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Synthesis and Antimicrobial Activity of Novel 7-(Heteroaryl)-1,2,4-triazolo[1,5-a]-pyrimidine Derivatives

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The synthesis, characterization and antimicrobial activity of novel 1,2,4-triazolo[1,5-a]pyrimidines have been reported. The compounds were prepared by acid catalyzed condensation of 3-amino-1,2,4-triazole with 3-(dialkylamino)acryloalkanone.

Key Words: 1,2,4-Triazolo[1,5-a]pyrimidines, 3-Amino-1,2,4-triazole, 3-(Dialkylamino)acryloalkanone, Antifungal activity.

INTRODUCTION

Fungal infections have recently emerged as a growing threat to human health, especially in persons whose immune systems are compromised in some way. Clinicians are particularly concerned that the increasing use of antifungal drugs will lead to drug-resistant fungi, especially in settings such as hospitals where hospital-acquired infections are a growing problem. Fungal diseases are called mycoses and those affecting humans can be divided into four groups based on the level of penetration into the body tissues. These are superficial mycoses, cutaneous mycoses or dermatomycoses, subcutaneous mycoses and systemic or deep mycoses. Unlike bacteria, both fungi and humans are eukaryotes. Thus fungal and human cells are similar at the molecular level. This makes it more difficult to find or design drugs that target fungi without affecting human cells.

Different classes of antifungal agents are disclosed in the literature. Those are polyenes, imidazoles, triazoles, thiazoles and allylamines. Among the triazoles, fluconazole, voriconazole and posaconazole are promising antifungal reagents[1-3] (Fig. 1). As antifungal drugs remain in focus of therapeutic interest, the search for even more active triazole derivatives is of great significance and utility.

John and coworkers[4] reported the antianxiolytic activity of 7-(substituted phenyl)-1,2,4-triazolo[1,5-a]pyrimidine compounds. Our substantial interest in pharmaceutically important heterocycles motivates us to synthesize novel triazole derivatives and to evaluate their antimicrobial activity. In this connection we aimed to synthesize novel fused 7-(heteroaryl)-1,2,4-triazolo[1,5-a]pyrimidines by replacing the substituted

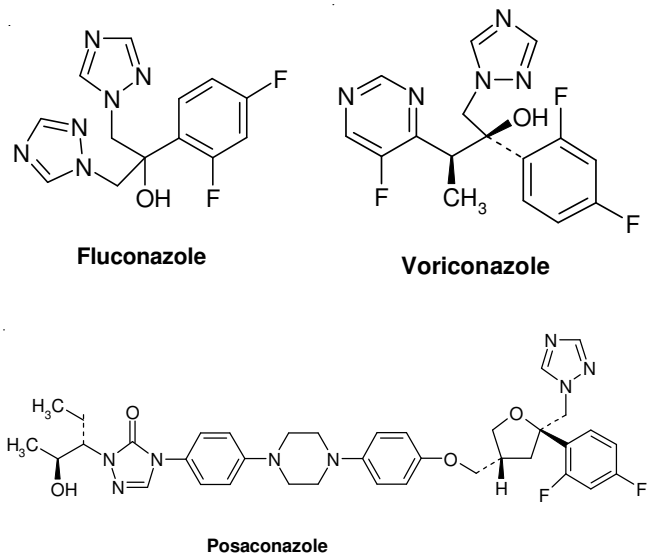


Fig. 1. Popular antifungal drugs

phenyl group of above triazolo pyrimidines with various 5-and 6-membered heterocyclic moieties. The antimicrobial activity results were compared with respect to voriconazole and sulfamethoxazole as reference drug candidates. The heteroaryl moieties of these 7-(heteroaryl)-1,2,4-triazolo[1,5-a]pyrimidines have been chosen from 5- and 6-membered heterocyclic compounds, like pyridine, pyrazine, thiophene, bromothiophene and furan.

The novel 7-(heteroaryl)-1,2,4-triazolo[1,5-a]pyrimidines **1(a-n)** have been synthesized by reacting heteroaromatic ketone (**4**) with dimethylformamide dimethyl acetal or

dimethylacetamide dimethyl acetal (**5**) in refluxing methanol[5] to yield the key intermediates, 3-(dimethylamino) acryloalkanones **2(a-n)**. These intermediates, **2(a-n)** were condensed with 3-amino-1,2,4-triazole (**3**) in the presence of acetic acid at elevated temperatures to afford 7-(heteroaryl)-1,2,4-triazolo[1,5-a]pyrimidines **1(a-n)**.

The novel 7-(heteroaryl)-1,2,4-triazolo[1,5-a] pyrimidines **1(a-n)** were evaluated for antifungal and antibacterial activity by employing voriconazole and sulfamethoxazole as reference drugs. Their antifungal activity was tested against *Aspergillus niger* (NCIM 1196) and *Candida albicans* (NCIM3471) by cup plate method. Voriconazole, a popular antifungal drug was employed as reference drug for this antifungal activity. Solutions of test compounds were made in methanol at a concentration of 2.5 mg/mL under aseptic conditions. All the solutions were sterilized by membrane filtration[6]. Solid agar media were used for all the test organisms. The activity was expressed in terms of zone of inhibition[7]. And their antibacterial activity was tested against *E. coli* (NCIM 2065), *Staphylococcus aureus* (NCIM 2079) and *Pseudomonas aeruginosa* (NICM 2200) by cup plate method using sulfamethoxazole as reference drug.

EXPERIMENTAL

All reactions were monitored by thin layer chromatography carried out on 0.2 mm E. Merck silica gel plates (60F-254) using UV light as a visualizing agent. All solvents were obtained from commercial sources and freshly distilled before use. The ^{1}H and ^{13}C NMR spectra were recorded on Buker 400 MHz spectrometer. The chemical shifts are reported in δ parts per million (ppm) relative to TMS. The IR spectra were recorded in solid state as KBr dispersion using Perkin-Elmer FT-IR spectrometer. The mass spectra were recorded on Waters Quattro Micro LC/MS/MS. Analytical HPLC was performed on a Waters system equipped with a UV detector set at 225 nm. Compounds were dissolved in mobile phase and injected through a 100 µL loop. The following eluent system was used: 0.01M H_3PO_4 solution and acetonitrile (750:250 ratio). HPLC retention times (t_R) were obtained, at flow rates of 1.0 mL/min, using Kromasil C_{18} 5 µ (250 mm × 4.6 mm) column and isocratically run up to 1 h.

Synthesis of 3-dimethylaminoacryloalkanones 2(a-n)

Synthesis of 3-dimethylamino-1-(3-pyridyl)-2-propen-1-one (2a): 3-Acetylpyridine (20 g, 0.16 mol), N,N-dimethylformamide dimethyl acetal (25 g, 0.21 mol) and methanol (40 mL) were charged into a 250 mL three-necked round-bottom flask and stirred at reflux temperature for 10 h. Methanol was evaporated under reduced pressure to get a brown crystalline residue (33.9 g). The resulting residue was crystallized from a solvent mixture of ethyl acetate:isopropyl ether (1:3) (100 mL) at 5-10 ºC to afford a yellow coloured crystalline solid (20 g).

Yield: 60 %, m.p. 80.2 ºC; purity (HPLC): 99.89 %; IR (KBr, ν_{max}, cm^{-1}): 3423, 2918, 1640, 1578, 1514. ^{1}H NMR ($CDCl_3$/TMS): δ 2.95 (s, 3H), 3.18 (s, 3H), 5.66 (d,1H, *J* = 12.4 Hz), 7.35 (dd,1H, *J* = 4.8 Hz, 8.0 Hz), 7.85 (d, 1H, *J* = 12.0 Hz), 8.17 (dt, 1H, *J* = 8.0Hz), 8.65 (dd, 1H, *J* = 1.6 Hz, 4.8 Hz), 9.07 (s, 1H). ^{13}C NMR ($CDCl_3$/TMS): δ 37.01, 44.84, 91.38, 122.89, 134.62, 135.25, 148.52, 151.03, 154.33 and 185.84. MS (m/z): 177.4 (M + 1) (100 %), 132.2 (42 %).

Synthesis of 3-dimethylamino-1-(3-pyridyl)-2-buten-1-one (2b): Compound (**2b**) was prepared in the same manner as described above using N,N-dimethylacetamide dimethyl acetal instead of N,N-dimethylformamide dimethyl acetal crystallized from ethyl acetate:*n*-hexane (1:1), yield: 40 %, m.p. 51.3-56.5 ºC; purity (HPLC): 99.56 %; IR (KBr, ν_{max}, cm^{-1}): 3432, 1610, 1568, 1539, 1405. ^{1}H NMR ($CDCl_3$/TMS): δ 2.68 (s, 3H), 3.11 (s, 6H), 5.61 (s, 1H), 7.33 (m, 1H), 8.15 (dd, 1H, *J* = 2.0 Hz, 8.0 Hz), 8.63 (dd, 1H, *J* = 1.6 Hz, 4.8 Hz), 9.05 (s, 1H). ^{13}C NMR ($CDCl_3$/TMS): δ 16.37, 40.00, 91.77, 122.92, 134.61, 137.87, 148.38, 150.51, 164.62, 185.23. MS (m/z): 191.5 (M + 1) (100 %), 146.3 (55 %).

Synthesis of 3-dimethylamino-1-(4-pyridyl)-2-propen-1-one (2c): Compound (**2c**) was prepared in the same manner by employing 4-acetylpyridine and N,N-dimethylformamide dimethyl acetal as substrate and reactant, respectively.

Crystallized from ethyl acetate:isopropyl ether (1:3), yield: 43 %, m.p. 114 ºC; purity (HPLC): 99.98 %; IR (KBr, ν_{max}, cm^{-1}): 3424, 3027, 1640, 1564, 1525. ^{1}H NMR ($CDCl_3$/TMS): δ 2.96 (s, 3H), 3.19 (s, 3H), 5.64 (d, 1H, *J* = 12.4 Hz), 7.68 (dd, 2H, *J* = 1.2 Hz, 4.8 Hz), 7.86 (d, 1H, *J* = 12.4 Hz), 8.70 (dd, 2H, *J* = 1.2 Hz, 4.4 Hz). ^{13}C NMR ($CDCl_3$/TMS): δ 37.25, 45.13, 91.50, 121.02, 147.03, 150.06, 155.03, 186.36. MS (m/z): 177.4 (M + 1) (100 %), 159.4 (24 %), 134.3 (5 %).

Synthesis of 3-dimethylamino-1-(4 -pyridyl)-2-buten-1-one (2d): Compound (**2d**) was synthesized in the same manner by employing 4-acetylpyridine and N,N-dimethylacetamide dimethyl acetal.

Crystallized from ethyl acetate:*n*-hexane (1:1), yield: 38 %, m.p. 91.6 ºC; purity (HPLC): 99.89 %; IR (KBr, ν_{max}, cm^{-1}): 3422, 3015, 1601, 1529, 1487. ^{1}H NMR ($CDCl_3$/TMS): δ 2.68 (s, 3H), 3.11 (s, 6H), 5.58 (d, 1H), 7.64 (dd, 2H, *J* = 1.6 Hz, 4.4 Hz), 8.66 (dd, 2H, *J* = 1.6 Hz, 4.8 Hz). ^{13}C NMR ($CDCl_3$/TMS): δ 16.47, 40.09, 91.53, 120.91, 149.59, 149.86, 165.25, 185.31. MS (m/z): 191.5 (M + 1) (100 %), 174.5 (8 %), 173.5 (51 %), 146.3 (8 %).

Synthesis of 3-dimethylamino-1-(2-pyridyl)-2-propen-1-one (2e): Compound (**2e**) was prepared in the same manner by employing 2-acetylpyridine and N,N-dimethylformamide dimethyl acetal.

Crystallized from ethyl acetate:isopropyl ether (1:3), yield: 70 %, m.p. 122.6 ºC; purity (HPLC): 99.85 %; IR (KBr, ν_{max}, cm^{-1}): 3431, 3015, 1638, 1565, 1534. ^{1}H NMR ($CDCl_3$/TMS): δ 2.99 (s, 3H), 3.18 (s, 3H), 6.46 (d, 1H, *J* = 12.4 Hz), 7.35 (m, 1H), 7.80 (m, 1H), 7.90 (d, 1H, *J* = 12.4 Hz), 8.15 (d, 1H, *J* = 8.0 Hz), 8.63 (1H, m). ^{13}C NMR ($CDCl_3$/TMS): δ 37.27, 44.95, 90.94, 121.81, 125.21, 136.53, 148.07, 154.56, 156.03, 186.65. MS (m/z): 177.5 (M + 1) (100 %), 159.4 (16 %), 134.3 (23 %).

Synthesis of 3-dimethylamino-1-(2-pyridyl)-2-buten-1-one (2f): Compound (**2f**) was prepared in the same manner by employing 2-acetylpyridine and N,N-dimethylacetamide dimethyl acetal.

Crystallized from ethyl acetate:*n*-hexane (1:1), yield: 44 %, m.p. 69 ºC; purity (HPLC): 98.72 %; IR (KBr, ν_{max}, cm^{-1}):

3432, 2913, 1604, 1541, 1465. ^{1}H NMR ($CDCl_3$/TMS): δ 2.71 (s, 3H), 3.14 (s, 6H), 6.55 (s, 1H), 7.31 (m, 1H), 7.78 (m, 1H), 8.14 (dt, 1H, *J* = 8 Hz), 8.59 (ddd, 1H, *J* = 0.8 Hz, 1.6 Hz). ^{13}C NMR ($CDCl_3$/TMS): δ 16.40, 40.06, 90.74, 121.57, 124.64, 136.47, 147.79, 157.88, 164.91, 185.46. MS (m/z): 191.5 (M + 1) (100 %), 173.4 (33 %), 146.3(24 %).

Synthesis of 3-dimethylamino-1-(2-pyrazinyl)-2-propen-1-one (2g): Compound (**2g**) was prepared by employing acetylpyrazine and N,N-dimethylformamide dimethyl acetal.

Crystallized from ethyl acetate:*n*-hexane (1:1), yield: 38 %, m.p. 131.0 ºC; purity (HPLC): 99.74 %; IR (KBr, ν_{max}, cm^{-1}): 3433, 2946, 1637, 1582, 1544. ^{1}H NMR ($CDCl_3$/TMS): δ 3.01 (s, 3H), 3.21 (s, 3H), 6.34 (d, 1H, *J* = 12.4 Hz), 7.93 (d, 1H, *J* = 12.8 Hz), 8.56 (dd, 1H, *J* = 1.6 Hz, 2.8 Hz), 8.65 (d, 1H, *J* = 2.4 Hz), 9.34 (d, 1H, *J* = 1.6 Hz). ^{13}C NMR ($CDCl_3$/TMS): δ 37.50, 45.25, 90.92, 142.76, 144.29, 146.02, 150.45, 154.99, 185.35. MS (m/z): 178.5 (M + 1) (100 %), 160.4 (16 %).

Synthesis of 3-dimethylamino-1-(2-pyrazinyl)-2-buten-1-one (2h): The above compound (**2h**) was prepared using acetylpyrazine and N,N-dimethylacetamide dimethyl acetal.

Crystallized from ethyl acetate:*n*-hexane (1:1), yield: 53 %, m.p. 131.8 ºC; purity (HPLC): 99.14 %; IR (KBr, ν_{max}, cm^{-1}): 3422, 2922, 1617, 1537, 1444. ^{1}H NMR ($CDCl_3$/TMS): δ 2.73 (s, 3H), 3.15 (s, 6H), 6.44 (s, 1H), 8.52 (dd, 1H, *J* = 1.2 Hz, 2.4 Hz), 8.60 (d, 1H, *J* = 2.8 Hz), 9.32 (d, 1H, *J* = 1.6 Hz). ^{13}C NMR ($CDCl_3$/TMS): δ 16.48, 40.08, 90.37, 142.28, 143.99, 145.19, 152.06, 165.55, 183.57. MS (m/z): 192.5 (M + 1) (100 %), 174.4 (24 %).

Synthesis of 3-dimethylamino-1-(2-thienyl)-2-propen-1-one (2i): The above compound (**2i**) was synthesized using 2-acetylthiophene and N,N-dimethylformamide dimethyl acetal.

Crystallized from ethyl acetate:isopropyl ether (1:3), yield: 59 %, m.p. 110.9 ºC; purity (HPLC): 99.85 %; IR (KBr, ν_{max}, cm^{-1}): 3422, 3070, 1636, 1544, 1513. ^{1}H NMR ($CDCl_3$/TMS): δ 2.92 (s, 3H), 3.14 (s, 3H), 5.61 (d, 1H, *J* = 12.0 Hz), 7.07 (dd, 1H, *J* = 3.6 Hz, 4.8 Hz), 7.47 (dd, 1H, *J* = 1.2 Hz, 5.2 Hz), 7.63 (dd, 1H, *J* = 0.8 Hz, 3.6 Hz), 7.79 (d, 1H, *J* = 12.4 Hz). ^{13}C NMR ($CDCl_3$/TMS): δ 37.10, 44.86, 91.22, 127.94, 143.74, 147.18, 150.17, 154.60, 184.80. MS (m/z): 182.4 (M + 1) (100 %), 111.1 (60 %).

Synthesis of 3-dimethylamino-1-(2-thienyl)-2-buten-1-one (2j): Compound (**2j**) was synthesized by employing 2-acetylthiophene and N,N-dimethylacetamide dimethyl acetal.

Crystallized from ethyl acetate:*n*-hexane (1:1), yield: 70 %, m.p. 94.5 ºC; purity (HPLC): 99.93 %; IR (KBr, ν_{max}, cm^{-1}): 3417, 2952, 1588, 1544, 1515. ^{1}H NMR ($CDCl_3$/TMS) δ: d2.64 (s, 3H), 3.07 (s, 6H), 5.64 (s, 1H), 7.04 (dd, 1H, *J* = 3.6 Hz, 4.8 Hz), 7.41 (dd, 1H, *J* = 1.2 Hz, 4.8 Hz), 7.55 (dd, 1H, *J* = 1.2 Hz, 4.0 Hz). ^{13}C NMR ($CDCl_3$/TMS): δ 16.13, 39.73, 91.23, 126.86, 127.18, 129.12, 149.85, 163.44, 179.72. MS (m/z): 196.5 (M + 1) (100 %), 112.2 (21%), 111.1 (76 %).

Synthesis of 3-dimethylamino-1-(5-bromo-2-thienyl-)-2-propen-1-one (2k): Preparation of compound (**2k**) was prepared from 5-bromo-2-acetylthiophene and N,N-dimethylformamide dimethyl acetal.

Crystallized from ethyl acetate:isopropyl ether (1:3), yield: 70 %, m.p. 114.1 ºC; purity (HPLC): 100 %; IR (KBr, ν_{max}, cm^{-1}): 3415, 2900, 1631, 1548, 1521. ^{1}H NMR ($CDCl_3$/TMS): δ 2.91 (s, 3H), 3.15 (s, 3H), 5.5 (d, 1H, *J* = 12.4 Hz), 7.03 (d, 1H, *J* = 4.0 Hz), 7.34 (d, 1H, *J* = 4.0 Hz), 7.78 (d, 1H, *J* = 12.0 Hz). ^{13}C NMR ($CDCl_3$/TMS): δ 37.21, 45.01, 90.57, 118.12, 128.09, 130.57, 148.96, 153.73, 179.39. MS (m/z): 262.4 (M + 2) (97 %), 260.4 (M^+) (100 %), 189.3 (18 %).

Synthesis of 3-dimethylamino-1-(5-bromo-2-thienyl)-2-buten-1-one (2l): Compound (**2l**) was prepared by reacting 5-bromo-2-acetylthiophene with N,N-dimethylacetamide dimethyl acetal.

Crystallized from ethyl acetate:*n*-hexane (1:1), yield: 79 %, m.p. 126.7 ºC; purity (HPLC): 99.89 %; IR (KBr, ν_{max}, cm^{-1}): 2915, 1580, 1540, 1491. ^{1}H NMR ($CDCl_3$/TMS): δ 2.61 (s, 3H), 3.07 (s, 6H), 5.49 (s, 1H), 7.00 (d, 1H, *J* = 4.0 Hz), 7.28 (d, 1H, *J* = 4.0 Hz). ^{13}C NMR ($CDCl_3$/TMS): δ 16.32, 39.94, 90.34, 116.91, 126.76, 130.36, 151.53, 164.01, 178.44. MS (m/z): 276.4 (M + 2) (98 %), 274.4 (M^+) (100 %), 191.3 (29 %).

Synthesis of 3-dimethylamino-1-(2-furyl)-2-propen-1-one (2m): Compound (**2m**) was synthesized by using 2-acetylfuran and N,N-dimethylformamide dimethyl acetal.

Crystallized from ethyl acetate:isopropyl ether (1:3), yield: 68 %, m.p. 82.1 ºC; purity (HPLC): 99.94 %; IR (KBr, ν_{max}, cm^{-1}): 3421, 2918, 1641, 1576, 1541. ^{1}H NMR ($CDCl_3$/TMS): δ 2.92 (s, 3H), 3.14 (s, 3H), 5.66 (d, 1H, *J* = 12.4Hz), 6.48 (dd, 1H, *J* = 1.6 Hz, 3.6 Hz), 7.06 (dd, 1H, *J* = 0.8 Hz, 3.6 Hz), 7.49 (dd, 1H, *J* = 0.4Hz, 1.2 Hz), 7.79 (d, 1H, *J* = 12.4 Hz). ^{13}C NMR ($CDCl_3$/TMS): δ 37.10, 44.82, 91.24, 111.62, 113.12, 143.98, 153.3, 154.63, 177.24. MS (m/z): 166.4 (M + 1) (100 %), 98.0 (30 %), 95.0 (49 %).

Synthesis of 3-dimethylamino-1-(2-furyl)-2-buten-1-one (2n): The above compound (**2n**) was synthesized by using 2-acetylfuran and N,N-dimethylacetamide dimethyl acetal.

Crystallized from ethyl acetate:hexane (1:1), yield: 66 %, m.p. 76.7 ºC; purity (HPLC): 99.41 %; IR (KBr, ν_{max}, cm^{-1}): 3403, 3112, 1603, 1571, 1557. ^{1}H NMR ($CDCl_3$/TMS): δ 2.65 (s, 3H), 3.07 (s, 6H), 5.71 (s, 1H), 6.44 (dd, 1H, *J* = 1.6 Hz, 3.2 Hz), 7.00 (d, 1H, *J* = 3.6 Hz), 7.43 (dd, 1H, *J* = 0.8 Hz, 1.6 Hz). ^{13}C NMR ($CDCl_3$/TMS): δ 16.39, 39.89, 90.95, 111.54, 112.07, 143.16, 156.27, 163.84, 176.58. MS (m/z): 180.5 (M + 1) (100 %), 112.2 (25 %), 95.0 (26 %).

Synthesis of novel 7-(heteroaryl)-1,2,4-triazolo[1,5-a]-pyrimidine compounds 1(a-n)

7-(3-Pyridyl)-1,2,4-triazolo[1,5-a]pyrimidine (1a): 3-Dimethylamino-1-(3-pyridyl)-2-propen-1-one (**2a**) (5 g, 0.028 moles), 3-amino-1,2,4-triazole (2.4 g, 0.028 moles) and glacial acetic acid (30 mL) were charged into a 100 mL three-necked round-bottom flask and stirred for 6 h at 110 ºC. The reaction mass was concentrated under reduced pressure and the pH was adjusted to 8 using 10 % aq. sodium carbonate solution and the resulting precipitate was extracted into methylene chloride (40 mL × 2). The combined organic layer was dried over anhydrous sodium sulphate and the solvent was evaporated under reduced pressure to yield cream coloured solid. The solid was crystallized from acetone to afford the title compound. Yield: 55 %, m.p. 207.8 ºC; purity (HPLC):

99.54 %; IR (KBr, ν_{max}, cm^{-1}) 3051, 1610, 1587, 1542, 1279, 703; ^{1}H NMR (DMSO-d_6) δ 9.29 (s, 1H), 8.97 (d, 1H, *J* = 4.8 Hz), 8.8 (dd 1H, *J* = 1.6 Hz, 4.8 Hz), 8.74 (s, 1H), 8.60 (m, 1H), 7.73 (d, 1H, *J* = 4.8 Hz), 7.67 (m, 1H); ^{13}C NMR (DMSO-d_6) δ 155.58, 155.15, 152.04, 149.78, 144.91, 137.19, 125.97, 123.514, 110.01; m/z 198.4 (M + 1) (100 %), 153.3 (9 %), 102.9 (12 %), 88.8 (78 %). Anal. calcd. (%) for $C_{10}H_7N_5$: C, 60.90; H, 3.55; N, 35.50. Found (%): C, 60.72; H, 3.41; N, 35.28.

The following 7-(heteroaryl)-1,2,4-triazolo[1,5-a]pyrimidines have been prepared similarly using appropriate starting materials.

5-Methyl-7-(3-pyridyl)-1,2,4-triazolo[1,5-a]pyrimidine (1b): 3-Dimethylamino-1-(3-pyridyl)-2-buten-1-one (**2b**) was used as substrate. Reaction time: 5 h. Crystallized from ethyl acetate to get a brown solid. Yield: 81 %, melting point: 176.2 ºC; Purity (HPLC): 98.32 %; IR (KBr, ν_{max}, cm^{-1}) 3057, 1614, 1588, 1540, 1476, 1428, 1291, 704; ^{1}H NMR (DMSO-d_6) δ 9.29 (s, 1H), 8.8 (dd, 1H, *J* = 1.6 Hz, 3.2 Hz), 8.65 (s, 1H), 8.59 (dt, 1H, *J* = 2.0 Hz), 7.70 (m, 2H), 2.70 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 166.64, 155.80, 155.68, 152.42, 150.00, 144.37, 137.99, 126.68, 124.44, 111.57, 25.20; m/z 212.5 (M + 1) (100 %), 102.9 (13 %), 98.0 (28 %). Anal. calcd. (%) for $C_{11}H_9N_5$: C, 62.54; H, 4.26; N, 33.14. Found (%): C, 62.14; H, 4.08; N, 32.51.

7-(4-Pyridyl)-1,2,4-triazolo[1,5-a]pyrimidine (1c): 3-Dimethylamino-1-(4-pyridyl)-2-propen-1-one (**2c**) was used as substrate. Reaction time: 5 h. Crystallized from acetone, to get an off-white solid. Yield: 70 %, melting point: 213.6 ºC; Purity (HPLC): 99.55 %; IR (KBr, ν_{max}, cm^{-1}) 3419, 3055, 1614, 1595, 1542, 1491, 1280, 821, 588; ^{1}H NMR (DMSO-d_6) δ 9.04 (d, 1H, *J* = 4.4 Hz), 8.89 (dd, 2H, *J* = 1.6 Hz, 4.8 Hz), 8.79 (s 1H), 8.17 (dd, 2H, *J* = 1.6 Hz, 4.4 Hz), 7.78 (d, 1H, *J* = 4.4 Hz); ^{13}C NMR (DMSO-d_6) δ 155.88, 155.71, 150.99, 150.51, 145.09, 137.30, 123.58, 121.78, 110.71; m/z 198.4 (M + 1) (100 %), 153.3 (6 %), 88.9 (7 %), 88.3 (14 %). Anal. calcd. (%) for $C_{10}H_7N_5$: C, 60.90; H, 3.55; N, 35.50. Found (%): C, 60.54; H, 3.38; N, 35.25.

5-Methyl-7-(4-pyridyl)-1,2,4-triazolo[1,5-a]pyrimidine(1d): 3-Dimethylamino-1-(4-pyridyl)-2-buten-1-one (**2d**) was used as substrate. Reaction time: 4 h. Crystallized from ethyl acetate, to get an off-white solid. Yield: 55 %, m.p. 167.5 ºC; purity (HPLC): 96.7 %; IR (KBr, ν_{max}, cm^{-1}): 3058, 1621, 1543, 1293, 822, 643; ^{1}H NMR (DMSO-d_6) δ 8.88 (dd, 2H, *J* = 1.6 Hz, 4.8 Hz), 8.68 (s, 1H), 8.15 (dd, 2H, *J* = 1.6 Hz, 4.8 Hz), 7.72 (s, 1H), 2.71 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 166.53, 155.71, 150.62, 144.16, 137.68, 123.84, 111.79, 25.13; m/z 212.5 (M + 1) (100 %), 87.8 (15 %).

7-(2-Pyridyl)-1,2,4-triazolo[1,5-a]pyrimidine (1e): 3-Dimethylamino-1-(2-pyridyl)-2-propen-1-one (**2e**) was used as substrate. Reaction time: 4 h. Crystallized from ethyl acetate, to afford a grey solid. Yield: 38 %, m.p. 160.9 ºC; purity (HPLC): 96.8 %; IR (KBr, ν_{max}, cm^{-1}) 3072, 1607, 1571, 1538, 1514, 1265, 755, 637; ^{1}H NMR (DMSO-d_6) δ 9.05 (d, 1H, *J* = 4.4 Hz), 9.03 (d, 1H, *J* = 8.0 Hz), 8.89 (d, 1H, *J* = 4.0 Hz), 8.85 (s, 1H), 8.14-8.18 (td, 1H, *J* = 1.6 Hz), 8.07 (d, 1H, *J* = 4.8 Hz), 7.70 (dd, 1H, *J* = 4.8 Hz, 6.8 Hz); ^{13}C NMR (DMSO-d_6) δ 156.32, 156.04, 156.00, 150.93, 147.09, 145.93, 138.11, 127.22, 126.71, 110.57; m/z 198.3 (M + 1) (100 %), 157.3 (7 %).

5-Methyl-7-(2-pyridyl)-1,2,4-triazolo[1,5-a]pyrimidine (1f): 3-Dimethyl amino-1-(2-pyridyl)-2-buten-1-one (**2f**) was used as substrate. Reaction time: 4 h. Crystallized from methanol to get a pale brown solid. Yield: 45 %, melting point: 145.6 ºC; purity (HPLC): 90.6 %; IR (KBr, ν_{max}, cm^{-1}) 3105, 1609, 1570, 1553, 1468, 1284, 772; ^{1}H NMR (DMSO-d_6) δ 8.99 (d, 1H, *J* = 8 Hz), 8.88 (m 1H), 8.74 (s, 1H), 8.15 (td, 1H, *J* = 1.6 Hz), 7.96 (s, 1H), 7.70 (m, 1H), 2.74 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 165.84, 156.46, 156.02, 150.86, 147.31, 144.65, 138.00, 126.98, 126.60, 110.87, 25.41; m/z 212.6 (M + 1) (100 %), 170.4 (8 %).

7-(Pyrazinyl)-1,2,4-triazolo[1,5-a]pyrimidine (1g): 3-Dimethylamino-1-(2-pyrazinyl)-2-propen-1-one (**2g**) was used as substrate. Reaction time: 4 h. Crystallized from methanol, to get an off-white solid. Yield: 39 %, m.p. 218.8 ºC; purity (HPLC): 93.5 %; IR (KBr, ν_{max}, cm^{-1}) 3099, 1611, 1542, 1518, 1401, 1267, 1012, 856, 670; ^{1}H NMR (DMSO-d_6) δ 10.1(d, 1H, *J* = 1.6 Hz), 9.08 (d, 1H, 4.8 Hz), 9.00 (dd, 1H, *J* = 1.6 Hz, 2.0 Hz), 8.95 (d, 1H, *J* = 2.4 Hz), 8.89 (s, 1H), 8.07 (d, 1H, *J* = 4.4 Hz); ^{13}C NMR (DMSO-d_6) δ 155.99, 155.69, 147.39, 146.38, 145.56, 143.71, 143.27, 110.57; m/z 199.3 (M^+ + 1) (100 %), 157.3 (7 %).

5-Methyl-7-(pyrazinyl)-1,2,4-triazolo[1,5-a]pyrimidine (1h): 3-Dimethylamino-1-(2-pyrazinyl)-2-buten-1-one (**2h**) was used as substrate. Reaction time: 4 h. Crystallized from methanol to get a pale brown solid. Yield: 66 %, m.p. 276.4 ºC; purity (HPLC): 93.8 %; IR (KBr, ν_{max}, cm^{-1}) 3093, 1610, 1530, 1467, 1276, 865; ^{1}H NMR (DMSO-d_6) δ 10.05 (d, 1H, *J* = 1.2 Hz), 8.98 (m, 2H), 8.76 (s, 1H), 7.96 (s, 1H), 2.75 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 165.46, 155.55, 147.07, 146.11, 145.18, 143.32, 143.08, 142.29, 110.84, 24.79; m/z 213.3 (M + 1) (100 %), 171.4 (8 %).

7-(2-Thienyl)-1,2,4-triazolo[1,5-a]pyrimidine (1i): 3-Dimethylamino-1-(2-thienyl)-2-propen-1-one (**2i**) was used as substrate. Reaction time: 6 h. Crystallized from methanol to get a cream coloured solid. Yield: 58 %, m.p. 174.3 ºC; purity (HPLC): 98.6 %; IR (KBr, ν_{max}, cm^{-1}) 3099, 1590, 1546, 1497, 1265, 815, 738; ^{1}H NMR (DMSO-d_6) δ 8.9 (d, 1H, *J* = 5.2 Hz), 8.84 (s, 1H), 8.60 (d, 1H, *J* = 4.0 Hz), 8.21 (d, 1H, *J* = 4.8 Hz), 8.00 (d, 1H, *J* = 4.8 Hz), 7.44 (t, 1H, *J* = 4.4 Hz); ^{13}C NMR (DMSO-d_6) δ 155.42, 155.24, 141.04, 135.51, 133.11, 129.73, 128.32, 106.03; m/z 203.5 (M^+ + 1) (100 %), 193.4 (11 %), 161.3 (6 %), 87.8 (6 %). Anal. calcd. (%) for $C_9H_6N_4S$: C, 53.44; H, 2.96; N, 27.69; S, 15.85. Found (%): C, 53.22; H, 2.85; N, 26.91; S, 15.19.

5-Methyl -7-(2-thienyl)-1,2,4-triazolo[1,5-a]pyrimidine (1j): 3-Dimethylamino-1-(2-thienyl)-2-buten-1-one (**2j**) was used as substrate. Reaction time: 4 h. Crystallized from methanol to get a cream solid. Yield: 58 %, m.p. 145.8 ºC; purity (HPLC): 99.4 %; IR (KBr, ν_{max}, cm^{-1}) 3059, 1599, 1541, 1251, 739; ^{1}H NMR (DMSO-d_6) δ 8.72 (s, 1H), 8.5 (dd, 1H, *J* = 1.2 Hz, 4.0 Hz), 8.15 (dd, 1H, *J* = 0.8 Hz, 4.8 Hz), 7.91 (s, 1H), 7.72 (dd, 1H, *J* = 4.0 Hz, 5.2 Hz), 2.67(s, 3H); ^{13}C NMR (DMSO-d_6) δ 164.44, 155.17, 139.95, 134.92, 132.68, 129.85, 128.23, 106.45, 25.60; m/z 217.3 (M + 1) (100 %), 207.4 (4 %), 175.3 (5 %), 102.2 (4 %), 87.9 (4 %). Anal. calcd. (%) for $C_{10}H_8N_4S$:

C, 55.53; H, 3.7; N, 25.89; S, 14.82. Found (%): C, 54.99; H, 3.54; N, 25.45; S, 14.84.

7-(5-Bromo-2-thienyl)-1,2,4-triazolo[1,5-a]pyrimidine (1k): 3-Dimethylamino-1-(5-bromo-2-thienyl)-2-propen-1-one (**2k**) was used as substrate. Reaction time: 6 h. Crystallized from methanol to get an orange solid. Yield: 68 %, m.p. 207.3 ºC; purity (HPLC): 99.7 %; IR (KBr, ν_{max}, cm^{-1}) 3094, 1589, 1541, 1505, 1260, 814, 799; ^{1}H NMR (DMSO-d_6) δ 8.93 (d, 1H, *J* = 4.8 Hz), 8.85 (s, 1H), 8.41 (d, 1H, *J* = 4.0 Hz), 8.06 (d, 1H, *J* = 5.2 Hz), 7.61 (d, 1H, *J* = 4.4 Hz); ^{13}C NMR (DMSO-d_6) δ 155.61, 154.89, 140.31, 133.62, 131.89, 131.20, 122.72, 105.97; m/z 283.3 (M + 2), 281.3 (M^+) (100 %), 202.4 (6 %), 160.3 (8 %), 87.9 (4 %). Anal. calcd. (%) for $C_9H_5BrN_4S$: C, 38.44; H, 1.77; N, 19.91; S, 11.40, Found (%): C, 38.09; H, 1.74; N, 19.29; S, 11.43.

5-Methyl-7-(5-bromo-2-thienyl)-1,2,4-triazolo[1,5-a]pyrimidine(1l): 3-Dimethylamino-1-(2-thienyl-5-bromo)-2-buten-1-one (**2l**) was used as substrate. Reaction time: 4 h. Crystallized from methanol to get an off-white solid. Yield: 75 %, m.p. 188.6 ºC; purity (HPLC): 99.2 %; IR (KBr, ν_{max}, cm^{-1}) 3094, 1607, 1548, 1416, 1287, 799; ^{1}H NMR (DMSO-d_6) δ 8.73 (s, 1H), 8.34 (d, 1H, *J* = 4.4 Hz), 7.98 (s, 1H), 7.58 (d, 1H, *J* = 4.0 Hz), 2.67 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 155.14, 133.13, 131.71, 131.10, 106.38, 24.82; m/z 297.3 (M + 2) (100 %), 295.3 (M^+) (100 %), 216.4 (8 %), 175.3 (6 %), 174.3 (39 %), 141.3 (11 %), 109.0 (19 %), 99.1 (6 %).

7-(2-Furanyl)-1,2,4-triazolo[1,5-a]pyrimidine (1m): 3-Dimethylamino-1-(2-furyl)-2-propen-1-one (**2m**) was used as substrate. Reaction time: 4 h. Crystallized from *n*-hexane to get a brown solid. Yield: 82 %, m.p. 185.2 ºC; purity (HPLC): 98.0 %; IR (KBr, ν_{max}, cm^{-1}) 1620, 1569, 1541, 1281, 773; ^{1}H NMR (DMSO-d_6) δ 8.93 (d, 1H, *J* = 4.8 Hz), 8.83 (s, 1H), 8.25 (d, 1H, *J* = 1.6 Hz), 8.13 (d, 1H, *J* = 3.6 Hz), 7.70 (d, 1H *J* = 4.8 Hz) 6.96 (dd, 1H, *J* = 1.6 Hz, 3.6 Hz) 2.689 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 156.16, 155.09, 148.51, 142.76, 136.90, 120.95, 114.21, 105.02; m/z 187.6 (M^+ + 1) (100 %), 177.4 (25 %), 145.2 (7 %). Anal. calcd. (%) for $C_9H_6N_4O$: C, 58.05; H, 3.22; N, 30.07. Found (%): C, 57.26; H, 3.30; N, 29.69; S.

5-Methyl-7-(2-furanyl)-1,2,4-riazolo[1,5-a]pyrimidine (1n): 3-Dimethyl amino-1-(2-furyl)-2-buten-1-one (**2n**) was used as substrate. Reaction time: 4 h. Crystallized from *n*-hexane to get a brown solid. Yield: 74 % m.p. 161.6 ºC; purity (HPLC): 95.0 %; IR (KBr, ν_{max}, cm^{-1}) 3109, 1617, 1571, 1531, 1296, 1015, 789; ^{1}H NMR (DMSO-d_6) δ 8.72 (s, 1H), 8.22 (d, 1H, *J* = 0.8 Hz), 8.07 (d, 1H, *J* = 3.6 Hz), 7.60 (s, 1H), 6.94 (dd, 1H, *J* = 2.0 Hz, 3.6 Hz), 2.68 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 165.27, 155.83, 148.10, 142.72, 135.48, 120.35, 113.93, 105.30, 24.97; m/z 201.6 (M + 1) (100 %), 191.4 (27 %), 159.3 (23 %), 131.2 (21 %), 108.9 (14 %), 104.1 (11 %).

Antimicrobial activity

Evaluation of antifungal activity: *Aspergillus niger* (NCIM 1196), *Candida albicans* (NCIM3471) test strains were maintained on PDA (potato dextrose agar) and MGYP (malt extract, glucouse, yeast extract, bacto peptone, agar), media and were sub cultured. These fungi served as test pathogen for the assay. All experiments with *Aspergillus niger*, *Candida albicans* were carried in media as follows: Bactopeptone (9.4 g), yeast extract (4.7 g), beef extract (2.4 g), sodium chloride (10.0 g) and dextrose anhydrous (10.0 g), agar (23.5 g) were dissolved in 1000 mL of water and pH adjusted to 6.1.

A solution of test compounds was prepared in methanol at a concentration of 2.5 mg/mL under aseptic conditions. All the solutions were sterilized by membrane filtration. Solid agar media were used for all the test organisms. Antimicrobial activity of the compounds against different strains of fungi was determined by cup plate method and activity was expressed in terms of zone of inhibition. Inoculum was prepared by washing a medium slant of test organism with 5 mL of aq. sodium chloride and further diluting 1-10 mL to make an inoculum containing 106 CFU/mL. This suspension was added to 25 mL melted medium at temperature 45-50 ºC and plates were prepared. Holes of diameter of 6 mm were made in to the agar plates with sterile borer and filled with the experimental sample solutions. The plates were incubated at 28 ± 1 ºC temperature for 72 h. The results were compared with that of standard voricanazole. The diameters of zones of inhibition were measured in mms.

Compounds **1a-n** were employed to test antifungal properties. Among these, compound **1a**, **1k**, **1l**, **1m** and **1n** exhibited antifungal property.

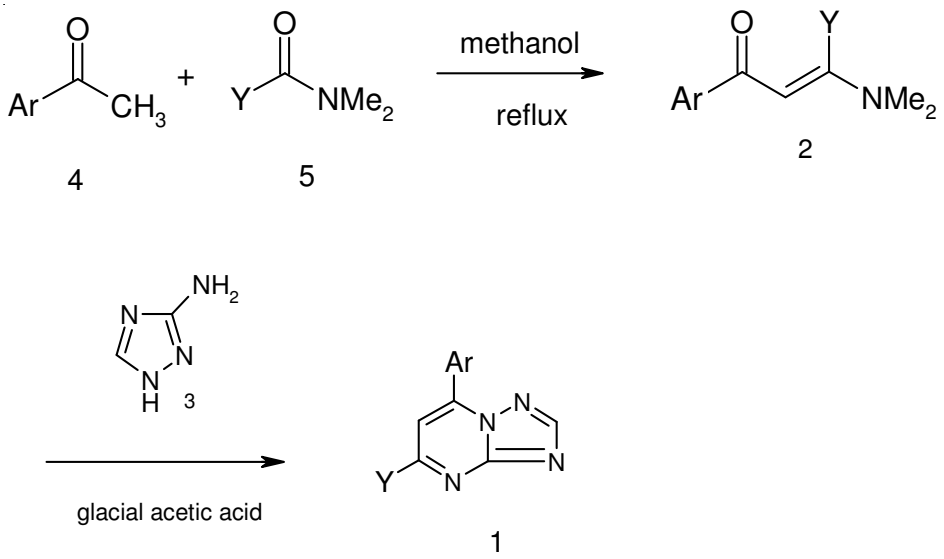
Evaluation of antibacterial activity: The compounds **1a**, **1k**, **1l**, **1m** and **1n** were also tested for their antibacterial activity against *E. coli* (NCIM 2065), *Staphylococcus aureus* (NCIM 2079) and *Pseudomonas aeruginosa* (NICM 2200) by cup plate method. Media preparation: bactopeptone (6 g), pancreatic digest of casein (4 g) yeast extract (3 g), beef extract (1.5 g), dextrose anhydrous (1 g), agar (15 g) dissolved in 1000 mL of distilled water and pH adjusted[8] to 6.6.

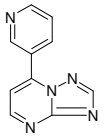
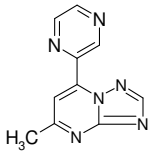
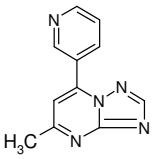
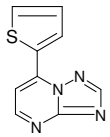
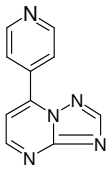
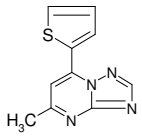
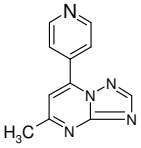
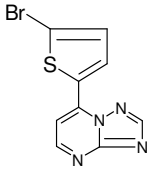
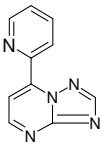
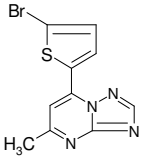
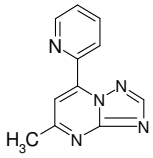
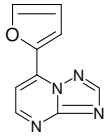
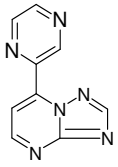
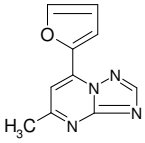
Solutions of the test compounds were made in methanol at a concentration of 2.5 mg/mL under aseptic conditions and the antibacterial activity was tested in similar manner as described under antifungal activity and the results were compared with that of standard sulfamethaxazole.

RESULTS AND DISCUSSION

The novel 7-(heteroaryl)-1,2,4-triazolo[1,5-a]pyrimidines **1(a-n)** were synthesized by reacting heteroaromatic ketone (**4**) with dimethylformamide dimethyl acetal or dimethylacetamide dimethyl acetal (**5**) in refluxing methanol to yield the key intermediates, 3-(dimethylamino) acryloalkanones **2(a-n)**, which were reacted with 3-amino-1,2,4-triazole (**3**) in the presence of acetic acid at at 110 ºC to yield 7-(heteroaryl)-1,2,4-triazolo[1,5-a]pyrimidines **1(a-n)**. The synthetic pathway and structural formulae of the novel 7-(heteroaryl)-1,2,4-triazolo[1,5-a]pyrimidine compounds **1(a-n)** are depicted in **Scheme-I**. The products were isolated by simple crystallization techniques. The yields of the products were found to be moderate to good.

The 7-(heteroaryl)-1,2,4-triazolo[1,5-a]pyrimidine compounds **1(a-n)** were tested for their antifungal activity against *Aspergillus niger* (NCIM 1196) and *Candida albicans* (NCIM3471) by cup plate method. Voriconazole, a popular antifungal drug was employed as reference drug for this antifungal activity. The compounds **1a**, **1k**, **1l**, **1m** and **1n** having



Entry	Ar	Y	Structure of the compound	Entry	Ar	Y	Structure of the compound
1a	3-pyridyl	H		**1h**	pyrazinyl	CH_3	
1b	3-pyridyl	CH_3		**1i**	2-thienyl	H	
1c	4-pyridyl	H		**1j**	2-thienyl	CH_3	
1d	4-pyridyl	CH_3		**1k**	(5-bromo)-2-thienyl	H	
1e	2-pyridyl	H		**1l**	(5-bromo)-2-thienyl	CH_3	
1f	2-pyridyl	CH_3		**1m**	2-furanyl	H	
1g	pyrazinyl	H		**1n**	2-furanyl	CH_3	

Scheme-I: Synthesis of novel 7-(heteroaryl)-1,2,4-triazolo[1,5-a]pyrimidine compounds **1(a-n)**

TABLE-2
ANTIBACTERIAL ACTIVITY OF SELECTED NOVEL 7-(HETEROARYL)-1,2,4-TRIAZOLO[1,5-a]-PYRIMIDINE COMPOUNDS AT 2.5 mg/mL

Compound	*Staphylococcus aureus* (zone of inhibition) (mm)	*E. coli* (zone of inhibition) (mm)	*Pseudomonas aeruginosa* (zone of inhibition) (mm)
1a	1.2 ± 0.2	2 ± 0.2	0.8 ± 0.2
1m	1 ± 0.2	0.9 ± 0.2	1.7 ± 0.2
1n	15 ± 0.2	19 ± 0.2	14 ± 0.2
Sulphamethoxazole (reference)	40 ± 0.2	25 ± 0.2	28 ± 0.2

3-pyridyl, (5-bromo)-2-thienyl and 2-furanyl moieties in the 7-position of the current series of 1,2,4-triazolo[1,5-a]pyrimidines were found to be active. Compound **1m** is most active of this series. The results are summarized in Table-1.

TABLE-1
ANTIFUNGAL ACTIVITY OF NOVEL 7-(HETEROARYL)-1,2,4-TRIAZOLO[1,5-a]PYRIMIDINE COMPOUNDS AT 2.5 mg/mL

Compound	*Aspergillus niger* (zone of inhibition)	*Candida albicans* (zone of inhibition) (mm)
1a	14 ± 0.2	10 ± 0.2
1k	11 ± 0.2	8 ± 0.2
1l	15 ± 0.2	10 ± 0.2
1m	23 ± 0.2	18 ± 0.2
1n	17 ± 0.2	11 ± 0.2
Voriconazole (reference)	25 ± 0.2	21 ± 0.2

These compounds **1a**, **1k**, **1l**, **1m** and **1n** were also tested for their antibacterial activity against *E. coli* (NCIM 2065), *Staphylococcus aureus* (NCIM 2079) and *Pseudomonas aeruginosa* (NICM 2200) by cup plate method. Sulfamethoxazole an antibacterial agent was used as reference drug. The compounds **1a** and **1m** exhibited less antibacterial activity, whereas compounds **1k**, **1l** did not show any zone of inhibition. The results are shown in Table-2.

Conclusion

We have reported here the synthesis and antimicrobial activity of novel 7-heteroaryl-1,2,4-triazolo[1,5-a]pyrimidine compounds (**1a-n**). These were tested against *Aspergillus niger* (NCIM 1196), *Candida albicans* (NCIM3471) for their antifungal activity employing voriconazole as reference drug. Compounds **1a**, **1k**, **1l**, **1m** and **1n** showed moderate to good antifungal activity, **1m** being the most active. The potencies of these molecules varied depending upon the nature of heteroaryl moiety substituted in the 7-position. This is in good agreement with the previous findings that triazoles possess pronounced antifungal activity. The compounds **1a**, **1k**, **1l**, **1m** and **1n** were also tested for their antibacterial activity against *E. coli* (NCIM 2065), *Staphylococcus aureus* (NCIM 2079) and *Pseudomonas aeruginosa* (NICM 2200) by cup plate method. The results were compared with reference antibacterial agent, sulfamethoxazole. Compound **1n** having 2-furyl moiety in the 7-position showed antibacterial activity. The compounds **1a** and **1m** exhibited less antibacterial activity, whereas compounds **1k**, **1l** did not show any zone of inhibition.

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