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Synthesis, antimicrobial activity and cytotoxicity of 2-substituted benzimidazole incorporated with thiazole

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Two new series 2-(2-(1-(1-methyl-1*H*-benzo[*d*]imidazol-2-yl)ethylidene)hydrazinyl)-4-(aryl)thiazoles **3a-k** and 2-(2-(1-(1-methyl-1*H*-benzo[*d*]imidazol-2-yl)ethyl)hydrazinyl)-4-(aryl)thiazoles **4a-k** encompassing benzimidazole incorporated with thiazole have been synthesized using rational approach. These thiazole derivatives **3a-k** have been reduced at the site of formation of Schiff base (>CH=N0) to obtain final compounds **4a-k**. Compounds **3a-k** and **4a-k** have been screened for their *in vitro* antibacterial and antifungal actions against four strains each. Among the screened compounds, **3d, 3e, 3f, 3g, 3j, 3k, 4d, 4e, 4f** and **4k** have emerged as highly effective antibacterial agents, while compounds **3d, 3e, 3f, 3k** and **4k** have come out as most potent antifungal molecules. Comparison of outcomes of antibacterial and antifungal screening of compounds **3a-k** and **4a-k** directed that antimicrobial potential of unreduced derivatives **3a-k** is higher than reduced derivatives **4a-k**. SAR study reveals that presence of halogen (-F, -Cl, -Br) substituents is accountable for significant antimicrobial potential. Also the results of preliminary MTT cytotoxicity assay on HeLa cells indicates that antimicrobial activity of **3e, 3f, 3g, 3j, 3k, 4e, 4f** and **4k** is accompanied by low extent of cytotoxic concentrations.

Keywords: 2-Substituted benzimidazole, thiazole, antibacterial activity, antifungal activity, cytotoxicity

As resistance to antimicrobial drugs is widespread; there is an increase necessity for the recognition of novel structures which could lead to the design of new, potent and less toxic antimicrobial agents. The chemistry and pharmacology of benzimidazoles have been of great interest to medicinal chemistry[1] because its derivatives possessed countless biological activities such as antioxidant[2,3], antimicrobial[4], anthelmintic[2], anticancer[5,6], antihypertensive[7], anti-HIV[8], anti-inflammatory[9], analgesic[10,11], antiprotozoal[12] and anti-hepatitis B virus activity[13]. A variety of benzimidazoles are in use, like thiabendazole and flubendazole (anthelmintic), omeprazole and lansoprazole (antiulcerative) and astemizole (antihistaminic).

On the other hand thiazole system has found broad application in drug development for the treatment of inflammation[14], hypertension[15], bacterial infection[16], HIV infections[17] and cancer[18,19]. The thiazolium ring present in vitamin B1 serves as an electron sink, and its coenzyme form is important for the decarboxylation of α-keto acids[20]. It has been reported that 2,4-disubstituted thiazoles possess very good antitumor[21], antioxidative[22], antiviral[22] and antifungal[23] activities.

The benzimidazoles and thiazoles still remain one of the most versatile classes of compounds against microbes and therefore, are useful substructures for further molecular exploration. Keeping the above facts in view, we deliberated it of interest to synthesize 2-substituted benzimidazole incorporated with thiazoles for their antimicrobial activity. We have synthesized a series of thiazoles bearing *N*-methyl benzimidazole by replacing the quinoline motif in our previously synthesized compounds[24] **5a-g** and **6a-g** and screened them for their antibacterial property. In this attempt, we got excellent antibacterial results. Structural relevance of title compounds **3a-k** and **4a-k** with previously synthesized compounds is shown in Figure 1.

Induced by above indicated observations, we have planned to synthesize molecules having benzimidazole and thiazole entities possessing noteworthy pharmacological properties. In continuation of our research for new, discerning, nontoxic, safe and efficient antimicrobial agents, we studied the antimicrobial potential of the title molecules against vast range of different human pathogenic micro-organisms to evaluate concrete SAR results. Additionally cytotoxicity studies were

also carried out on HeLa cell lines to evaluate the capability of these compounds to obstruct the cell proliferation.

Result and Discussion

The multiple reaction sequences employed to synthesize title compounds are shown in Scheme I. Compound **1** which is starting material was prepared by following already reported experimental procedure[25,26]. Treatment of 1-(1-methyl-1*H*-benzo[*d*]imidazol-2-yl)ethenone **1** with thiosemicarbazide in presence of methanol as a solvent under stirring at 50°C gave intermediate 2-(1-(1-methyl-1*H*-benzo[*d*]imidazol-2-yl)ethylidene) hydrazinecarbothioamide**2** in good yield and high purity.

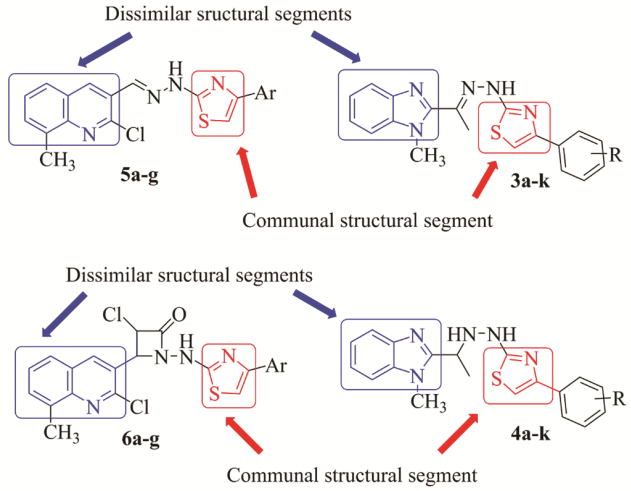
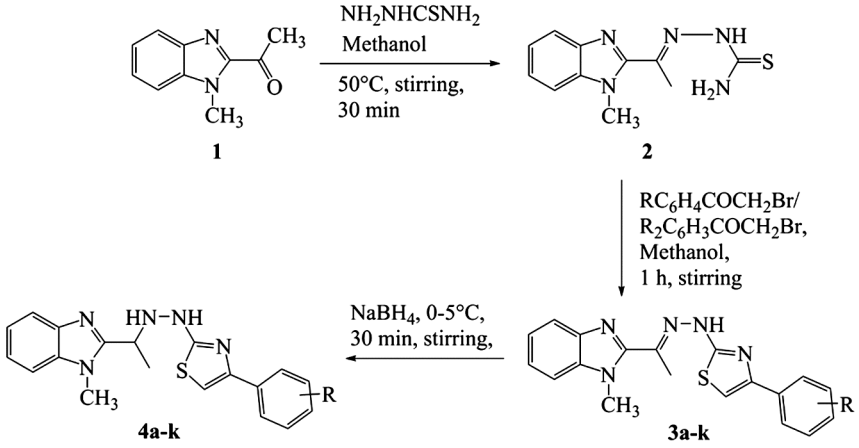


Figure 1 — Structural relevance of title compounds **3a-k** and **4a-k** with previously synthesized compounds **5a-g** and **6a-g**respectively

Presence of *N*-substituted thiourea or thiosemicarbazone as a terminal fragment in **2** makes it a versatile precursor for the Hantzsch thiazole synthesis with aromatic α-haloketones in alcoholic medium under stirring for 1 h to obtain 2-(2-(1-(1-methyl-1*H*-benzo[*d*]imidazol-2-yl)ethylidene)hydrazinyl)-4-(aryl)thiazoles **3a-k** in high yield without any further purification.

Finally the title molecules 2-(2-(1-(1-methyl-1*H*-benzo[*d*]imidazol-2-yl)ethyl)hydrazinyl)-4-(aryl)thiazoles **4a-k** was conveniently obtained by reduction of compounds **3a-k** using sodium borohydride as a selective reducing agent under stirring for half an hour at 0-5°C to subside exothermic addition of sodium borohydride.Synthetic route and reaction conditions employed for the synthesis of compounds **3a-k** and **4a-k** has been given in Scheme I. The structures of all the newly synthesized compounds were established by elemental analysis, FTIR, ^{1}H and ^{13}C NMR and mass spectral studies.

The investigation of antibacterial and antifungal screening data revealed that all the tested compounds **3a-k** and **4a-k** showed very good to moderate inhibition at 6.25–12.5µg/mL in DMSO. The compounds **3d, 3e, 3f, 3g, 3k, 4e, 4f** and **4k** showed



3a. R = H
3b. R = *p*-CH_3
3c. R = *p*-OCH_3
3d. R = *o*-Cl
3e. R = *p*-Cl
3f. R = *p*-Br
3g. R = *p*-F
3h. R = *p*-NO_2
3i. R = *p*-CN
3j. R = 2,4-Cl
3k. R = 2,4-F

4a. R = H
4b. R = *p*-CH_3
4c. R = *p*-OCH_3
4d. R = *o*-Cl
4e. R = *p*-Cl
4f. R = *p*-Br
4g. R = *p*-F
4h. R = *p*-NO_2
4i. R = *p*-CN
4j. R = 2,4-Cl
4k. R = 2,4-F

Scheme I — Synthetic route for compounds **3a-k** and **4a-k**

comparatively very good activity against all the bacterial strains with respect to standard drug ciprofloxacin. This high activity profile is attributed to the presence of halogen functions (-F, -Cl, -Br) to the phenyl ring at position 4 of thiazole ring. The compounds **3c,3i, 4g, 4h, 4i** and **4j** exhibited moderate antibacterial activity compared to that of standard against all the bacterial strains. Compound **3j**showed very good activity against *Escherichia coli*. The compound **4d** showed very good activity against *Pseudomonas aeruginosa* and *Pseudomonas aureus*. The compounds **3d, 3e, 3f, 3g, 3k** and **4k** showed comparatively very good activity against all the fungal strains. The compounds **3b, 3c, 3i, 3j, 4d, 4e, 4f,** and **4j** exhibited moderate activity against all fungal strains. Results showed that combination of benzimidazole with thiazole ring gave an enhanced biological effect against all the bacterial strains. Overall decrease in antibacterial and antifungal activity with respect to compounds **3a-k** has been observed in compounds **4a-k** which were obtained by reduction of the CH=N- fragment of compounds **3a-k**. Comparison of MIC for antibacterial activity with respect to standard drug ciprofloxacin and antifungal activity with respect to standard drug ciclopiroxolamine of compounds **3a-k** and **4a-k** is shown in Figure 2 and Figure 3 respectively.

Result of Cytotoxicity Studies

The synthesized novel molecules **3a-k** and **4a-k** were evaluated for *in vitro* cytotoxicity against human cervical cancer cell line (HeLa) by the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay[31], which measures the reduction of tetrazolium bromide salt into a formazan dye by mitochondrial dehydrogenases in treated *versus* untreated cells. Outcome of this assay unquestionably indicate that compounds **3e, 3f, 3g, 3j, 3k, 4e, 4f** and **4k** do not acquire cytotoxicityatconcentration of 100 µM (IC50 >100 µM). All other molecules own moderate toxicity against HeLa cell lines. It was ensured that none of the tested molecules acquire noteworthy cytotoxicity effect on HeLa cell lines, indicating that compounds

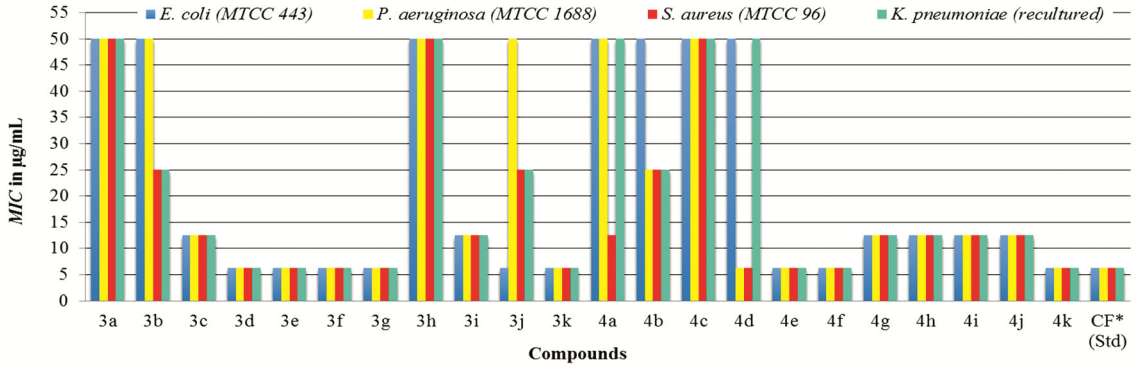


Figure 2 — Comparison of *MIC* (µg/mL) for antibacterial activity of the compounds **3a-k** and **4a-k** with respect to standard drug ciprofloxacin.

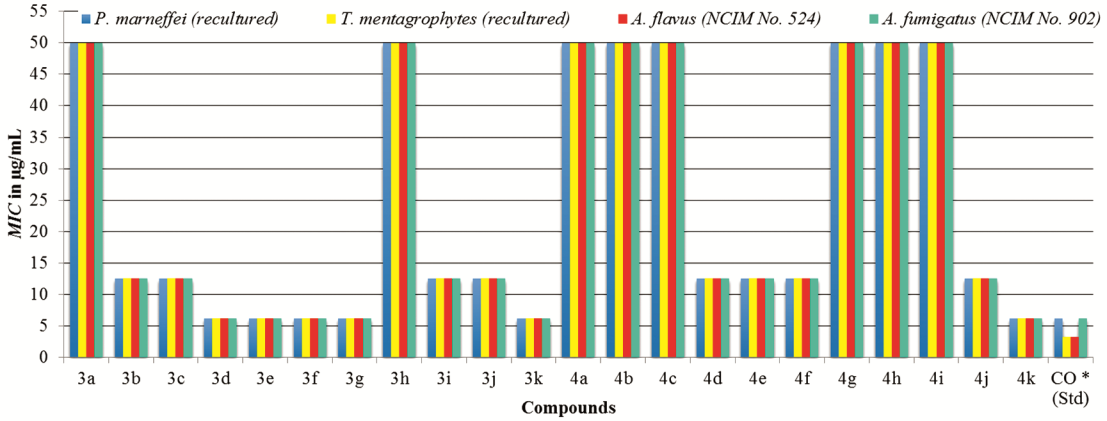


Figure 3 — Comparison of *MIC* (µg/mL) for antifungal activity of the compounds **3a-k** and **4a-k** with respect to standard drug Ciclopiroxolamine

were promising candidates for *in vivo* use as antimicrobial agents.

Structure activity relation study

From the results of antimicrobial potential, it can be concluded that reduced derivatives possess lover antimicrobial activity than unreduced derivatives. A study of structure–activity relationship revealed that the presence of a halogen (-F, -Cl, -Br) function at 2nd and 4th position of the phenyl ring as seen with compounds **3d, 3e,3f, 3j, 3k, 4e, 4f** and **4k** was found to be the main structural requirement for retaining antimicrobial profile. Antimicrobial potential of these compounds is also supported by lower level of cytotoxicity. Further compound **3d** is found to be very good active against microbial strain but possess moderate cytotoxicity. Based on this observation it can be summerise that presence of halogen substituent at *ortho* position is responsible for moderate cytotoxicity and the same on either *para* or *ortho* and *para* are accounted for lower level of cytotoxicity.

Material and Method

All the chemicals were purchased from Sigma Aldrich, Merck and S. D. Fine chemicals-India. Commercial grade solvents were used and were distilled before use. Solvents used were extra dried. Elemental analysis (% C, H, N) data were obtained by using Perkin Elmer 2400-CHN analyzer, IR spectra were recorded on IR Thermo nicolet 200 using KBr pellets technique. ^{1}H NMR and spectra were recorded on BrukerAvance(400 MHz) and ^{13}C NMR were recorded on Varian Mercury-400, 100 MHzNMR Spectrometer by using TMS as an internal standard and $CDCl_3$ as solvent. Mass spectra were recorded on Schimadzu LC-MS 2010 spectrophotometer. Melting points were recorded on Gallenkamp apparatus and were uncorrected. TLC experiments were performed on alumina backed silica gel 60 F_{245} plates (E. Merck). Iodine and Ultraviolet (UV) lamp was used for visualisation of the spots on TLC.Chemical shifts are reported in ppm (δ). The signals are designated as follows: s, singlet; d, doublet; t, triplet; q, quartate; m, multiplet.

Experimental Section

Preparation of1-(1-methyl-1*H*-benzo[*d*]imidazol-2-yl)ethanone (1)

Already reported compound **1** as a starting material was synthesized according to literature method[25,26].

Preparation of 2-(1-(1-methyl-1*H*-benzo[*d*] imidazol-2-yl)ethylidene)hydrazinecarbothioamide (2)

To a solution of compound **1** (0.01 mole) in methanol was added thiosemicarbazide (0.01 mole) and the mixture was refluxed for 1 hr. The mixture was allowed to attain RT and poured onto crushed ice with stirring. The separated solid was filtered and washed with water. The solid was recrystallized from methanol.

Yield 80%; mp 168-170°C. IR (KBr) υ_{max}/cm^{-1}: 3355, 3460 (N-H, NH_2), 3129 (C-H, aromatic), 2930, 2855 (C-H, -CH_3), 1620 (C=N), 1250 (C=S); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.61 (s, 3H), 3.94 (s, 3H), 6.64 (s, 2H), 7.26 (d, *J* = 8.0 Hz, 2H), 7.60 (d, *J* = 7.6 Hz, 2H), 8.57 (s, 1H,); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 180, 150, 138.5, 136.8, 131.3, 121.2, 120.3, 112.4, 108.6, 32.1, 18.2; LC-MS *(m/z)*: 247 (M^+). Anal. calcd for $C_{11}H_{13}N_5S$: C-53.42, H-5.30, N-28.32; found: C-53.39, H-5.32, N-28.22.

General procedure for the synthesis of 2-(2-(1-(1-methyl-1*H*-benzo[*d*]imidazol-2-yl)ethylidene)hydrazinyl)-4-(aryl)thiazoles (3a-k)

To a solution of compound **2** (0.01 mole) and corresponding 2-bromo-arylethanone (0.01 mole) in methanol was stirred for 1 hr. The separated solid was filtered, air dried and recrystallized from chloroform as pale yellow solid. Reaction completion was monitored by TLC using 7:3 (n-hexane:ethyl acetate) as mobile phase. Characterization data of compounds **3a-k** are given below.

2-(2-(1-(1-methyl-1*H*-benzo[*d*]imidazol-2-yl)ethylidene)hydrazinyl)-4-phenylthiazole (3a)

Yield 77%; mp 130-132°C. IR (KBr) υ_{max}/cm^{-1}: 3373 (N-H, broad), 3150 (C-H, aromatic), 2955, 2886 (C-H, -CH_3), 1623 (C=N); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.63 (s, 3H, C-CH_3), 3.97 (s, 3H, N-CH_3), 6.72 (s, 1H, Ar-H of thiazole), 7.24 -7.61 (m, 9H, Ar-H), 8.93 (s, 1H, -NH);^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 15.6, 36.3, 107.0, 114.0, 119.4, 123.0, 125.3, 132.5, 133.4, 135.2, 138.4, 140.9, 145.5, 154.3, 159.4, 176.9; LC-MS *(m/z)*: 347 (M^+). Anal. calcd. For $C_{19}H_{17}N_5S$: C-65.60, H-4.85, N-20.10; Found: C-65.68, H-4.93, N-20.16%.

2-(2-((1-methyl-1*H*-benzo[*d*]imidazol-2-yl)ethylidene)hydrazinyl)-4-*p*-tolylthiazole (3b)

Yield 71%; mp 132-134°C. IR (KBr) υ_{max}/cm^{-1}: 3375 (N-H stretching, broad), 3151 (C-H, aromatic), 2953, 2884 (C-H, -CH_3), 1624 (C=N); ^{1}H NMR (400

MHz, $CDCl_3$, δ, ppm): 2.34 (s, 3H), 2.79 (s, 3H), 3.79 (s, 3H), 6.79 (s, 1H), 7.28 -7.67 (m, 8H), 8.50 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 15.4, 21.5, 36.5, 107.2, 114.4, 119.2, 123.3, 125.5, 132.3, 134.0, 135.4, 138.2, 140.4, 145.7, 154.1, 159.4, 176.9; LC-MS *(m/z)*: 361 (M^+). Anal. calcd. For $C_{20}H_{19}N_5S$: C-66.50, H-5.38, N-19.41; Found: C-66.46, H-5.30, N-19.37%.

4-(4-methoxyphenyl)-2-(2-((1-methyl-1*H*-benzo[*d*]imidazol-2-yl)ethylidene)hydrazinyl)thiazole (3c)

Yield 72%; mp 137-139°C. IR (KBr) υ_{max}/cm^{-1}: 3374 (N-H, broad), 3152 (C-H, aromatic), 2959, 2885 (C-H, -CH_3), 1626 (C=N); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.70 (s, 3H), 2.83 (s, 3H), 3.67 (s, 3H), 6.70 (s, 1H), 7.30-7.65 (m, 8H), 8.52 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 15.5, 36.3, 55.8, 107.0, 114.4, 116.2, 119.6, 123.4, 125.1, 126.3, 138.6, 140.4, 145.2, 154.2, 159.2, 176.2; LC-MS *(m/z)*: 377 (M^+). Anal. calcd. For $C_{20}H_{19}N_5OS$: C-63.62, H-5.10, N-18.50; Found: C-63.64, H-5.07, N-18.55%.

4-(2-chlorophenyl)-2-(2-((1-methyl-1*H*-benzo[*d*]imidazol-2-yl)ethylidene)hydrazinyl)thiazole (3d)

Yield 70%; mp 148-150°C. IR (KBr) υ_{max}/cm^{-1}: 3376 (N-H, broad), 3159 (C-H, aromatic), 2960, 2889 (C-H, -CH_3), 1629 (C=N), 736 (C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.61 (s, 3H), 3.65 (s, 3H), 6.65 (s, 1H), 7.21-7.64 (m, 8H), 8.55 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 15.6, 36.4, 107.1, 114.3, 119.3, 123.2, 128.7, 129.5, 132.3, 132.9, 134.2, 134.5, 136.6, 140.4, 145.6, 150.8, 159.5, 176.5, LC-MS *(m/z)*: 381 (M^+), 383 (M^{+2}). Anal. calcd. For $C_{19}H_{16}ClN_5S$: C-59.71, H-4.20, N-18.35; Found: C-59.76, H-4.22, N-18.34%.

4-(4-chlorophenyl)-2-(2-((1-methyl-1*H*-benzo[*d*]imidazol-2-yl)ethylidene)hydrazinyl)thiazole (3e)

Yield 70%; mp 151-153°C. IR (KBr) υ_{max}/cm^{-1}: 3379 (N-H, broad), 3157 (C-H, aromatic), 2953, 2887 (C-H, -CH_3), 1624 (C=N), 737 (C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.80 (s, 3H), 3.66 (s, 3H), 6.67 (s, 1H), 7.23-7.62 (m, 8H), 8.59 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 15.6, 36.1, 107.0, 114.4, 119.6, 123.4, 126.3, 131.1, 133.4, 134.2, 134.3, 140.9, 145.5, 154.3, 159.4, 176.9; LC-MS *(m/z)*: 381 (M^+), 383 (M^{+2}). Anal. calcd. For $C_{19}H_{16}ClN_5S$: C-59.72, H-4.19, N-18.36; Found: C-59.76, H-4.22, N-18.34%.

4-(4-bromophenyl)-2-(2-((1-methyl-1*H*-benzo[*d*]imidazol-2-yl)ethylidene)hydrazinyl) thiazole (3f)

Yield 69%; mp 150-152°C. IR (KBr) υ_{max}/cm^{-1}: 3372 (N-H, broad), 3156 (C-H, aromatic), 2952, 2885 (C-H, -CH_3), 1624 (C=N), 600 (C-Br); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.60 (s, 3H), 3.67 (s, 3H), 6.73 (s, 1H), 7.29-7.70 (m, 8H), 8.84 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 15.4, 36.2, 107.1, 114.3, 119.2, 123.0, 125.4, 130.6, 132.2, 132.3, 134.2, 140.0, 145.5, 154.2, 159.3, 176.6; LC-MS *(m/z)*: 427 (M^+), 429 (M^{+2}). Anal. calcd. For $C_{19}H_{16}BrN_5S$: C-53.60, H-3.80, N-16.40; Found: C-53.53, H-3.78, N-16.43%.

4-(4-fluorophenyl)-2-(2-((1-methyl-1*H*-benzo [*d*]imidazol-2-yl)ethylidene)hydrazinyl)thiazole (3g)

Yield 65%; mp 149-151°C. IR (KBr) υ_{max}/cm^{-1}: 3380 (N-H, broad), 3155 (C-H, aromatic), 2957, 2882 (C-H, -CH_3), 1633 (C=N), 1020 (C-F); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.75 (s, 3H), 3.68 (s, 3H), 6.80 (s, 1H), 7.26-7.64 (m, 8H), 8.81 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 15.2, 36.4, 107.0, 114.3, 117.2, 119.4, 123.1, 130.4, 132.8, 134.8, 140.2, 145.6, 154.2, 159.2, 164.7, 1768; LC-MS *(m/z)*: 365 (M^+), 367 (M^{+2}). Anal. calcd. For $C_{19}H_{16}FN_5S$: C-62.50, H-4.37, N-19.13; Found: C-62.45, H-4.41, N-19.16%.

2-(2-((1-methyl-1*H*-benzo[*d*]imidazol-2-yl)ethylidene)hydrazinyl)-4-(4-nitrophenyl)thiazole (3h)

Yield 66%; mp 162-164°C. IR (KBr) υ_{max}/cm^{-1}: 3369 (N-H, broad), 3154 (C-H, aromatic), 2960, 2891 (C-H, -CH_3), 1620 (C=N), 1344, 1451 (-NO_2); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.66 (s, 3H), 3.67 (s, 3H), 6.82 (s, 1H), 7.32-7.72 (m, 8H), 8.82 (s, NH, 1H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 15.6, 36.4, 107.2, 114.4, 119.6, 123.2, 126.6, 128.4, 138.0, 141.1, 145.3, 147.7, 154.2, 159.4, 176.5; LC-MS *(m/z)*: 392 (M^+). Anal. calcd. For $C_{19}H_{16}N_6O_2S$: C-58.10, H-4.09, N-21.36; Found: C-58.15, H-4.11, N-21.42%.

4-(2-(2-((1-methyl-1*H*-benzo[*d*]imidazol-2-yl)ethylidene)hydrazinyl)thiazol-4-yl)benzonitrile (3i)

Yield 71%; mp 136-138°C. IR (KBr) υ_{max}/cm^{-1}: 3368 (N-H, broad), 3160 (C-H, aromatic), 2950, 2881 (C-H, -CH_3), 2241 (CN, sharp), 1636 (C=N);

^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.63 (s, 3H), 3.66 (s, 3H), 6.66 (s, 1H), 7.27-7.64 (m, 8H), 8.70 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 15.8, 36.5, 107.4, 114.6, 116.2, 119.8, 123.4, 126.3, 132.9, 138.6, 137.5, 140.7, 145.2, 154.4, 159.6, 176.2; LC-MS *(m/z)*: 372 (M^+). Anal. calcd. For $C_{20}H_{16}N_6S$: C-64.45, H-4.29, N-22.50; Found: C-64.50, H-4.33, N-22.56%.

4-(2,4-dichlorophenyl)-2-(2-((1-methyl-1*H*-benzo[*d*]imidazol-2-yl)ethylidene)hydrazinyl)thiazole (3j)

Yield 78%; mp 140-142°C. IR (KBr) υ_{max}/cm^{-1}: 3381 (N-H, broad), 3158 (C-H, aromatic), 2956, 2892 (C-H, -CH_3), 1637 (C=N), 743 (C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.45 (s, C-3H), 3.67 (s, 3H), 6.69 (s, 1H), 7.33-7.71 (m, 7H), 8.72 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 15.4, 36.3, 107.1, 114.3, 119.4, 123.2, 129.4, 130.2, 133.3, 133.9, 134.9, 136.4, 138.2, 140.0, 145.5, 151.0, 159.6, 176.0; LC-MS *(m/z)*: 415 (M^+), 417 (M^{+2}), 419 (M^{+4}). Anal. calcd. For $C_{19}H_{15}Cl_2N_5S$: C-54.78, H-3.60, N-16.78; Found: C-54.81, H-3.63, N-16.82%.

4-(2,4-difluorophenyl)-2-(2-((1-methyl-1*H*-benzo[*d*]imidazol-2-yl)ethylidene) hydrazinyl)thiazole (3k)

Yield 70%; mp 138-140°C. IR (KBr) υ_{max}/cm^{-1}: 3378 (N-H, broad), 3165 (C-H, aromatic), 2953, 2883 (C-H, -CH_3), 1635 (C=N), 1025 (C-F); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.50 (s, 3H), 3.69 (s, 3H), 6.71 (s, 1H), 7.27-7.80 (m, 7H), 8.88 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 15.8, 36.7, 104.1, 107.2, 114.2, 115.2, 119.5, 121.3, 123.3, 132.7, 136.4, 140.5, 141.7, 147.6, 154.3, 159.4, 161.1, 176.9; LC-MS *(m/z)*: 383 (M^+), 385 (M^{+2}). Anal. calcd. For $C_{19}H_{15}F_2N_5S$: C-59.48, H-3.90, N-18.30; Found: C-59.52, H-3.94, N-18.27%.

General procedure for the synthesis of 2-(2-(1-(1-methyl-1*H*-benzo[*d*]imidazol-2-yl) ethyl)hydrazinyl)-4-(aryl)thiazole (4a-k)

In a conical flask, compound **3a-k** (0.01 mole) was taken in methanol (50 mL) and cooled to 0-5°C in an ice bath on a magnetic stirrer. To this mixture, sodium borohydride (0.01 mole) was added portion wise within half an hour at 0-5°C. The resulting solid was filtered, washed with cold methanol (10 mL), dried and recrystallized from methanol. Reaction completion was monitored by TLC using 7:3 (n-hexane:ethyl acetate) as mobile phase. Characterization data of compounds **4a-k** are given below.

2-(2-(1-(1-methyl-1*H*-benzo[*d*]imidazol-2-yl)ethyl)hydrazinyl)-4-phenylthiazole (4a)

Yield 76%; mp 177-179°C. IR (KBr) υ_{max}/cm^{-1}: 3382 (N-H, broad), 3171 (C-H, aromatic), 2962, 2899 (C-H, -CH_3), 1591 (C=N, benzimidazole ring); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 1.54 (d, J = 12 Hz, 3H), 3.84 (s, 3H), 4.22 (q, J = 10 Hz, 1H), 4.65 (s, 1H), 6.62 (s, 1H), 6.85-7.31 (m, 9H), 7.52 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 21.6, 34.2, 60.3, 103.3, 114.0, 117.6, 123.0, 129.3, 130.7, 131.4, 135.0, 137.9, 145.7, 146.6, 154.8, 177.3; LC-MS *(m/z)*: 349 (M^+). Anal. calcd. For $C_{19}H_{19}N_5S$: C-65.40, H-5.45, N-20.11; Found: C-65.30, H-5.48, N-20.04%.

2-(2-(1-(1-methyl-1*H*-benzo[*d*]imidazol-2-yl)ethyl)hydrazinyl)-4-p-tolylthiazole (4b)

Yield 73%; mp 212-214°C. IR (KBr) υ_{max}/cm^{-1}: 3379 (N-H, broad), 3170 (C-H, aromatic), 2960, 2895 (C-H, -CH_3), 1594 (C=N, benzimidazole ring); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 1.57 (d, J = 11.8 Hz, 3H), 2.34 (s, 3H), 3.83 (s, 3H), 4.22 (q, J = 9.8 Hz, 1H), 4.64 (s, 1H), 6.67 (s, 1H), 7.23-7.58 (m, 8H), 7.62 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 21.8, 23.7, 34.4, 60.1, 103.3, 114.2, 117.8, 123.2, 127.7, 129.5, 132.4, 135.1, 137.7, 145.3, 146.5, 154.6, 177.1; LC-MS *(m/z)*: 363 (M^+). Anal. calcd. For $C_{20}H_{21}N_5S$: C-66.11, H-5.90, N-19.30; Found: C-66.09, H-5.82, N-19.27%.

4-(4-methoxyphenyl)-2-(2-(1-(1-methyl-1*H*-benzo[d]imidazol-2-yl)ethyl)hydrazinyl)thiazole (4c)

Yield 78%; mp 235-137°C. IR (KBr) υ_{max}/cm^{-1}: 3375 (N-H, broad), 3167 (C-H, aromatic), 2958, 2891 (C-H, -CH_3), 1592 (C=N, benzimidazole ring); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 1.56 (d, J = 11.5 Hz, 3H), 3.27 (s, 3H), 3.66 (s, 3H), 4.27 (q, J= 10.2 Hz, 1H), 4.61 (s, 1H), 6.63 (s, 1H), 6.81-7.22 (m, 8H), 7.55 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm):.6, 34.5, 55.4, 60.2, 105.3, 114.8, 116.6, 121.2, 123.0, 127.5, 132.3, 139.9, 145.7, 148.4, 154.6, 164.2, 177.0; LC-MS *(m/z)*: 379 (M^+). Anal. calcd. For $C_{20}H_{21}N_5OS$: C-63.36, H-5.55, N-18.41; Found: C-63.30, H-5.58, N-18.46%.

4-(2-chlorophenyl)-2-(2-(1-(1-methyl-1*H*-benzo[d]imidazol-2-yl)ethyl)hydrazinyl)thiazole (4d)

Yield 69%; mp 238-140°C. IR (KBr) υ_{max}/cm^{-1}: 3380 (N-H, broad), 3173 (C-H, aromatic), 2964, 2897 (C-H, -CH_3), 1594 (C=N, benzimidazole ring), 731 (C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 1.55 (d, J = 11.4 Hz, 3H), 3.66 (s, 3H), 4.29 (q, J = 10.1

Hz, 1H), 4.61 (s, 1H), 6.65 (s, 1H), 6.81-7.22 (m, 8H), 7.55 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 21.8, 34.6, 60.6, 105.8, 114.2, 117.6, 123.0, 130.1, 131.9, 132.3, 132.8, 134.4, 134.7, 137.9, 143.7, 146.2, 147.6, 177.2; LC-MS *(m/z)*: 383 (M^+), 385 (M^{+2}). Anal. calcd. For $C_{19}H_{18}ClN_5S$ C-59.37, H-4.65, N-18.18; Found: C-59.44, H-4.73, N-18.24%.

4-(4-chlorophenyl)-2-(2-(1-(1-methyl-1*H*-benzo [d]imidazol-2-yl)ethyl)hydrazinyl)thiazole (4e)

Yield 69%; mp 230-232°C. IR (KBr) υ_{max}/cm^{-1}: 3376 (N-H stretching, broad), 3168 (C-H, aromatic), 2959, 2892 (C-H, -CH_3), 1597 (C=N, benzimidazole ring), 729 (C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 1.73 (d, *J* = 11.5 Hz, 3H), 3.67 (s, 3H), 4.22 (q, *J*= 10.2 Hz, 1H), 4.62 (s, 1H), 6.64 (s, 1H), 6.80-7.20 (m, 8H), 7.53 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 21.6, 34.7, 60.6, 105.6, 114.8, 117.2, 123.2, 130.8, 131.5, 133.2, 136.3, 137.7, 145.5, 146.6, 154.4, 177.2; LC-MS *(m/z)*: 383 (M^+), 385 (M^{+2}). Anal. calcd. For $C_{19}H_{18}ClN_5S$ C-59.40, H-4.81, N-18.30; Found: C-59.44, H-4.73, N-18.24.

4-(4-bromophenyl)-2-(2-(1-(1-methyl-1*H*-benzo[d]imidazol-2-yl)ethyl)hydrazinyl)thiazole (4f)

Yield 68%; mp 297-300°C. IR (KBr) υ_{max}/cm^{-1}: 3386 (N-H stretching, broad), 3175 (C-H, aromatic), 2961, 2896 (C-H, -CH_3), 1593 (C=N, benzimidazole ring), 610 (C-Br); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 1.74 (d, *J* = 11.2 Hz, 3H), 3.61 (s, 3H), 4.21 (q, *J* = 10.4 Hz, 1H), 4.63 (s, 1H), 6.67 (s, 1H), 6.80-7.18 (m, 8H), 7.56 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 21.8, 36.2, 60.3, 105.2, 114.6, 117.2, 123.2, 123.7, 130.7, 134.2, 134.9, 137.7, 143.3, 144.6, 154.3, 177.0; LC-MS *(m/z)*: 429 (M^+), 431 (M^{+2}). Anal. calcd. For $C_{19}H_{18}BrN_5S$ C-53.25, H-4.30, N-16.29; Found: C-53.28, H-4.24, N-16.35%.

4-(4-fluorophenyl)-2-(2-(1-(1-methyl-1*H*-benzo [d]imidazol-2-yl)ethyl)hydrazinyl)thiazole (4g)

Yield 72%; mp 288-290 oC. IR (KBr) υmax/cm-1: 3384 (N-H stretching, broad), 3176 (C-H, aromatic), 2965, 2900 (C-H, -CH_3), 1598 (C=N, benzimidazole ring), 1018 (C-F); 1H NMR (400 MHz, $CDCl_3$, δ, ppm): 1.74 (d, J = 11.5 Hz, 3H), 3.65 (s, 3H), 4.22 (q, J = 10.5 Hz, 1H), 4.62 (s, 1H), 6.69 (s, 1H), 6.82-7.24 (m, 8H), 7.57 (s, 1H); 13C NMR (100 MHz, $CDCl_3$, δ, ppm): 21.2, 36.6, 60.9, 107.2, 114.8, 118.6, 121.2, 123.5, 130.8, 132.4, 139.3, 143.9, 146.4, 154.3, 166.7, 177.8; LC-MS (m/z): 367 (M^+), 369 (M^{+2}). Anal. calcd. For $C_{19}H_{18}FN_5S$ C-62.22, H-5.00, N-19.13; Found: C-62.11, H-4.94, N-19.06%.

2-(2-(1-(1-methyl-1*H*-benzo[*d*]imidazol-2-yl)ethyl)hydrazinyl)-4-(4-nitrophenyl)thiazole (4h)

Yield 78%; mp 299-301°C. IR (KBr) υ_{max}/cm^{-1}: 3390 (N-H stretching, broad), 3173 (C-H, aromatic), 2968, 2895 (C-H, -CH_3), 1600 (C=N, benzimidazole ring), 1340, 1448 (-NO_2); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 1.74 (d, *J* = 11.6 Hz, 3H), 3.67 (s, 3H), 4.25 (q, *J* = 10.6 Hz, 1H), 4.63 (s, 1H), 6.68 (s, 1H), 6.86-7.25 (m, 8H), 7.56 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 21.8, 36.7, 60.0, 105.6, 114.8, 118.6, 123.8, 126.4, 128.4, 139.5, 141.7, 143.7, 146.2, 149.7, 154.7, 177.4; LC-MS *(m/z)*: 394 (M^+). Anal. calcd. For $C_{19}H_{18}N_6O_2S$ C-57.80, H-4.51, N-21.27; Found: C-57.85, H-4.60, N-21.31%.

4-(2-(2-(1-(1-methyl-1*H*-benzo[*d*]imidazol-2-yl)ethyl)hydrazinyl)thiazol-4-yl)benzonitrile (4i)

Yield 67%; mp 257-259°C. IR (KBr) υ_{max}/cm^{-1}: 3383 (N-H stretching, broad), 3180 (C-H, aromatic), 2962, 2896 (C-H stretching, -CH_3), 2238 (CN stretching, sharp), 1599 (C=N, benzimidazole ring); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 1.72 (d, *J* = 11.3 Hz, 3H), 3.66 (s, 3H), 4.23 (q, *J* = 10.3 Hz, 1H), 4.61 (s, 1H), 6.67 (s, 1H), 6.81-7.22 (m, 8H), 7.55 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 21.6, 36.6, 60.4, 105.6, 114.4, 116.2, 117.5, 118.8, 123.7, 126.5, 135.5, 137.6, 139.1, 145.4, 146.2, 154.8, 177.7; LC-MS *(m/z)*: 374 (M^+). Anal. calcd. For $C_{20}H_{18}N_6S$ C-64.21, H-4.80, N-22.40; Found: C-64.15, H-4.85, N-22.44%.

4-(2,4-dichlorophenyl)-2-(2-(1-(1-methyl-1*H*-benzo[*d*]imidazol-2-yl)ethyl)hydrazinyl) thiazole (4j)

Yield 65%; mp 261-263°C. IR (KBr) υ_{max}/cm^{-1}: 3387 (N-H stretching, broad), 3179 (C-H, aromatic), 2967, 2899 (C-H, -CH_3), 1595 (C=N, benzimidazole ring),738 (C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 1.72 (d, *J* = 11.9 Hz, 3H), 3.66 (s, 3H), 4.23 (q, *J* = 10.1 Hz, 1H), 4.63 (s, 1H), 6.68 (s, 1H), 6.82-7.28 (m, 7H), 7.57 (s, 1H);^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 21.4, 36.8, 60.2, 105.8, 114.2, 117.2, 123.2, 129.2, 130.0, 132.1, 132.9, 135.9, 137.9, 136.1, 143.3, 144.2, 147.8, 177.3; LC-MS *(m/z)*: 417 (M^+), 419 (M^{+2}), 420 (M^{+4}). Anal. calcd. For $C_{19}H_{17}Cl_2N_5S$ C-54.58, H-4.14, N-16.70; Found: C-54.55, H-4.10, N-16.74%.

4-(2,4-difluorophenyl)-2-(2-(1-(1-methyl-1*H*-benzo [*d*]imidazol-2-yl)ethyl)hydrazinyl)thiazole (4k)

Yield 71%; mp 285-287°C. IR (KBr) υ_{max}/cm^{-1}: 3388 (N-H stretching, broad), 3178 (C-H, aromatic), 2977, 2896 (C-H, -CH_3), 1598 (C=N, benzimidazole ring),1024 (C-F); ^{1}H NMR (400 MHz, $CDCl_3$,

δ, ppm): 1.78 (d, *J* = 11.8 Hz, 3H), 3.68 (s, 3H), 4.27 (q, *J* = 9.9 Hz, 1H), 4.62 (s, 1H), 6.72 (s, 1H), 7.10-7.74 (m, 7H), 7.57 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 21.8, 37.0, 60.9, 104.7, 105.9, 114.8, 113.2, 117.8, 118.6, 123.6, 132.7, 137.9, 143.9, 144.8, 149.6, 161.1, 165.3, 179.0; LC-MS *(m/z)*: 385 (M^+), 387 (M^{+2}). Anal. calcd. For $C_{19}H_{17}F_2N_5S$ C-59.17, H-4.40, N-18.13; Found: C-59.21, H-4.45, N-18.17%.

Biological activities

Antibacterial studies

The series of newly synthesized compounds were screened for their antibacterial activity against *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 1688), *Staphylococcus aureus* (MTCC 96), and *Klebsiella pneumoniae* (recultured) bacterial strains by serial plate dilution method[27,28]. Serial dilutions of the drug in Mueller–Hinton broth were taken in tubes and their *p*H was adjusted to 5.0 using phosphate buffer. A standardized suspension of the test bacterium was inoculated and incubated for 16–18 h at 37°C. The minimum inhibitory concentration (MIC) was noted by seeing the lowest concentration of the drug at which there was no visible growth. A number of antimicrobial discs were placed on the agar for the sole purpose of generating zones of inhibition in the bacterial lawn. Twenty milliliters of agar media was poured into each Petri dish. Excess of suspension was decanted and plates were dried by placing in an incubator at 37°C for an hour. Using an agar punch, wells were made on these seeded agar plates and minimum inhibitory concentrations of the test compounds in dimethyl sulfoxide (DMSO) were added into each labeled well. A control was also prepared for the plates in the same way using solvent DMSO. The Petri dishes were prepared in triplicate and maintained at 37°C for 3–4 days. Antibacterial activity was determined by measuring the diameter of inhibition zone. Activity of each compound was compared with ciprofloxacin as standard. Zone of inhibition was determined for **3a–k** and **4a–k** and the results are summarized in Table I.

Antifungal studies

A series of newly synthesized compounds were screened for their antifungal activity against

Table I — Antibacterial activity of the title compounds **3a-k** and **4a-k**

Compd	MIC † in μg/mL and zone of inhibition in mm			
	*E. coli*MTCC 443	*P. aeruginosa*MTCC 1688	*S. aureus*MTCC 96	*K. pneumoniae (recultured)*
3a	50 (<10)	50 (<10)	50 (<10)	50 (<10)
3b	50 (10–15)	50 (20-25)	25 (20-25)	25 (<10)
3c	12.5 (10–15)	12.5 (10–15)	12.5 (18-22)	12.5 (<10)
3d	6.25 (14–18)	6.25 (14-18)	6.25 (14–18)	6.25 (14–18)
3e	6.25 (14–18)	6.25 (20-25)	6.25 (14–18)	6.25 (14–18)
3f	6.25 (14–18)	6.25 (20-25)	6.25 (18-22)	6.25 (14–18)
3g	6.25 (14–18)	6.25 (20-25)	6.25 (14–18)	6.25 (14–18)
3h	50 (14–18)	50 (14-18)	50 (<10)	50 (<10)
3i	12.5 (10–15)	12.5 (10–15)	12.5 (14–18)	12.5 (14-18)
3j	6.25 (20–25)	50 (<10)	25 (14-18)	25 (10–15)
3k	6.25 (14–18)	6.25 (20-25)	6.25 (14–18)	6.25 (14–18)
4a	50 (14-18)	50 (<10)	12.5 (14-18)	50 (<10)
4b	50 (14–18)	25 (20-25)	25 (14–18)	25 (20-25)
4c	50 (<10)	50 (20-25)	50 (<10)	50 (<10)
4d	50 (14-18)	6.25 (20–25)	6.25 (20-25)	50 (14-18)
4e	6.25 (14–18)	6.25 (14-18)	6.25 (14–18)	6.25 (14–18)
4f	6.25 (14–18)	6.25 (20-25)	6.25 (14–18)	6.25 (20-25)
4g	12.5 (14–18)	12.5 (14-18)	12.5 (10–15)	12.5 (14-18)
4h	12.5 (10–15)	12.5 (14-18)	12.5 (14–18)	12.5 (<10)
4i	12.5 (14-18)	12.5 (20–25)	12.5 (14-18)	12.5 (<10)
4j	12.5 (14-18)	12.5 (10–15)	12.5 (14-18)	12.5 (14–18)
4k	6.25 (20–25)	6.25 (20–25)	6.25 (20-25)	6.25 (20-25)
Ciprofloxacin (Standard)	6.25 (30–40)	6.25 (25–33)	6.25 (30–40)	6.25 (23–27)

† The MIC values were evaluated at concentration range, 6.25–50 μg/mL. Above table shows the MIC values in μg/mL and the corresponding zone of inhibition in mm.

Aspergillus flavus (NCIM No. 524), *Aspergillus fumigates* (NCIM No. 902), *Penicillium marneffei* (recultured) and *Trichophyton mentagrophytes* (recultured) in DMSO by serial plate dilution method[29,30]. Sabourands agar media was prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 mL) and adjusting the *p*H to 5.7. Normal saline was used to make a suspension of spore of fungal strains for lawning. A loopful of particular fungal strain was transferred to 3 mL saline to get a suspension of corresponding species. Twenty milliliters of agar media was poured into each Petri dish. Excess of suspension was decanted and plates were dried by placing in incubator at 37°C for 1 h. Using a punch, wells were made on these seeded agar plates minimum inhibitory concentrations of the test compounds in DMSO were added into each labeled well. A control was also prepared for the plates in the same way using solvent DMSO. The Petri dishes were prepared in triplicate and maintained at 37°C for 3–4 days. Antifungal activity was determined by measuring the diameter of inhibition zone. Activity of each compound was compared with ciclopiroxolamine as standard. Zones of inhibition were determined for **3a-k** and **4a–k** and the results are summarized in Table II.

In vitro cytotoxicity assay

In vitro cytotoxicity activity of compounds **3a–k** and **4a-k** was measured by means of the IC_{50} using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay method. The IC_{50} determination was performed according to the National Committee for Clinical Laboratory Standards (NCCLS) recommendations. All compounds were dissolved in 0.1% DMSO with the stock concentration of 10 g/L, and diluted with medium freshly before drug administration. Cell lines were seeded into 96-well plates at density of 8×10^3 cells/well. After 24 h seeding, each compound dilution was added in duplicate and incubation continued at 37°C in a humidified atmosphere containing 10% FBS, 1% glutamine, and 50 µM/mL gentamicin sulfate in a 5% CO2 and 95% air. After 24

Table II — Antifungal activity of title compounds **3a-k** and **4a-k**

Compd	MIC† in µg/mL and zone of inhibition in mm			
	P. marneffei (recultured)	*T. mentagrophytes (recultured)*	*A. flavus (NCIM No. 524)*	*A. fumigatus (NCIM No. 902)*
3a	50 (<10)	50 (<10)	50 (<10)	50 (<10)
3b	12.5 (18-22)	12.5 (18-22)	12.5 (18-22)	12.5 (18-22)
3c	12.5 (10–15)	12.5 (10–15)	12.5 (18-22)	12.5 (<10)
3d	6.25 (14–18)	6.25 (14-18)	6.25 (14–18)	6.25 (14–18)
3e	6.25 (14–18)	6.25 (20-25)	6.25 (14–18)	6.25 (14–18)
3f	6.25 (14–18)	6.25 (20-25)	6.25 (18-22)	6.25 (14–18)
3g	6.25 (14–18)	6.25 (20-25)	6.25 (14–18)	6.25 (14–18)
3h	50 (<10)	50 (<10)	50 (<10)	50 (<10)
3i	12.5 (18-22)	12.5 (18-22)	12.5 (18-22)	12.5 (18-22)
3j	12.5 (18-22)	12.5 (18-22)	12.5 (18-22)	12.5 (18-22)
3k	6.25 (14–18)	6.25 (20-25)	6.25 (14–18)	6.25 (14–18)
4a	50 (<10)	50 (<10)	50 (<10)	50 (<10)
4b	50 (14–18)	50 (20-25)	50 (14–18)	50 (20-25)
4c	50 (<10)	50 (<10)	50 (<10)	50 (<10)
4d	12.5 (18-22)	12.5 (18-22)	12.5 (20-25)	12.5 (18-22)
4e	12.5 (18-22)	12.5 (18-22)	12.5 (18-22)	12.5 (18-22)
4f	12.5 (18-22)	12.5 (18-22)	12.5 (18-22)	12.5 (18-22)
4g	50 (<10)	50 (<10)	50 (<10)	50 (<10)
4h	50 (<10)	50 (<10)	50 (<10)	50 (<10)
4i	50 (<10)	50 (<10)	50 (<10)	50 (<10)
4j	12.5 (<10)	12.5 (<10)	12.5 (<10)	12.5 (<10)
4k	6.25 (25–30)	6.25 (25–30)	6.25 (30-37)	6.25 (30-37)
Ciclopiroxolamine (standard)	6.25 (20–27)	3.25 (27–33)	3.25 (25–30)	6.25 (25–30)

† The MIC values were evaluated at concentration range, 3.25–50 µg/mL. Above table shows the MIC values in µg/mL and the corresponding zone of inhibition in mm.

Table III — Results of cytotoxic activities of the tested compounds **2**, **3a-k** and **4a-k**

Compd	Cytotoxicity (IC_{50}) Hela	Compd	Cytotoxicity (IC_{50}) Hela
2	2.2214	**4a**	68
3a	57.20	**4b**	60.20
3b	62.30	**4c**	62
3c	80	**4d**	71
3d	65.30	**4e**	>100
3e	>100	**4f**	>100
3f	>100	**4g**	73
3g	75	**4h**	82
3h	79	**4i**	90
3i	80	**4j**	62
3j	>100	**4k**	>100
3k	>100	Doxorubicin	3.24

h, 20 μL MTT reagent was added at 5 mg/mL in PBS (filter sterilized, light protected, and stored at 4°C) per well, and after 4 h of incubation at 37°C, MTT was converted to a blue formazan product by mitochondrial succinated hydrogenase. This product was eluted from cells by addition of 150 mL of DMSO. The absorbance at 570 nm was determined by an ELISA using a ELX800 microplate spectrophotometer. The IC_{50} value was defined as the concentration at which 50% of the cells could survive. IC_{50} values obtained for these compounds are shown in Table III.

Conclusion

By choosing proper experimental conditions we have been able to synthesize 2-(2-(1-(1-methyl-1*H*-benzo[*d*]imidazol-2-yl)ethylidene)hydrazinyl)-4-(aryl) thiazoles **3a-k** and 2-(2-(1-(1-methyl-1*H*-benzo[*d*]imidazol-2-yl)ethyl)hydrazinyl)-4-(aryl)thiazoles **4a-k** in good yield and less reaction time under simple reaction condition without any auxiliary purification and investigate for antimicrobial assay and cytotoxicity assay with the hope of discovering new structure leads serving aspotential broad spectrum pharmacological agents. Results of their antibacterial and antifungal screening studies revealed that all the newly synthesized compounds exhibited moderate to very good activity against pathogenic strains. Among all the synthesized compounds, **3d, 3e, 3f, 3g, 3k, 4e, 4f** and **4k** acquire very good antibacterial potential, while compounds **3d, 3e, 3f, 3g, 3k** and **4k** emerged as highly potential antifungal agents at low level of cytotoxic concentration. Structure activity relationship studies indicate that antimicrobial potential of unreduced derivatives **3a-k** is higher than reduced derivatives **4a-k** andpresence of halogen substituents (-F -Cl, -Br) in phenyl ring which is attached at 4th position of the thiazole ring results in enhancement of the antibacterial and antifungal activity. Cytotoxicity study pointed out that location of halogen substituent either on *para* or *ortho* and *para* position make them safe candidate without any cytotoxicity effect, whereas the same on *ortho* position has lower IC_{50} value. These results conclude that incorporation of two distinct heterocycles *viz* benzimidazole and thiazole has enhanced the activity and hence they are ideally fitted for further remodelling to attain more adequate antibacterial as well antifungal compounds.Further structural alterations might lead to the discovery of more efficient antimicrobial candidates.

Supplementary Information

Supplementary information is available in the website http://nopr.niscair.res.in/handle/123456789/60.

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