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Research Article

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

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In the present study, a novel series of 2-substituted benzimidazole derivatives were synthesized and characterized by means of IR, ^{1}H-NMR, ^{13}C- 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 (C*andida albicans* ATCC 2091 and *Aspergillus niger* ATCC 9029) activities. The Minimum Inhibitory Concentrations was determined by agar streak dilution method. 1-(4-(1*H*-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, ^{1}H-NMR, ^{13}C-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 Bomen FTIR spectrometer MB 104 with KBr Pellets. ^{1}H-NMR spectra were recorded on 300 MH_z – 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 SiO_2 gel (HF_{254} 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^{o}C - 211^{0}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 benzylidine)-4-(1*H*-benzo[*d*]imidazol-2-yl) benzenamine **(2a-2e)** recrystallized from ethanol.

General method of synthesis of azetidinones (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^{o}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) azetidin-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-(1H-benzo[d]imidazol-2-yl)phenyl)-2-(4-substituted phenyl)thiazolidin-4-one **(4a-4e)** recrystallized from absolute ethanol.

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

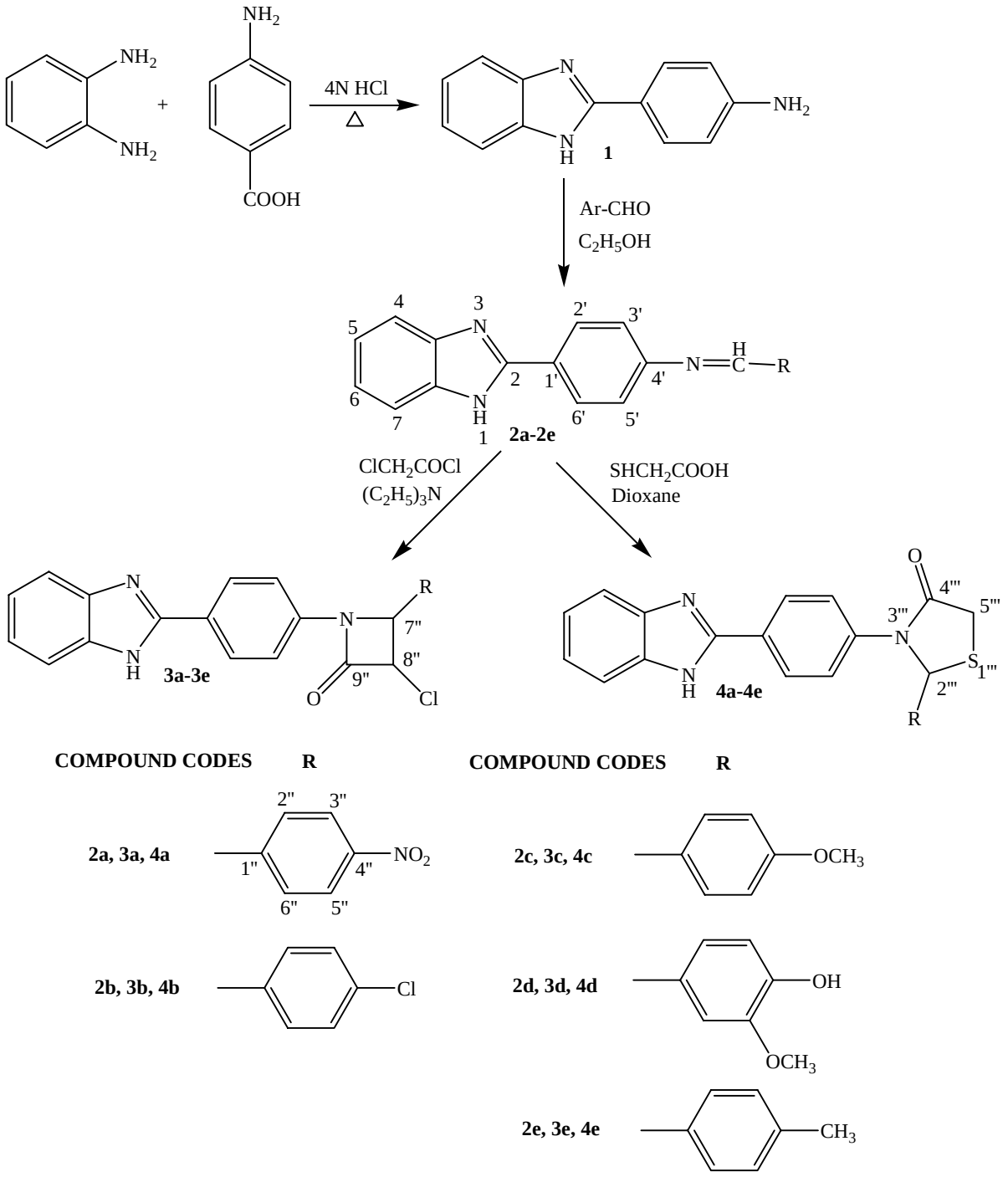
This compound was obtained as a yellow solid; Yield 91%; mp 265^{o}C-267^{o}C; IR (KBr) cm^{-1}; 3048 (Ar-H), 3338 (N-H), 1423 (C-N), 1601 (C=N), 1518, 1344 (Ar-NO_2), ^{1}H-NMR ($CDCl_3$) δ: 7.2-8.2 (m, 12H Ar-H); 10.1 (1H, NH,) 8.39 (s, 1H, N-CH); ^{13}C- NMR ($CDCl_3$) δ: 160.1 (N-CH), 152.9 (C-2), 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 $C_{20}H_{14}N_4O_2$: 342.35). Anal calcd for $C_{20}H_{14}N_4O_2$: C, 70.17; H, 4.12; N, 16.37, Found: C, 69.97; H, 4.13; N, 16.28.

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

This compound was obtained as a pale yellow solid; Yield 78%; mp 215^{o}C-217^{o}C; IR (KBr) cm^{-1}; 2998 (Ar-H), 3358 (N-H), 1382 (C-N), 1601 (C=N), 778 (Ar-Cl), ^{1}H-NMR($CDCl_3$) δ : 7.0-8.1 (m, 12H, Ar-H), 9.9 (1H, NH,) 8.35 (s, 1H, N-CH); ^{13}C- NMR ($CDCl_3$) 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 $C_{20}H_{14}ClN_3$: 331.79). Anal calcd for $C_{20}H_{14}ClN_3$: C, 72.40; H, 4.25; N, 12.66, Found: C, 72.51; H, 4.22; N, 12.68.

Scheme 1



N-(4-methoxybenzylidine)-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-OCH_3); ^{1}H- NMR ($CDCl_3$) δ :7.0-7.8 (m, 12H, Ar-H), 9.8 (^{1}H, NH), 3.9 (3H, OCH_3); ^{13}C- NMR ($CDCl_3$) 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 $C_{21}H_{17}ClN_3O$: 327.37). Anal calcd for $C_{21}H_{17}ClN_3O$: C, 77.04; H, 5.23; N, 12.84, Found: C, 77.19; H, 5.21; N, 12.81.

4-(4-(1H-benzo[d]imidazolo-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-OCH_3), 3319 (Ar-OH); ^{1}H- NMR ($CDCl_3$) δ: 6.6-7.8 (m, 12H, Ar-H), 9.8 (1H, NH), 8.1 (s, 1H, N-CH), 3.9 (3H, Ar-OCH_3), 11.9 (1H Ar-OH); ^{13}C- NMR ($CDCl_3$) 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 $C_{21}H_{17}N_3O_2$: 343.37). Anal calcd for $C_{21}H_{17}N_3O_2$: C, 73.45; H, 4.99; N, 12.24, Found: C, 73.29; H, 4.96; N, 12.17.

N-(4-methyl benzylidine)-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-CH_3), ^{1}H-NMR ($CDCl_3$) δ: 7.1-7.9 (m, 12H, Ar-H), 9.9 (1H, NH), CH_3 (3H, CH_3), 8.4 (s, 1H, N-CH); ^{13}C-NMR ($CDCl_3$) 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 $C_{21}H_{17}N_3$: 311.37). Anal calcd for $C_{21}H_{17}N_3$: 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)azetidin-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-NO_2), 812 (CH-Cl) 1686 (β-Lactam, C=O); ^{1}H-NMR ($CDCl_3$) δ: 7.3-8.7 (m, 12H, Ar-H), 10.1 (s, 1H, NH), 3.1 (d, 1H, aze.CH); ^{13}C- NMR ($CDCl_3$) 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 $C_{22}H_{15}ClN_4O_3$: 418). Anal calcd for $C_{22}H_{15}ClN_4O_3$: 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)azetidin-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); ^{1}H- NMR ($CDCl_3$) δ: 7.0-8.4 (m, 12H, Ar-H), 9.9 (s, 1H, NH), 3.3 (d, 1H, aze.CH); ^{13}C- NMR ($CDCl_3$) 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 $C_{22}H_{15}Cl_2N_3O$: 408.28) Anal calcd for $C_{22}H_{15}Cl_2N_3O$: 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)azetidin-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- OCH_3), 799 (CH-Cl) 1681 (β-Lactam C=O); ^{1}H- NMR ($CDCl_3$) δ: 7.0-7.8 (m, 12H, Ar-H), 9.8 (s, 1H, NH), 3.9 (d, 1H, aze.CH); ^{13}C- NMR ($CDCl_3$) 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 $C_{23}H_{18}ClN_3O_2$: 403.86). Anal calcd for $C_{23}H_{18}ClN_3O_2$: 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) azetidin-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-OCH_3), 795 (CHCl) 1675 (β-Lactam C=O); ^{1}H-NMR ($CDCl_3$) δ: 6.6.-7.8 (m, 12H, Ar-H), 9.8(s, 1H, NH), 3.1 (d, 1H, aze.CH); ^{13}C- NMR ($CDCl_3$) 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 $C_{23}H_{18}ClN_3O_3$: 419.86). Anal calcd for $C_{23}H_{18}ClN_3O_3$: 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 azetidin-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-CH_3), 817 (Ar-Cl), 1678 (β-Lactam C=O); ^{1}H- NMR ($CDCl_3$) δ: 7.1-7.8 (m, 12H, Ar-H), 9.9 (s, 1H, NH), 2.4 (3H, CH_3), 3.1 (d, 1H, Ar-H), ^{13}C- 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 $C_{23}H_{18}ClN_3O$: 387.86). Anal calcd for $C_{23}H_{18}ClN_3O$: 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-NO_2), 1701 (C=O), 1105 (C-S); ^{1}H- NMR ($CDCl_3$) δ: 7.1-8.0 (m, 12H, Ar-H), 9.4 (s, 1H, NH), 3.5 (2H, thiazolidinone CH_2), 3.9 (1H, thiazolidinone CH); ^{13}C- 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 $C_{22}H_{16}N_4O_3S$: 416.18). Anal calcd for $C_{22}H_{16}N_4O_3S$: 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); ^{1}H-NMR ($CDCl_3$) δ: 7.1-7.7(m, 12H, Ar-H), 8.9 (s, 1H, NH), 3.9 (2H– thiazolidinone CH_2), 3.7 (1H, thiazolidinone CH) ^{13}C- NMR ($CDCl_3$) 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 $C_{22}H_{16}ClN_3OS$: 405.89). Anal calcd for $C_{22}H_{16}ClN_3OS$: 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-OCH_3), 1684 (C=O), 1109 (C-S); ^{1}H- NMR ($CDCl_3$) δ: 6.6-7.9 (m, 12H, Ar-H), 8.7 (s, 1H, NH), 3.36 (3H,OCH_3), 3.81 (2H, thiazolidinone CH_2), 5.9 (1H, thiazolidinone CH), ^{13}C- NMR ($CDCl_3$) 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 $C_{23}H_{19}N_3O_2S$: 401.48). Anal calcd for $C_{23}H_{19}N_3O_2S$: 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-OCH_3), 3356 (Ar-OH), 719 (C=O), 1108 (C-S); ^{1}H- NMR ($CDCl_3$) δ: 6.5-7.8 (m, 12H, Ar-H), 8.6 (s, 1H,NH), 3.46 (3H, OCH_3), 11.6 (1H, ArOH), 4.4 (2H, thiazolidinone CH_2), 3.56 (1H, thiazolidinone CH); ^{13}C- NMR ($CDCl_3$) 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 $C_{23}H_{19}N_3O_3S$: 417.48). Anal calcd for $C_{23}H_{19}N_3O_3S$: 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-CH_3), 1700 (C=O), 1117 (C-S); ^{1}H-NMR ($CDCl_3$) δ: 7.0-8.0 (m, 12H, Ar-H), 10.3 (s, 1H, NH), 2.6 (3H,CH_3), 3.9 (2H, thiazolidinone CH_2), 3.5 (1H, Thiazolidinone CH), ^{13}C- NMR ($CDCl_3$) 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 $C_{23}H_{19}N_3OS$:385.48). Anal calcd for $C_{23}H_{19}N_3OS$: 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, *Staphylococuss 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 (10^5 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^{o}4 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 dimethyl-formamide 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^{o}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 10^5 Cfu/mL and applied to plates with serially diluted compounds in dimethylformamide to be tested and incubated at 37^{o}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 great of bacteria or fungi, on the plate. The observed MIC is presented in Table 1.

Table 1 Antimicrobial activity of the synthesized compounds

Compound	*In vitro* **activity – Zone of inhibition (MIC)**					
	E. coli	*K. pneumoniae*	*S. aureus*	*S. epidermidis*	*C. albicans*	*A. niger*
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 stains 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 -NO_2, -Cl, and electron donating group like -OCH_3, -OH were found to increase the antimicrobial properties, whereas electron donating group like -CH_3 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 (-NO_2 and -Cl) and electron-donating (-OCH_3, -OH and -CH_3) groups are enhanced biological activity.

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