.4 (C-2''), 63.4 (C-7''), 62.0 (C-8''), 56.2 (C-10''); EI-MS m/z (M⁺): 419 (calcd for C₂₃H₁₈ClN₃O₃: 419.86). Anal calcd for C₂₃H₁₈ClN₃O₃: C, 65.79; H, 4.32; N, 10.01, Found: C, 65.81; H, 4.29; N, 10.08.

1-(4-(1H-benzo[d]imidazol-2-yl)phenyl)-3-chloro-4-p-tolyl azetid-2-one (3e)

This compound was obtained as a pale brown solid; Yield 32%; mp 130°C-132°C; IR (KBr) cm^{-1} : 3028 (Ar-H), 3376 (NH), 1411 (C-N), 1603 (C=N), 2918, 2859 (Ar-CH₃), 817 (Ar-Cl), 1678 (β -Lactam C=O); ¹H- NMR (CDCl₃) δ :

7.1-7.8 (m, 12H, Ar-H), 9.9 (s, 1H, NH), 2.4 (3H, CH₃), 3.1 (d, 1H, Ar-H), ¹³C- NMR δ : 162.2 (C-9''), 152.9 (C-2), 141.7 (C-4'), 140.5 (C-1''), 138.9 (C-8&C-9), 136.4 (C-4''), 128.9 (C-5''&C-3''), 127.7 (C-2'&C-6'), 126.9 (C-2''&C-6''), 126.3 (C-1'), 123.0 (C-5&C-6), 122.1 (C-5'&C-3'), 115.3 (C-4&C-7), 63.1 (C-7''), 62.0 (C-8''), 24.3 (C-10''); EI-MS m/z (M⁺): 387 (calcd for C₂₃H₁₈ClN₃O: 387.86). Anal calcd for C₂₃H₁₈ClN₃O: C, 71.22; H, 4.68; N, 10.83, Found: C, 71.33; H, 4.65; N, 10.79.

3-(4-(1H-benzo[d]imidazol-2-yl)phenyl)-2-(4-nitro phenyl)thiazolidin-4-one (4a)

This compound was obtained as a white crystals; Yield 36%; mp 256°C-258°C; IR (KBr) cm^{-1} : 3077 (Ar-H), 3340 (NH), 1417 (C-N) 1598 (C=N), 1515, 1343 (Ar-NO₂), 1701 (C=O), 1105 (C-S); ¹H- NMR (CDCl₃) δ : 7.1-8.0 (m, 12H, Ar-H), 9.4 (s, 1H, NH), 3.5 (2H, thiazolidinone CH₂), 3.9 (1H, thiazolidinone CH); ¹³C- NMR δ : 171.2 (C-4''), 152.9 (C-2), 146.8 (C-4), 145.3 (C-1''), 141.3 (C-4'), 138.9 (C-8&C-9), 129.7 (C-2''&C-6''), 127.7 (C-2'&C-6'), 126.8 (C-1'), 123.0 (C-5&C-6), 122.2 (C-5'&C-3'), 121.0 (C-3''&C-5''), 115.3 (C-4&C-7), 65.6 (C-1'''), 33.6 (C-5'''). EI-MS m/z (M⁺): 416 (calcd for C₂₂H₁₆N₄O₃S: 416.18). Anal calcd for C₂₂H₁₆N₄O₃S: C, 63.45; H, 3.87; N, 13.45, Found: C, 63.37; H, 3.91; N, 13.51.

3-(4-(1H-benzo[d]imidazol-2-yl)phenyl)-2-(4-chloro phenyl)thiazolidin-4-one (4b)

This compound was obtained as a brown solid; Yield 22%; mp 208°C-210°C; IR (KBr) cm^{-1} : 3350 (NH), 1403 (C-N), 1590 (C=N), 783 (Ar-Cl), 1693 (C=O), 1118 (C-S); ¹H-NMR (CDCl₃) δ : 7.1-7.7(m, 12H, Ar-H), 8.9 (s, 1H, NH), 3.9 (2H- thiazolidinone CH₂), 3.7 (1H, thiazolidinone CH) ¹³C- NMR (CDCl₃) δ : 171.2 (C-4''), 152 (C-2), 141.7 (C-4'), 138.9 (C-8&C-9), 137.3 (C-1''), 130.2 (C-2''&C-6''), 128.8 (C-3''&C-5''), 127.7 (C-2'&C-6'), 126.3 (C-1'), 123.0 (C-5&C-6), 122.1 (C-3' ' &C-5'), 115.3 (C-4&C-7), 65.6 (C-2'''), 33.6 (C-5'''); EI-MS m/z (M⁺): 405 (calcd for C₂₂H₁₆ClN₃OS: 405.89). Anal calcd for C₂₂H₁₆ClN₃OS: C, 65.10; H,

3.97; N, 10.35, Found: C, 65.13; H, 4.03; N, 10.41.

3-(4-(1H-benzo[d]imidazol-2-yl)phenyl)-2-(4-methoxy phenyl)thiazolidin-4-one (4c)

This compound was obtained as a pale yellow solid; Yield 30%; mp-190°C-192°C; IR (KBr) cm⁻¹; 3011 (Ar-H) 3198 (NH), 1420 (C-N) 1602 (C=N), 1214 (Ar-OCH₃), 1684 (C=O), 1109 (C-S); ¹H- NMR (CDCl₃) δ: 6.6-7.9 (m, 12H, Ar-H), 8.7 (s, 1H, NH), 3.36 (3H,OCH₃), 3.81 (2H, thiazolidinone CH₂), 5.9 (1H, thiazolidinone CH), ¹³C- NMR (CDCl₃) δ: 171.2 (C-4'''), 159.1 (C-4''), 152 (C-2), 141.7 (C-4'), 138.9 (C-8&C-9), 131.5 (C-1''), 129.8 (C-2''&C-6''), 127.7 (C-2'&C-6'), 126.3 (C-1'), 123.0 (C-5&C-6), 122.1 (C-3'&C-5'), 115.3 (C-4&C-7), 114.2 (C-3''&C-5''), 65.6 (C-2'''), 55.9 (C-7''), 33.6 (C-5'''); EI-MS m/z (M⁺): 401 (calcd for C₂₃H₁₉N₃O₂S: 401.48). Anal calcd for C₂₃H₁₉N₃O₂S: C, 68.81; H, 4.77; N, 10.47, Found: C, 68.79; H, 4.80; N, 10.44.

3-(4-(1H-benzo[d]imidazol-2-yl)phenyl)-2-(4-hydroxy-3-(methoxyphenyl)thiazolidine-4-one (4d)

This compound was obtained as a pale brown solid; Yield 46%; mp 290°C-292°C; IR (KBr) cm⁻¹; 3033 (Ar-H), 3124 (NH), 1394 (C-N), 1604 (C-N), 1208 (Ar-OCH₃), 3356 (Ar-OH), 719 (C=O), 1108 (C-S); ¹H- NMR (CDCl₃) δ: 6.5-7.8 (m, 12H, Ar-H), 8.6 (s, 1H,NH), 3.46 (3H, OCH₃), 11.6 (1H, ArOH), 4.4 (2H, thiazolidinone CH₂), 3.56 (1H, thiazolidinone CH); ¹³C- NMR (CDCl₃) δ: 171.2 (C-4'''), 152 (C-2), 151.3 (C-3'') 144.1 (C-4''), 141.7 (C-4'), 138.9 (C-8&C-9), 132.8 (C-1''), 127.7 (C-2'&C-6'), 126.3 (C-1), 122.5 (C-2''), 122.1 (C-3&C-5'), 23.6 (C-5&C-6), 116.8 (C-5''), 115.3 (C-4&C-7), 114.2 (C-2''), 65.9 (C-2'''), 56.2 (C-7''), 33.6 (C-5'''); EI-MS m/z (M⁺) 417 (calcd for C₂₃H₁₉N₃O₃S: 417.48). Anal calcd for C₂₃H₁₉N₃O₃S: C, 66.17; H, 4.59; N, 10.07, Found: C, 66.13; H, 4.61; N, 10.01.

3-(4-(1H-benzo[d]imidazol-2-yl)phenyl)-2-p-tolylthiazolidin-4-one (4e)

This compound was obtained as a white crystals; Yield 24%; mp 235°C-237°C; IR (KBr) cm⁻¹; 3023 (Ar-H), 3359 (NH), 1393 (C-N) 1604 (C=N), 2919, 2857 (Ar-CH₃), 1700 (C=O), 1117 (C-S); ¹H-NMR (CDCl₃) δ: 7.0-8.0 (m, 12H, Ar-H), 10.3 (s, 1H, NH), 2.6 (3H,CH₃), 3.9 (2H, thiazolidinone CH₂), 3.5 (1H, Thiazolidinone CH), ¹³C- NMR (CDCl₃) δ: 171.2 (C-4'''), 152 (C-2), 141.7 (C-4') 138.9 (C-8&C-9), 136.8 (C-4''), 136.2 (C-1''), 129.0 (C-3''&C-5''), 128.7 (C-2''&C-6''), 127.7 (C-2'&C-6'), 126.3 (C-1'), 123.0 (C-5&C-6), 122.1 (C-3'&C-5'), 115.3 (C-4&C-7), 65.6 (C-2'''), 33.6 (C-5'''), 24.3 (C-7''); EI-MS m/z (M⁺): 385 (calcd for C₂₃H₁₉N₃OS:385.48). Anal calcd for C₂₃H₁₉N₃OS: C71.66; H, 4.97; N, 10.90, Found: C, 71.58; H, 4.92; N, 10.81.

Antimicrobial Screening

The *in vitro* antibacterial (*Staphylococcus aureus* ATCC9144, *Staphylococcus epidermidis* ATCC 155, *Klebsiella pneumoniae* ATCC 29665 and *Escherichia coli* ATCC 25922) and antifungal (*Candida albicans* ATCC 2091 and *Aspergillus niger* ATCC 9029) activities of the compounds were evaluated by paper disc diffusion method. The minimum inhibitory concentrations of the compounds were also determined by agar streak dilution method.

Paper disc diffusion method

The sterilized (Gilles, 1994) (autoclaved at 120°C for 30 min) medium (40-50°C) was inoculated (1mL/100mL of medium) with the suspension (10⁵ cfu/mL) of the micro organism (matched to McFarland barium sulphate standard) and poured into a petridish to give a depth of 3-4 mm. The paper impregnated with the test compounds (25, 50, 100 µg/mL in dimethyl formamide) was placed on the solidified medium. The plates were preincubated for 1h at room temperature and incubated at 37°C for 24 h and 48h for anti bacterial and antifungal activity respectively. Ciprofloxacin (Dr.

Reddy's Laboratories, Batch no. IC 666 E^o4 India) and ketoconazole (Wuhan Shengmao Corporation Batch no: SBML/403, China) was used as standard for antibacterial and anti fungal activity respectively. The observed zone of inhibition is presented in Table 1.

Minimum Inhibitory concentration

Minimum inhibitory concentration (Hawkey,1994) (MIC) of the test compounds were determined by agar streak dilution method. A stock solution of the synthesized compound [50 µg/mL] in dimethylformamide was prepared and graded quantities of the test compounds were incorporated in specified quantity of molten sterile agar (nutrient agar for antibacterial

activity and sabouraud dextrose agar medium for anti fungal activity) A specified quantity of the medium (40-50°C) containing the compound was poured into a petridish to give a depth of 3-4mm and allowed to solidify suspension of the microorganism were prepared to contain approximately 10⁵ Cfu/mL and applied to plates with serially diluted compounds in dimethylformamide to be tested and incubated at 37°C for 14h and 48h for bacteria and fungi respectively. The MIC was considered to be lowest concentration of the test substance exhibiting no visible growth of bacteria or fungi, on the plate. The observed MIC is presented in Table 1.

Table 1 Antimicrobial activity of the synthesized compounds

| Compound | <i>In vitro</i> activity - Zone of inhibition (MIC) | | | | | |
|---------------|---|----------------------|------------------|-----------------------|--------------------|-----------------|
| | <i>E. coli</i> | <i>K. pneumoniae</i> | <i>S. aureus</i> | <i>S. epidermidis</i> | <i>C. albicans</i> | <i>A. niger</i> |
| 2a | 22(19) | 16(21) | 16(21) | 30(9) | 22(14) | 15(16) |
| 2b | 25(14) | 15(19) | 12(31) | 25(14) | 17(18) | 13(21) |
| 2c | 20(23) | 13(27) | 15(22) | 23(19) | 15(23) | 9 (26) |
| 2d | 21(17) | 15(22) | 14(26) | 28(12) | 24(10) | 12(19) |
| 2e | 13(30) | 13(31) | 12(31) | 18(22) | 18(20) | 10(32) |
| 3a | 28(15) | 20(17) | 17(19) | 25(9) | 24(11) | 14(15) |
| 3b | 14(31) | 26(12) | 13(27) | 25(12) | 21(15) | 12(20) |
| 3c | 17(21) | 14(26) | 18(16) | 21(18) | 20(18) | 9(31) |
| 3d | 19(19) | 18(20) | 12(32) | 23(14) | 18(21) | 11(24) |
| 3e | 15(12) | 10(29) | 13(25) | 17(24) | 15(26) | 9(28) |
| 4a | 23(16) | 16(19) | 15(22) | 26(13) | 24(9) | 13(19) |
| 4b | 25(12) | 15(24) | 19(13) | 16(21) | 22(13) | 12(23) |
| 4c | 21(19) | 12(29) | 13(28) | 14(24) | 20(19) | 15(12) |
| 4d | 25(9) | 15(22) | 16(19) | 18(20) | 24(11) | 8(32) |
| 4e | 20(21) | 14(24) | 11(32) | 15(26) | 11(25) | 12(26) |
| Ciprofloxacin | 29 | 27 | 25 | 34 | - | - |

Zone of inhibition in mm, MIC in µg/mL

Results and Discussion

All the synthesized compounds exhibited significant antibacterial and moderate to potent antifungal activity. 2-substituted benzimidazole schiff bases and its

azetidinone and thiazolidinone derivatives were found to exhibit most potent antimicrobial activity against all the microbial strains tested. All the compounds were active against all tested micro organism with a

range of MIC values for *S. aureus* (13-32 µg/mL), *S. epidermidis* (9-26 µg/mL), *K. pneumoniae* (12-31 µg/mL), *E.coli* (9-31µg/mL), *C.albicans* (9-25 µg/mL) and *A.niger* (12-32 µg/mL). Compounds **2a**, **4a** and **4d** exhibited potent antimicrobial activity (MIC: 9 µg/mL) against *S. epidermidis* (*E.coli* and *C. albicans* compounds **3b** and **4c** showed significant activity (12 µg/mL) against *K. pneumoniae* and *A. niger*). Compounds **3e** and **4b** were found to be active (MIC: 12 µg/mL) against *E. coli*. Compounds **2d** and **3a** exhibited significant activity against *S. epidermidis* (MIC: 12 µg/mL) and *C. albicans* (MIC: 11 µg/mL) respectively. Compounds **2b**, **2c**, **2e**, **3c**, **3d** and **4e** were found to exhibit moderate to potent antimicrobial properties. The data revealed that electron withdrawing groups like -NO₂, -Cl, and electron donating group like -OCH₃, -OH were found to increase the antimicrobial properties, whereas electron donating group like -CH₃ group found to have moderate activity. The most of the synthesized compounds exhibited significant antibacterial activity and moderate antifungal activity.

Structure-activity relationship studies

Structure-activity relationship (SAR) studies revealed that different substitutions on the benzimidazole schiff bases and its azetidinone and thiazolidinone derivatives exerted varied biological activity. The electronic nature of the substituent groups at 4' positions in benzimidazole nucleus, 7'' azetidinone and 2''' thiazolidinone led to significant variation in antimicrobial activity. Among the series compounds substituted by electron-withdrawing (-NO₂ and -Cl) and electron-donating (-OCH₃, -OH and -CH₃) groups are enhanced biological activity.

Reference

- David, W., Zhijan, K., and Christine, C. The Synthesis of Dicationic Extended Bis-Benzimidazoles. 2004. *Molecules.*, 9, 158-163.
- Gilles, S. H. 1994. Medical Microbiology-Illustrated, Butterworth Heinemann Ltd, United kingdom. 234-247.
- Hawkey, P. M., and Lewis, D. A.1994. Medical Bacteriology-A Practical approach, OxfordUniversity Press., United Kingdom. 181-194.
- Jitendar, S.S., Rishi, S.H., and Vipin, K. 2002. Benzimidazoles with biphenyls: Synthesis of 5-substituted-2-npropyl-1-[(2'-carboxybiphenyl-4-yl)- methyl] benzimidazoles. *J. Ind. Inst. Sci.*, 82, 177-182.
- Kohara, Y., Kubo, K., Imamiya, E., Wada, T., Inada Y., and Naka, T. 1996. Synthesis and angiotensin II receptor antagonistic activities of benzimidazole derivatives bearing acidic heterocycles as novel tetrazole bioisosteres. *J. Med. Chem.*, 39, 5228-5235.
- Khyati, A.P., Pares, S.O., Bhatt, S.B., and Parikh, A.R. 2000. Synthesis of some 2-azetidinones as a potential anti-tubercular agents. *Ind. J. Chem.*, 39B, 716-718.
- Nare, B., Prichard, R. K., and Georges, E. 1994. Benzimidazoles, potent anti-mitotic drugs: Substrates for the P-glycoprotein transporter in multidrug-resistant cells. *Bio Chem Pharmacol.*, 48, 2215-2222.
- Shiva, P.S., Surendra, S.P., Krishna, R., and Virgil, I. S. 1981. Chemistry and biological activity of thiazolidinones. *Chem. Rev.*, 81, 175-203.