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Research Article

Synthesis, Characterization, and Biological Activity of Novel Schiff and Mannich Bases of 4-Amino-3-(N-phthalimidomethyl)-1,2,4-triazole-5-thione

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The present work describes the syntheses and antimicrobial activity studies of a series of novel Schiff bases (**4a–4i**) and their Mannich bases (**5a–5h**) starting from 4-amino-3-(N-phthalimido-methyl)-1,2,4-triazole-5-thione (**3**). All the synthesized compounds were characterized by IR, ^{1}H-NMR, ^{13}C-NMR, and MS. All the synthesized compounds were screened for four Gram-negative strains, one Gram-positive strain of bacteria, and one diploid fungal strain. In general the antimicrobial activity increased remarkably on the introduction of azomethine functionality in parent triazole (**3**). The antimicrobial activity further improved when morpholine group was added to them except for *Enterobacter cloacae*, where loss of activity was observed. The results are promising and show that the fine tuning of the structures (**5a, 5b, 5e, 5f,** and **5h**) can lead to some new antimicrobial compounds.

1. Introduction

Many of the antibiotics presently in use are becoming ineffective due to the emergence of resistant microbial strains. It proves that the microbes are more intelligent than what is being anticipated by human beings, as they quickly develop mechanisms to intercept the antibiotic, thus making them ineffective. This situation demands the development of new antimicrobial agents which can deprive the microbes of their pathogenicity by novel multisite mechanisms of action [1–4]. The 1,2,4-triazole nucleus is the main structural unit of many medicines currently in market. Ribavirin (**1**), letrozole (**2**), fluconazole (**3**), itraconazole (**4**), and anastrozole (**5**) are a few to name which are currently in use as medicines (Figure 1). Many other 1,2,4-triazole derivatives are also known to possess pharmacological activities like antitubercular, anticonvulsant, anti-inflammatory, and analgesic activities [5–14]. It has been reported that triazoles are less susceptible to metabolic degradation and have higher target specificity and wider spectrum of activities as compared to imidazoles [15, 16]. Many heterocyclic systems having azomethine functionality are known to possess cytotoxic, antimicrobial, anticancer, and antifungal activities [17–21].

The literature reveals that the presence of morpholine or piperazine ring on a heterocyclic system contributes to enhanced pharmacological activities in many cases [22–24]. This could be attributed to the increased solubility of the compounds in aqueous solvents because of the formation of aminium salt [25]. These wide applications of 1,2,4-triazole Schiff and Mannich bases motivated us to synthesize new derivatives of 4-amino-3-(N-phthalimidomethyl)-1,2,4-triazole-5-thione and to test their potential as antibacterial and antifungal agents.

2. Experimental

General Comments. All reagents were purchased from Acros, Fluka, and Aldrich and used without further purification. All reactions were performed in standard glassware. All reactions were monitored by TLC plates precoated with silica gel Si 60 F_{254} from Merck and were visualized under UV lamp at $\lambda = 254$ nm. Melting points were determined with electrothermal apparatus, Gallenkamp and are uncorrected. IR spectra were recorded on PerkinElmer spectrophotometer by ATR technique. ^{1}H-NMR and ^{13}C-NMR spectra were

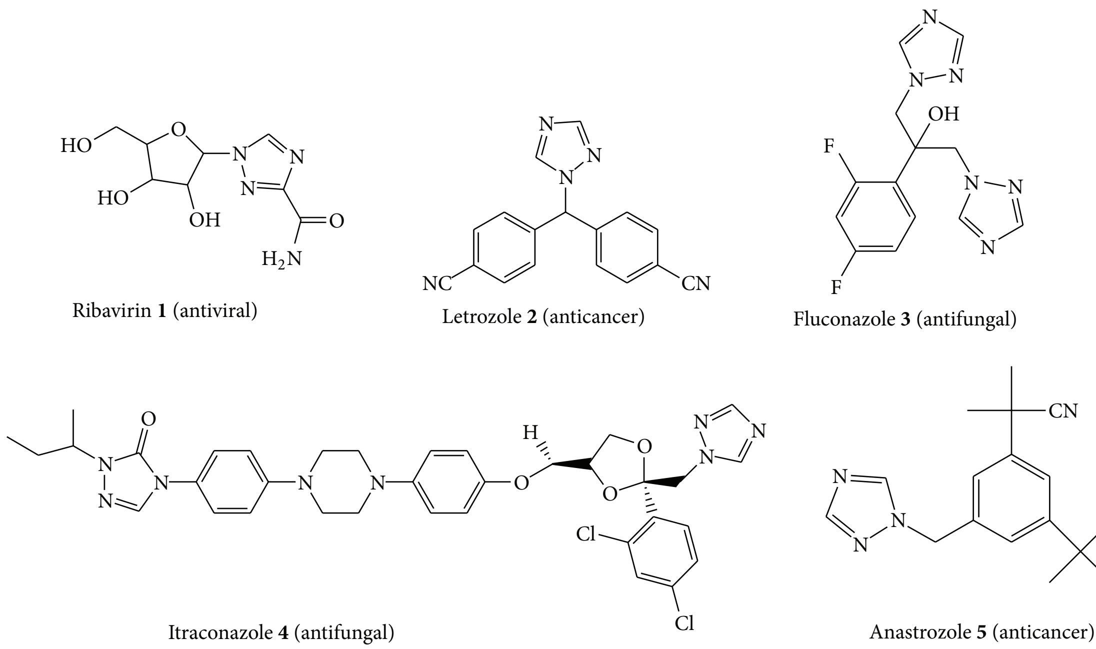


Figure 1: Some 1,2,4-triazole based medicine currently in use.

recorded on Burker 300 MHz/400 MHz instruments and 75 MHz/100 MHz instruments, respectively; the solvent used is specified along with data. Mass spectra were recorded on Jeol mass spectrometer in electron ionization mode. Leco 3200CHNS analyzer was used for elemental analysis. The thiocarbohydrazide (**2**) and the 4-amino-3-(N-phthalimidomethyl)-1,2,4-triazole-5-thione (**3**) were synthesized by the previously reported method [26, 27]. All manipulations of microbial activity were performed in laminar flow chamber (LFC) with disposable surgical gloves, all standard biosafety measures were taken, and contaminated materials after experimentation were collected in autoclave bags and autoclaved at 120°C before disposal.

*2.1. 4-Amino-3-(N-phthalimidomethyl)-1,2,4-triazole-5-thione (**3**).* To a clean test tube were added 0.01 mol (2.05 g) of N-phthaloylglycine and 0.01 mol (1.06 g) of thiocarbohydrazide, and the test tube was kept in a preheated oil bath. The molten mixture was kept at 145°C for 25 minutes. On cooling the mixture a solid mass was formed which was triturated with ethanol and filtered. The crude solid was recrystallized in acetonitrile and ethanol (1 : 1). Yield: 69%, white crystals, m.p. 189–192°C, IR (ATR) (ν cm^{-1}), 3305.08, 3152.47 (NH), 2982.12 (Ar-H), 2952.46 (CH_2), 1767.06 (cyclic amide), 1605 (C=N), 1145 (C=S). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 13.98 (b, 1H, NH), 7.86–7.94 (m, 4H, C_6H_4), 5.63 (s, 2H, NH_2), 4.84 (s, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 167.53 (C=S), 167.24 (C=O), 147.81 (N=C), 135.24 (N=C), (131.91, 123.86 (Ar-C)), 32.99 (CH_2). MS-E1 (*M/z*), Relative intensity: 275.1 (M+1, 100%), 259.1 (65%), 160.1 (80%), 130.1 (30%), 104.0 (45%), 77.1 (30%); $C_{11}H_9N_5O_2S$: Calculated: C, 47.99; H, 3.30; N, 25.44; S, 11.65. Found: C, 48.10; H, 3.35; N, 25.49; S, 11.85.

*2.2. General Procedure for Synthesis of Schiff Bases of 1,2,4-Triazole (3) (**4a–4i**).* Aromatic aldehyde (15 mmol) was dissolved in 8.0 mL of glacial acetic acid, and 10 mmol of 1,2,4-triazole (**3**) was added. The reaction mixture was refluxed for 25 minute to one hour in a preheated oil bath. The mixture was cooled, and the solid formed was filtered and washed with cold ethanol. The solid was recrystallized from ethanol and Schiff bases (**4a–4i**) were obtained in good to excellent yields.

*2.3. 4-(Benzylidene)amino-3-(N-phthalimidomethyl)-1,2,4-trazole-5-thione (**4a**).* Yield 42.8%, Yellow crystals m.p. 198–200°C. IR (ATR, ν cm^{-1}): 3283.40, 3156.39 (N–H), 3012.31 (Ar-H), 2996.00 (CH_2), 1770.75 (cyclic amide), 1599.79 (C=N), 1243.82 (C–N), 1120.06 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 14.02 (s, 1H, NH), 9.92 (s, 1H, N=CH), 7.88 (m, 4H, Ph), 7.80 (d, 2H, J = 7.2 Hz, Ph), 7.59 (m, 1H, Ph), 7.50 (m, 2H, Ph), 4.99 (s, 2H, CH_2). ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 167.47 (C=S), 164.39 (C=O), 162.48 (N=CH), 146.52 (C=N), (135.33, 133.23, 132.29, 131.81, 129.52, 129.10, 123.90 (Ar-C)), 33.17 (CH_2). MS-E1: (*m/z*, Relative intensity, %): 363.3 (20%), 260.2 (100%), 242.2 (24%), 228.2 (19%), 203.2 (5%), 183.2 (15.9%), 160.2 (28.8%), 148.2 (18.2%), 130.1 (24%), 104.1 (87%), 89.1 (7.1%), 76.1 (45%). $C_{18}H_{13}N_5O_2S$: Calculated: C, 59.49; H, 3.61; N, 19.27; S, 8.82. Found: C, 59.48; H, 3.66; N19.30; S, 8.84.

*2.4. 4-(2-Hydroxybenzylidene)amino-3-(N-phthalimidomethyl)-1,2,4-triazole-5-thione (**4b**).* Yield 73%, white crystals, m.p. 239–241°C. IR (ATR, ν cm^{-1}): 3216.31 (O–H), 3055.35 (Ar-H), 2997.4 (CH_2), 1774.02 (cyclic amide), 1601.09 (C=N), 1253.65 (C–N), 1119.56 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 13.95 (s, 1H, NH), 10.40 (s, 1H, OH), 10.13 (s, 1H, N=CH), 7.89 (m, 4H, Ph), 7.70 (dd, 1H, J = 7.9, 1.5 Hz,

Ph), 7.40 (dt, 1H, J = 7.8 Hz, 1.5 Hz, Ph), 6.94 (d, 1H, J = 8.1 Hz, Ph), 6.85 (t, 1H, J = 7.8 Hz, Ph), 4.97 (s, 2H, CH_2). ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 167.49 (C=S), 162.37 (C=O), 160.72 (N=CH), 158.90 (C–OH), 146.56 (C=N), (135.31, 134.80, 131.82, 127.28, 123.88, 119.88, 118.62, 117.03 (Ar-C)), 33.19 (CH_2). MS-E1 (m/z, Relative intensity, %): 379.3 (M^+, 2.7%), 347.4 (2.8%), 330.3 (5.2%), 260.2 (100%), 242.2 (8.6%), 228.2 (27.1%), 160.2 (53.4%), 148.2 (32.5%), 130.1 (57.3%), 119.1 (84.2%), 104.1 (85.3%), 91 (88%). $C_{18}H_{13}N_5O_3S$: Calculated: C, 56.98; H, 3.45; N, 18.46; S, 8.45. Found: C, 57.07; H, 3.54; N, 18.57; S, 8.57.

*2.5. 3-(N-Phthalimidomethyl)-4-(3-pyridine)amino-1,2,4-triazole-5-thione (**4c**).* Yield 76.05%, off-white crystals, m.p. 219–221°C. IR (ATR, ν cm^{-1}): 3041.73 (Ar-H), 2905.69 (CH_2), 1771.38 (cyclic amide), 1591.89 (C=N), 1302.07 (C–N), 1112.59 (C=S). ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 14.07 (s, 1H, NH), 10.13 (s, 1H, N=CH), 8.95 (d, 1H, J = 1.8 Hz, Ph), 8.75 (dd, 1H, J = 4.8, 1.5 Hz, Ph), 8.22 (d, 1H, J = 8.1 Hz, Ph), 7.88 (m, 4H, Ph), 7.55 (dd, 1H, J = 7.8 Hz, 4.8 Hz, Ph), 5.01 (s, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 167.0 (C=S), 162.05 (C=O), 160.88 (HC=N), 152.0 (Py-C), 151.0 (Py-C), 149.0 (C=N), (133.7, 132.2, 130.4, 123.9, 123.6, 123.0 (Ar-C)), 33.5 (CH_2). MS-E1 (m/z, Relative intensity, %): 364.0 (12.8%), 260.0 (100%), 242.0 (8.1%), 228.0 (4.6%), 183.0 (6.4%), 160.0 (16.9%), 148.0 (11.1%), 130.0 (16.1%), 104.0 (46.7%). $C_{17}H_{12}N_6O_2S$: Calculated: C, 56.04; H, 3.32; N, 23.06; S, 8.80. Found: C, 56.16; H, 3.45; N 23.10; S, 8.82.

*2.6. 4-(3-Nitrobenzylidine)amino-3-(N-phthalimidomethyl)-1,2,4-triazole-5-thione (**4d**).* Yield 48%, yellow powder, m.p. 258–260°C. IR (ATR, ν cm^{-1}): 3200 (N–H), 3041.73 (Ar-H), 2919 (CH_2), 1770.38 (cyclic amide), 1591.89 (C=N), 1302.07 (C–N), 1112.59 (C=S). ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 14.06 (s, 1H, NH), 10.22 (s, 1H, N=CH), 8.61 (s, 1H, Ph), 8.40 (d, J = 6.8 Hz, 1H, Ph), 8.24 (d, J = 6.5 Hz, 1H, Ph), 7.85 (m, 5H, Ph), 5.01 (s, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 166.87 (C=S), 162.08 (C=O), 160.84 (N=CH), 148.21 (C=N), (146.09, 134.71, 134.61, 133.58, 131.23, 130.66, 126.65, 123.29, 122.47 (Ar-C)), 32.58 (CH_2). MS-E1: m/z, (Relative intensity, %): 407.9 (7.3%), 260.0 (100%), 242 (5.1%), 228.0 (3.5%), 160.0 (13.3%), 148.0 (17.3%), 130.0 (11.0%), 104.0 (26.2%). $C_{18}H_{12}N_6O_4S$: Calculated: C, 52.94; H, 2.96; N, 20.58; S, 7.85. Found: C, 53.0; H, 3.10; N, 20.78; S, 8.02.

*2.7. 4-(4-Chlorobenzylidine)amino-3-(N-phthalimidomethyl)-1,2,4-triazole-5-thione (**4e**).* Yield 64.52%, white crystals, m.p. 241–244°C. IR (ATR, ν cm^{-1}): 3035.73 (Ar-H), 2956 (CH_2), 1772.18 (cyclic amide), 1611.24 (C=N), 1306.75 (C–N), 1118.19 (C=S). ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 13.99 (s, 1H, NH), 9.99 (s, 1H, N=CH), 7.84 (m, 4H, Ph), 7.81 (d, 2H, J = 8.4 Hz, Ph), 7.57 (d, 2H, J = 8.4 Hz, Ph), 4.97 (s, 2H, CH_2). ^{13}C NMR: (100 MHz, DMSO-d_6, δ ppm): 166.87 (C=S), 162.05 (C=O), 162.02 (N=CH), 145.99 (C=N), (137.29, 134.74, 131.25, 130.73, 130.14, 129.12, 123.32 (Ar-C)), 32.56 (CH_2). MS-E1: (m/z, Relative intensity, %): 397.3 (14.1%), 260.2 (100%), 242.2 (23.1%), 228.2 (14.7%), 183.2 (15.7%), 160.2 (26.6%), 148.1 (15%), 137.1 (77.6%), 104.1 (61.7%), 76.1 (24.8%). $C_{18}H_{12}ClN_5O_2S$: Calculated: C, 54.34; H, 3.04; N, 17.60; S, 8.06. Found: C, 54.45; H, 3.16; N, 17.62; S, 8.08.

*2.8. 4-(4-Bromobenzylidine)amino-3-(N-phthalimidomethyl)-1,2,4-triazole-5-thione (**4f**).* Yield 66.13%, white crystals, m.p. 215–217°C. IR (ATR, ν cm^{-1}): 3201 (N–H), 3041.73 (Ar-H), 2950.09 (CH_2), 1772.54 (cyclic amide), 1620.89 (C=N), 1302.07 (C–N), 1112.07 (C=S), 710 (C–Br). ^1H NMR (400 MHz, DMSO-d_6, δ ppm) 14.04 (s, 1H, NH), 9.73 (s, 1H, N=CH), 7.79 (m, 4H, Ph), 7.57 (d, 2H, J = 8.4 Hz, Ph), 7.40 (d, 2H, J = 8.4 Hz, Ph), 5.0 (s, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 167.12 (C=S), 163.68 (C=O), 159.10 (N=CH), 144.94 (C=N), (134.39, 132.24, 131.85, 131.32, 130.08, 127.15, 123.75 (Ar-C)), 32.88 (CH_2). MS-E1: (m/z, Relative intensity, %): 442.9 (3.7%), 274.0 (100%), 260.0 (84.1%), 241 (16.2%), 182.9 (28.7%), 148.0 (26.3%), 127.0 (51.1%), 104 (47.4%). $C_{18}H_{12}BrN_5O_2S$: Calculated: C, 48.88; H, 2.73; N, 15.8; S, 7.25. Found: C, 49.00; H, 2.88; N, 15.86; S, 7.29.

*2.9. 3-(N-Phthalimidomethyl)-4-(4-pyridine)amino-1,2,4-triazole-5-thione (**4g**).* Yield 48.02%, amorphous solid, m.p. 217–218°C. IR (ATR, ν cm^{-1}): 3012.18 (Ar-H), 2896.83 (CH_2), 1771.78 (cyclic amide), 1603.02 (C=N), 1268.54 (C–N), 1119.49 (C=S). ^1H NMR (300 MHz, DMSO-d_6, δ ppm): 13.90 (s, 1H, NH), 9.70 (s, 1H, N=CH), 8.66 (d, 2H, J = 7.9, Ph), 7.98 (d, 2H, J = 7.9 Hz, Ph), 7.80 (m, 4H, Ph), 4.98 (s, 2H, CH_2). ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 168.20 (C=S), 162.70 (C=O), 159.00 (N=CH), 152.20 (Py-C), 149.00 (Py-C), 144.30 (C=N), (132.2, 123.70, 123.10, 120.41 (Ar-C)), 37.23 (CH_2). MS-E1 (m/z, Relative intensity, %): 364.0 (10.8%), 260.0 (100%), 242.0 (6.0%), 228.0 (3.1%), 183.0 (5.1%), 160.0 (12.9%), 148.0 (6.8%), 130.0 (8.6%), 104.0 (34.3%). $C_{17}H_{12}N_6O_2S$: Calculated: C, 56.04; H, 3.32; N, 23.06; S, 8.80. Found: C, 56.13; H, 3.51; N, 23.10; S, 8.81.

*2.10. 4-(4-Methoxybenzylidine)amino-3-(N-phthalimidomethyl)-1,2,4-triazole-5-thione (**4h**).* Yield 36% white crystals, m.p. 239°C. IR (ATR, ν cm^{-1}): 3200 (Ar-H), 2919 (sp3 C–H), 1767 (C=O of anhydride), 1602 (C=N), 1279 (C=S) and 1255 (C–O). ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 13.91 (s, 1H, NH), 9.69 (s, 1H, N=CH), 7.87 (m, 4H, Ph), 7.7 (d, 2H, J = 8.8 Hz, Ph), 7.33 (2H, J = 8.8 Hz, Ph), 4.94 (s, 2H, CH_2), 3.83 (s, 3H, O–CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 166.87 (C=S), 163.95 (C=O), 162.84 (N=CH), 161.99 (C–OCH_3), 145.78 (C=N), (134.72, 131.26, 130.54, 124.16, 123.30, 114.46 (Ar-C)), 55.47 (O–CH_3), 32.61 (CH_2), MS-E1: (m/z, Relative intensity, %): 393.4 (26.0%), 361.4 (4.7%), 260.2 (100%), 242.2 (8.7%), 228.3 (17.8%), 186.2 (9.8%), 160.2 (32.2%), 148.2 (10.8%), 133.1 (100%), 104 (66.4%). $C_{19}H_{15}N_5O_3S$: Calculated: C, 58.01; H, 3.84; N, 17.80; S, 8.15. Found: C, 58.10; H, 4.01; N, 17.85; S, 8.20.

*2.11. 4-(4-Fluorobenzylidine)amino-3-(N-phthalimidomethyl)-1,2,4-triazole-5-thione (**4i**).* Yield 35%, light yellow crystals, m.p. 231–233°C. IR (ATR, ν cm^{-1}): 3200 (Ar-H), 2912 (sp^3 C–H), 1770 (C=O of anhydride), 1597 (C=N), 1279 (C=S). ^1H NMR (300 MHz, DMSO-d_6, δ ppm): 14.00 (s, 1H, NH),

9.93 (s, 1H, N=CH), 7.85 (m, 6H, Ph), 7.33 (t, 2H, J = 8.4 Hz, Ph), 4.98 (s, 2H, CH_2). ^{13}C NMR: (75 MHz, DMSO-d_6, δ ppm): 166.91 (C=S), 164.62 (d, $^1J_{C-F}$ = 249 Hz, C–F), 162.43 (C=O), 162.02 (N=CH), 145.99 (C=N), 134.76, 131.27, 131.17 ($^3J_{C-F}$ = 15 Hz), 128.46, 123.35, 116.21 ($^2J_{C-F}$ = 21.7 Hz), (Ar-C), 32.62 (CH_2). MS-E1 (m/z, Relative intensity, %): 381.0 (22.4%), 260.0 (100%), 242.0 (10.1%), 228.0 (4.5%), 183.0 (7.7%), 160.0 (18.4%), 148.0 (9.1%), 132.0 (9.3%), 104.0 (36.6%). $C_{18}H_{12}FN_5O_2S$: Calculated: C, 56.69; H, 3.17; N, 18.36; S, 8.41. Found: C, 56.74; H, 3.37; N, 18.39; S, 8.44.

*2.12. General Procedure for the Synthesis of Mannich Bases (**5a–5h**).* The corresponding Schiff bases (**4a–4h**) (10 mmol) were dissolved in acetonitrile: dioxane (2 : 1) at RT. Then, a solution of formaldehyde (37%, 0.5 mL) and morpholine (10 mmol) in ethanol was added dropwise with vigorous stirring. The reaction mixture was stirred at RT for 2 hours and left over night. The solid product was filtered and washed with ethanol and recrystallized from acetonitrile: dioxane (2 : 1) to yield title compound.

*2.13. 4-(Benzylideneamino)-1-(morpholinomethyl)-3-(N-phthalimidomethyl)-1,4-dihydro-5H-1,2,4-triazole-5-thione (**5a**).* Yield 72%, m.p. 208–210°C IR (ATR, ν cm^{-1}) 2958 (CH_2, asym), 2855 (CH_2, sym), 1771 (cyclic amide), 1591 (C=N), 1112 (C=S). ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 10.50 (s, 1H, N=CH), 7.91 (dd, 2H, J = 5.7 Hz, J = 3.0 Hz, Ph), 7.80 (m, 2H, Ph), 7.77 (dd, 2H, J = 5.7 Hz, J = 3.0 Hz, Ph), 7.44–7.57 (m, 3H, Ph), 5.10 (s, 4H, 2 × CH_2, N–CH_2–N, N–CH_2–C), 3.69 (b, 4H, morpholine), 2.79 (b, 4H, CH_2, morpholine). ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 167.20 (C=S), 163.70 (C=O), 161.06 (N=CH), 145.05 (C=N), (134.40, 132.52, 132.28, 131.87, 128.93, 128.88, 123.73 (Ar-C)), 69.08 (N–CH_2–N), (66.85, 50.68 (CH_2, morpholine)), 32.96 (CH_2). MS-E1: (m/z, Relative intensity, %): 462.1 (M+1, 20.6%), 363.0 (10.5%), 260.0 (76.6%), 242.0 (4.6%), 228.0 (5.7%), 185 (4.9%), 160.0 (27.5%), 100.1 (100%). $C_{23}H_{22}N_6O_3S$: Calculated: C, 59.73; H, 4.79; N, 18.17; S, 6.93. Found: C, 59.82; H, 4.85; N, 18.20; S, 6.99.

*2.14. 4-((2-Hydroxybenzylidene)amino)-1-(morpholinomethyl)-3-(N-phthalimidomethyl)-1,4-dihydro-5H-1,2,4-triazole-5-thione (**5b**).* Yield 74%, m.p. 214–216°C. IR (ATR, ν cm^{-1}): 2942 (CH_2, asym), 2849 (CH_2, sym), 1774 (cyclic amide), 1618 (C=N), 1111 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm) 10.43 (s, 1H, N=CH), 10.00 (s, 1H, OH), 7.88 (m, 4H, Ph), 7.66 (dd, 1H, J = 8.1 Hz, 1.5 Hz, Ph), 7.40 (t, 1H, J = 7.6 Hz, Ph), 6.94 (d, 1H, J = 8.1 Hz, Ph), 6.83 (t, 1H, J = 7.5 Hz, Ph), 5.03 (s, 2H, N–CH_2–N), 5.02 (s, 2H, N–CH_2–C), 3.53 (b, 4H, CH_2, morpholine), 2.64 (b, 4H, CH_2, morpholine). ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 167.48 (C=S), 163.17 (C=O), 161.99 (N=CH), 159.00 (C–OH), 145.35 (C=N), (135.30, 134.98, 131.82, 127.24, 123.90, 119.86, 118.41, 117.05 (Ar-C)), 69.00 (N–CH_2–N), (66.51, 50.63 (CH_2, morpholine)), 32.27 (CH_2). MS-E1 (m/z, Relative intensity, %): 478 (M^+, 3%), 359 (5%), 260 (31.8%), 185 (3.8%), 160 (12%), 119 (18.1%), 110.1 (100%). $C_{23}H_{22}N_6O_4S$: Calculated: C, 57.73; H, 4.63; N, 17.56; S, 6.70. Found: C, 57.8; H, 4.73; N, 17.57; S, 6.79.

*2.15. 1-(Morpholinomethyl)-4-((pyridin-3-ylmethylene)amino)-3-(N-phthalimidomethyl)-1,4-dihydro-5H-1,2,4-triazole-5-thione (**5c**).* Yield 69% m.p. 188–190°C IR (ATR, ν cm^{-1}): 2953 (CH_2, asym), 2848 (CH_2, sym), 1772 cyclic amide, 1591 (C=N), 1115 (C=S). ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 10.13 (s, 1H, N=CH), 8.94 (s, 1H, Ph), 8.73 (dd, 1H, J = 6.2 Hz, 1.5 Hz, Ph), 7.93–7.83 (m, 5H, Ph) 5.07 (s, 2H, N–CH_2–N), 5.03 (s, 2H, N–CH_2–C), 3.64 (b, 4H, CH_2, morpholine), 2.71 (b, 4H, CH_2, morpholine), ^{13}C NMR (300 MHz, $CDCl_3$, δ ppm): 167.13 (C=S), 163.75 (C=O), 157.17 (N=CH), 152.98 (Py-C), 150.62 (Py-C), 145.00 (C=N), (134.82, 134.45, 131.86, 128.57, 123.86, 123.73 (Ar-C)), 69.17 (N–CH_2–N), (66.87, 50.73 (CH_2, morpholine)), 32.85 (CH_2). MS-E1 (m/z, Relative intensity, %): 463 (M+1, 7.5%), 363.9 (5.0%), 260.0 (40.0%), 228.0 (5.3%), 160.0 (14.0%), 100.1 (100%). $C_{22}H_{21}N_7O_3S$: Calculated: C, 57.01; H, 4.57; N, 21.15; S, 6.92. Found: C, 57.10; H, 4.68; N, 21.20; S, 7.02.

*2.16. 1-(Morpholinomethyl)-4-((3-nitrobenzylidene)amino)-3-(N-phthalimidomethyl)-1,4-dihydro-5H-1,2,4-triazole-5-thione (**5d**).* Yield 80% m.p. 193–195°C (decomposed), IR (ATR, ν cm^{-1}): 2940 (CH_2, asym), 2840 (CH_2, sym), 1774 (amide), 1610 (C=N), 1115 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 10.01 (s, 1H, N=CH), 8.62 (t, 1H, J = 1.5 Hz, 1.8 = Hz, Ph), 8.42 (ddd, 1H, J = 8.1, 2.1 and 0.6 Hz, Ph), 8.23 (d, 1H, J = 7.8 Hz, Ph), 7.78–7.92 (m, 4H, Ph) 7.79 (d, 1H, J = 8.2 Hz, Ph), 5.07 (2H, N–CH_2–N), 5.06 (s, 2H, N–CH_2–C), 3.54 (b, 4H, CH_2, morpholine), 2.66 (b, 4H, CH_2, morpholine). ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 167.46 (C=S), 163.20 (C=O), 162.93 (N=CH), 148.71 (C=N), (145.42, 135.31, 133.91, 131.76, 131.25, 127.43, 123.91, 123.13 (Ar-C)), 69.12 (N–CH_2–N), (66.51, 50.62 (CH_2, morpholine)), 33.20 (CH_2). MS-E1: (m/z, Relative intensity, %): 507. (M^+, 1.9%), 408.0 (2.5%), 260.0 (21.6%), 185.0 (2.7%), 160.0 (12.2%), 130.0 (6.1%), 100.1 (100%). $C_{23}H_{21}N_7O_5S$: Calculated: C, 54.43; H, 4.17; N, 19.32; S, 6.32. Found: C, 54.53; H, 4.28; N, 19.35; S, 6.33.

*2.17. 4-((4-Chlorobenzylidene)amino)-1-(morpholinomethyl)-3-(N-phthalimidomethyl)-1,4-dihydro-5H-1,2,4-triazole-5-thione (**5e**).* Yield 79% m.p. 178–180°C IR (ATR, ν cm^{-1}): 2958 (CH_2, asym), 2855 (CH_2, sym), 1771 (cyclic amide), 1593 (C=N), 1112 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 9.85 (s, 1H, N=CH) 7.88 (m, 4H, Ph), 7.80 (d, 2H, J = 8.7 Hz, Ph), 7.57 (d, 2H, J = 8.4 Hz, Ph), 5.02 (s, 4H, 2 × CH_2, N–CH_2–N, N–CH_2–C), 3.52 (b, 4H, CH_2, morpholine), 2.63 (b, 4H, CH_2, morpholine). ^{13}C NMR (300 MHz, DMSO-d_6, δ ppm): 166.95 (C=S), 163.62 (C=O), 162.73 (N=CH), 144.84 (C=N), (137.53, 134.84, 131.30, 130.60, 130.21, 129.21, 123.43 (Ar-C)), 68.60 (N–CH_2–N), (66.01, 50.14 (CH_2, morpholine)), 32.69 (CH_2). MS-E1: (m/z Relative intensity, %): (496.0, M^+, 5.7%), 396.9 (5.1%), 260.0 (56.5%), 242.0 (2.7%), 228.0 (3.5%), 185.0 (4.1%), 160.0 (20.8%), 137.0 (29.0%), 100.1 (100%). $C_{23}H_{21}ClN_6O_3S$: Calculated: C, 55.59; H, 4.26; N, 16.91; S, 6.45. Found: C, 55.74; H, 4.37; N, 16.99; S, 6.47.

*2.18. 1-(Morpholinomethyl)-4-((4-bromobenzylidene)amino)-3-(N-phthalimidomethyl)-1,4-dihydro-5H-1,2,4-triazole-5-thione (**5f**).* Yield 39% m.p. 189–191°C IR (ATR, ν cm^{-1}): 2958

(CH_2, asym), 2854 (CH_2, sym), 1772, 1719 (cyclic amide), 1607 (C=N), 1112 (C=S). ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 10.56 (s, 1H, N=CH), 7.86 (dd, 2H, J = 5.4 Hz, 3.0 Hz, Ph), 7.74 (dd, 2H, J = 5.4 Hz, 3.0 Hz, Ph), 7.66 (d, 2H, J = 8.4 Hz, Ph), 7.56 (d, 2H, J = 8.4 Hz, Ph), 5.05 (s, 2H, N–CH_2–N), 5.04 (s, 2H, N–CH_2–C), 3.63 (b, 4H, CH_2, morpholine), 2.73 (b, 4H, CH_2, morpholine), ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 167.12 (C=S), 163.68 (C=O), 159.10 (N=CH), 144.94 (C=N), (134.39, 132.24, 131.85, 131.85, 131.32, 130.08, 127.15, 123.75, 123.68 (Ar-C)), 69.12 (N–CH_2–N), (66.84, 50.70 (CH_2, morpholine)), 32.88 (CH_2). MS-E1 (*m/z*, Relative intensity, %): 540 (M^+, 3.1%), 441 (3.1%), 260.0 (84.4%), 242.0 (3.7%), 182.9 (22.6%), 160.0 (26.7%), 100.1 (100%). $C_{23}H_{21}BrN_6O_3S$: Calculated: C, 51.02; H, 3.91; N, 15.52; S, 5.92. Found: C, 51.00; H, 3.99; N, 15.59; S, 6.02.

*2.19. 1-(Morpholinomethyl)-4-((pyridin-4-ylmethylene)amino)-3-(N-phthalimidomethyl)-1,4-dihydro-5H-1,2,4-triazole-5-thione (**5g**).* Yield 69% m.p. 201–203°C IR (ATR, ν cm^{-1}): 2953 (CH_2, asym), 2848 (CH_2, sym), 1770 (cyclic amide), 1602 (C=N), 1112 (C=S). ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 10.10 (s, 1H, N=CH), 8.65 (d, 2H, J = 7.8 Hz, Ph), 7.97 (d, 2H, J = 7.8 Hz, Ph), 7.80 (m, 4H, Ph), 5.07 (s, 2H, N–CH_2–N), 5.03 (s, 2H, N–CH_2–C), 3.64 (b, 4H, CH_2, morpholine), 2.71 (b, 4H, CH_2, morpholine). ^{13}C NMR (300 MHz, $CDCl_3$, δ ppm): 167.23 (C=S), 163.72 (C=O), 157.17 (N=CH), 152.97 (Py-C), 149.02 (Py-C), 145.40 (C=N), (133.82, 134.54, 131.80, 128.77, 122.96, 123.73 (Ar-C)), 70.02 (N–CH_2–N), (66.89, 50.77 (CH_2, morpholine)), 32.65 (CH_2). MS-E1 (*m/z*, Relative intensity, %): 463 (M+1), 363.9 (5.8%), 260.0 (72.4%), 228.0 (2.8%), 160.0 (32.2%), 104 (26.8%), 100.1 (100%). $C_{22}H_{21}N_7O_3S$: Calculated: C, 57.01; H, 4.57; N, 21.15; S, 6.92. Found: C, 57.09; H, 4.63; N, 21.26; S, 7.03.

*2.20. 4-((4-Methoxybenzylidene)amino)-1-(morpholinomethyl)-3-(N-phthalimidomethyl)-1,4-dihydro-5H-1,2,4-triazole-5-thione (**5h**).* Yield 82% m.p. 218–220°C IR (ATR, ν cm^{-1}): 2939 (CH_2, asym), 2840 (CH_2, sym), 1770 (amide), 1598 (C=N), 1171 (C=S). ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 10.21 (s, 1H, N=CH), 7.86 (dd, 2H, 5.4 Hz and 3.3 Hz, Ph), 7.72–7.74 (m, 4H, Ph), 6.93 (d, 2H, J = 8.4 Hz, Ph), 5.04 (s, 4H, 2 × CH_2, N–CH_2–N, N–CH_2–C), 3.85 (s, 3H, OCH_3), 3.65 (b, 4H, CH_2, morpholine), 2.73 (b, 4H, CH_2, morpholine), ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 167.15 (C=S), 163.68 (C=O), 162.64 (N=CH), 161.99 (C–OCH_3), 144.85 (C=N), (134.38, 134.31, 131.93, 130.79, 124.83, 123.74, 123.65, 114.38 (Ph-C)), (69.14, 66.87 (CH_2, morpholine)), 55.48 (O–CH_3), 50.71 (CH_2), 32.9. MS-E1: (*m/z*, Relative intensity, %): 492 (M^+, 5.9%), 393.0 (9.6%), 358.0 (3.8%), 260.0 (57.2%), 228.0 (2.9%), 185.0 (3.1%), 160.0 (23%), 133.0 (71.1%), 100.1 (100%). $C_{24}H_{24}N_6O_4S$: Calculated: C, 58.52; H, 4.91; N, 17.06; S, 6.51. Found: C, 58.59; H, 4.92; N, 17.08; S, 6.53.

3. Microbiology

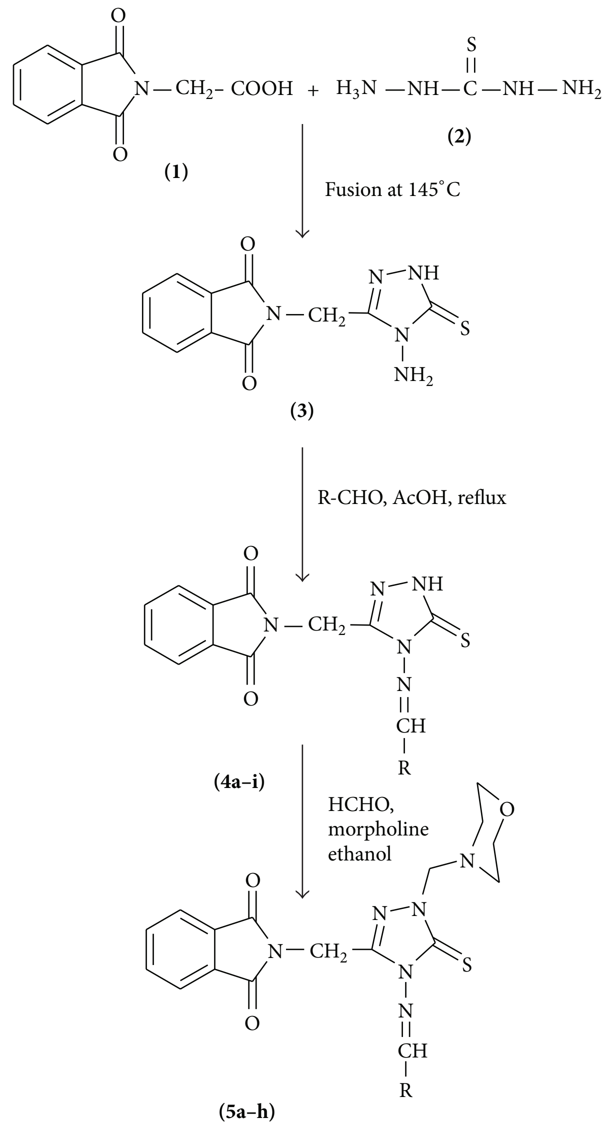
The antimicrobial activity of the compounds **3**, **4a–4i,** and **5a–5h** was tested on one Gram-positive strain (*Staphylococcus aureus*) ATCC25923, four Gram-negative strains (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 9027, *Enterobacter cloacae* 13047, and *Klebsiella pneumoniae* ATCC 13883), and one diploid fungus (*Candida albicans* ATCC SC5314). Levofloxacin, amikacin, and fluconazole were used as standards. Filter paper disc method was used to evaluate the microbial activity.

3.1. Methodology. 10 gm of nutrient agar broth was dissolved in 400 mL of distilled water. The media was autoclaved at 120°C for 2 hrs. The media was poured in sterilized petri plates up to 40 mL and then covered the plates with lids. The agar was allowed to set and harden. Filter papers of 5 mm diameter were cut and dipped in dilution of 100 μg/mL of each sample that is, **3**, **4a–4i**, **5a–5h**, and standards, and were placed on seeded agar. All stock solutions of tested compounds were made in DMSO. The bacterial culture was kept at 37°C, and fungal plates were kept at 18°C for 3-4 days. After incubation, the diameter of clear zone around the discs was measured and compared against zone of inhibition formed by solutions of known concentrations of standard antibiotics. All the tests were carried out in triplicate, and the results were averaged out.

4. Results and Discussion

4.1. Chemistry. The 1,2,4-triazole (**3**) was synthesized by the fusion of N-phthaloylglycine and thiocarbohydrazide by the method reported earlier by our group [27]. The Schiff bases were synthesized by refluxing the triazole (**3**) with corresponding aldehydes in glacial acetic acid; Figure 2 describes the synthetic scheme. All the synthesized compounds were characterized by spectroscopic analysis. In the H NMR spectra of Schiff bases (**4a–4i**), the two protons of CH_2 group of glycine gave a singlet in the range of 4.90–5.10 ppm. The most downfield signal around 14 ppm (NH) proves that the triazole exists in thione form when in solution. The proton of azomethine linkage (N=CH) gave a singlet downfield around 9.9–10.2 ppm. The chemical shifts for the four protons of phthalimido group are in the range of 7.89–7.79 ppm as a multiplet due to $AA'BB'$ spin system, whereas the para-disubstituted Schiff bases (**4f–4i**) give a doublet for each proton characteristic of an AB spin system in the range of 8.66–7.33 ppm. In C-13, the signal around 163 ppm is of carbonyl carbon of imide (C=O), and most down field signals around 167 ppm are for thione form (C=S). All other carbons are also well justified. The molecular ion could be seen in all the Schiff bases (**4a–4i**). In the IR spectra, characteristic absorption bands are visible for C=S and C=N groups in the range of 1110–1120 cm^{-1} and 1590–1600 cm^{-1}, respectively. The absorption for the C=O of cyclic amide appears around 1770 cm^{-1} in all compounds.

The Schiff bases (**4a–4h**) were reacted with formaldehyde in presence of morpholine to obtain the corresponding Mannich bases (**5a–5h**). The ^{1}H NMR spectra showed identical chemical shifts for the two methylene protons of phthalimidomethylene and the two morpholino methylene protons. The four protons either appeared as a one singlet in case of **5a**, **5e**, and **5h** or two singlets with a chemical shift



R:

4a, **5a** = phenyl
4b, **5b** = 2-hydroxyphenyl
4c, **5c** = 3-pyridyl
4d, **5d** = 3-nitrophenyl
4e, **5e** = 4-chlorophenyl
4f, **5f** = 4-bromophenyl
4g, **5g** = 4-pyridyl
4h, **5h** = 4-methoxyphenyl
4i = 4-fluorophenyl

Figure 2: Scheme for the synthesis of (**5a–5h**).

difference of only 0.01–0.03 ppm as in **5d**, **5f**, and **5g**. The methylenes of morpholine appeared as a set of two broad signals as shown in Figure 3. The methylenes of morpholine constitute A_2M_2 spin system which gives complex second order splitting pattern and is not easy to interpret. In case of morpholine ring, the rapid ring flipping at room temperature makes axial and equatorial protons of morpholine ring almost equivalent; therefore a broad signal showing some splitting as well is observed; however, it cannot be called a triplet as neither line intensity nor coupling constant values are justified for triplet. The C-13 data IR and mass spectral data are consistent with the structure.

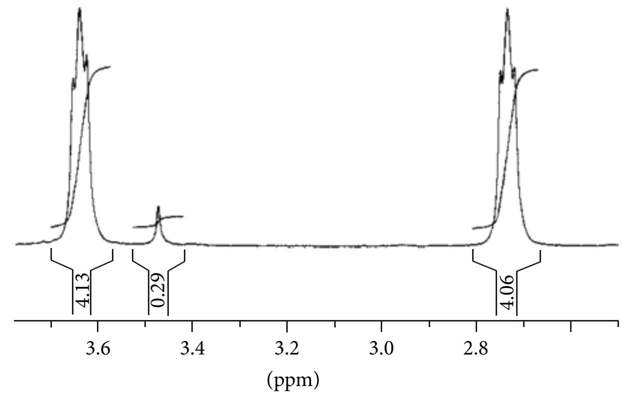


Figure 3: ^{1}H NMR signals for methylene protons of morpholine group.

4.2. Antimicrobial Activity Test. The antimicrobial activity of the 1,2,4-triazole (**3**) and the nine Schiff bases (**4a–4i**) derived from triazole (**3**) were tested on a Gram-positive strain (*Staphylococcus aureus*), four Gram-negative strains, (*Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, and *Klebsiella pneumoniae*), and one diploid fungus (*Candida albicans*). Levofloxacin, amakacine, and fluconazole were used as standards. A comparison of the microbial activity of the triazole (**3**) and its Schiff base derivatives (**4a–4i**) has been made. Furthermore, the Mannich bases (**5a–5h**) were also screened against the above mentioned strains. The results are shown in Table 1. The triazole (**3**) did not show any significant activity against the strains mentioned here; however, the introduction of azomethine linkage in all cases has improved the antimicrobial activity exceptions being **4h** and **4i**, where the activity remained almost the same. The best activity comparable to Levofloxacin was shown by **4b** and **4e** against *Enterobacter cloacae*, whereas compounds (**4a**, **4c**, **4d,** and **4g**) have displayed moderate activity against the same bacterial strain. **4a** and **4b** have demonstrated good activity and **4c**, **4g,** and **4f** moderate activity against *Klebsiella pneumoniae* with reference to levofloxacin and amikacin. Compounds (**4b**, **4c**, **4d**, **4f**, and **4g**) have displayed moderate activity against fungal strain, *Candida albicans*, in comparison to fluconazole. It has been observed [23] that the introduction of morpholine or piprazine ring increases the antimicrobial activity in many heterocyclic systems. For instance, itraconazole, eperezolid, and linezolid antibiotics possess a morpholine or piperazine ring. These ring functions increase the solubility in aqueous solvents when transformed into iminium salts, thus increasing the bioavailability of the compound. Keeping this in mind a morpholine group was introduced in compounds (**4a–4h**) the resulting Mannich bases (**5a–5h**) were also tested against the above mentioned six strains. In general antimicrobial activity in all cases significantly increased except for *Enterobacter cloacae,* where loss of activity was observed. Compound (**5h**) showed comparable activity to levofloxacin against *Escherichia coli* and *Klebsiella pneumoniae*. **5b**, **5d**, **5e**, and **5f** showed activity very close to levofloxacin against *Pseudomonas aeruginosa*. **4d** showed compareable activity to levofloxacin, however, its Mannich

Table 1: In vitro antimicrobial screening of compounds (mm) conc. 100 μg/mL.

Compound no.	Microorganisms and inhibition zone (mm)					
	Escherichia coli	*Pseudomonas aeruginosa*	*Enterobacter cloacae*	*Klebsiella pneumoniae*	*Staphylococcus aureus*	*Candida albicans*
3	7.00	8.00	9.68	9.40	7.00	9.95
4a	10.25	8.60	11.65	12.35	9.10	10.00
4b	8.52	9.65	14.42	11.20	8.10	11.75
4c	6.75	8.10	8.00	10.20	7.25	11.25
4d	7.38	9.30	9.60	9.83	8.00	10.76
4e	7.30	8.37	12.20	9.42	7.67	9.72
4f	8.21	8.43	9.33	10.70	9.42	10.45
4g	7.22	10.90	7.30	7.10	7.10	12.30
4h	6.55	6.95	7.30	7.44	8.0	7.82
4i	7.1	8.11	8.63	7.50	7.43	10.28
5a	13.2	13.20	—	11.10	12.60	14.30
5b	13.0	14.10	—	11.70	11.60	15.10
5c	7.00	10.00	9.00	11.00	11.00	13.20
5d	11.60	13.80	—	12.40	14.00	12.40
5e	12.50	14.80	—	12.00	14.10	13.70
5f	11.30	14.10	—	11.10	12.20	13.20
5g	—	—	—	—	10.00	12.20
5h	14.70	11.40	—	13.20	14.20	14.60
Levofloxacin	16.50	13.85	14.80	13.95	18.32	—
Fluconazole	—	—	—	—	—	17.25
Amikacin	17.70	12.75	16.30	14.20	19.20	—

base lost all activity. **5d**, **5e**, and **5h** showed significant increase in antimicrobial activity against *S. aureus*.

All compounds (**5a–5h**) showed improved activity against fungal strain *Candida albicans*; however, **5b** demonstrated best activity, and **5a** and **5h** also showed good activity. The results show that the compound **5h** is the most promising among the tested compounds. Our results strengthen the earlier findings by others that the presence of morpholine ring in heterocyclic molecules increases the antimicrobial activity. In conclusion the Mannich bases with electron donating substituents (–OH, $-OCH_3$) on phenyl ring **5b** and **5h** or with halogens on phenyl ring (**5e** and **5f**) showed best activity among the tested compounds. The results are promising and show that the fine tuning of the structures can lead to some new antibacterial compounds.

5. Conclusion

A series of novel Schiff bases and their Mannich bases were synthesized to study the structure activity relationship. All the synthesized compounds were screened for their antimicrobial activities against six strains. In general the antimicrobial activity increased remarkably on the introduction of azomethine functionality in parent triazole (**3**). The antimicrobial activity further improved when morpholine group was added to them except for *Enterobacter cloacae*, where loss of activity was observed. The results show that the compound (**5h**) is the most promising among the tested compounds. Our results strengthen the earlier findings by others that the presence of morpholine ring in heterocyclic molecules increases the antimicrobial activity. In conclusion the Mannich bases with electron donating substituents (–OH, $-OCH_3$) on phenyl ring (**5b** and **5h**) or with halogens on phenyl ring (**5e** and **5f**) showed the best activity among the tested compounds. The results are promising and show that the fine tuning of the structures (**5a**, **5b**, **5e**, **5f**, and **5h**) can lead to some new antimicrobial compounds.

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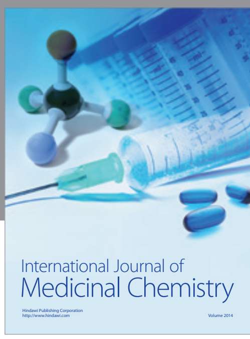

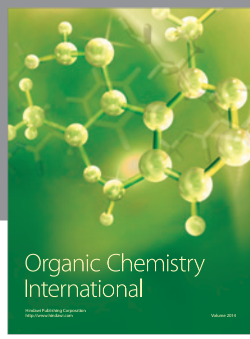

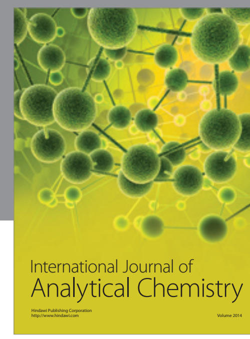

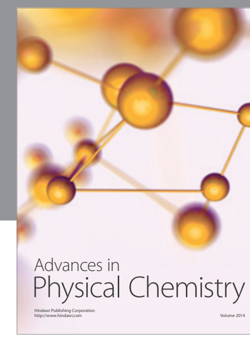

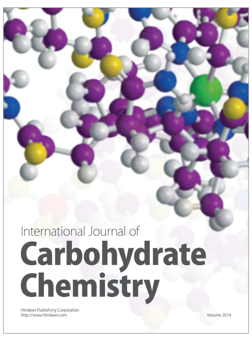

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