

An efficient solid phase one port synthesis of Novel triazolo[1,5-a]pyrimidine derivatives from 4-(4-aminophenyl)morpholin-3-one and evaluation of their antimicrobial activity

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ABSTRACT

A series of novel triazolo[1,5-a]pyrimidine derivatives was synthesized from 5-amino-1,2,4-triazole and biologically active morpholinone amine in excellent yield as promising class of antimicrobial agents. The antimicrobial activities were investigated against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Staphylococcus pyogen*, *Candida albicans*, *Aspergillusniger*, *Aspergillusclavatus* and compared with standard drugs Ampicillin, Chloramphenicol, Norfloxacin and Griseofulvin. All the synthesized compounds were characterized by IR, ¹H NMR, ¹³C and mass spectroscopy. The result of antimicrobial activity data revealed that compound 4f, 4g and 4i were found more active against bacterial species and compound 4c, 4d, 4g, 4i and 4j were found more active against fungal strain, while other compounds shows moderate to low activity against microbes.

Keywords: 1,2,4-triazole; 4-(4-aminophenyl)morpholin-3-one; antibacterial; antifungal; biological assay

1. INTRODUCTION

Various fused pyrimidines were studied in the past decade and were found to possess remarkable pharmacological properties like purines, pteridines, quinazolines, triazolopyrimidines, pyrazolopyrimidines, pyrimidoazepines, furopyrimidines, pyridopyrimidines and pyrrolopyrimidines.

The condensation of a pyrimidine ring with another ring of 1,2,4-triazole gives rise to the formation of bicyclic heterocycles known as 1,2,4-triazolopyrimidines. 1,2,4-triazolo[1,5-a]pyrimidine derivatives are thermodynamically more stable and, thus, the most studied ones [1], a few of them being commercially available.

Revisions surveying the synthesis, reactivity, spectroscopic characterization and crystallographic studies of 1,2,4-triazolo[1,5-c]pyrimidines [2], 1,2,4-triazolo[4,3-a]pyrimidines [3] and 1,2,4-triazolo[4,3-c]pyrimidines [4] have also been published.

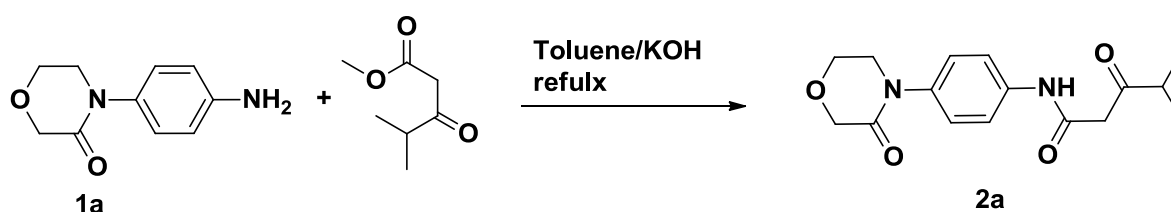
Recently, 1,2,4-triazolo[1,5-a]pyrimidines have aroused increasing attention from the chemical and biological points of view due to their diverse pharmacological activities such as

anti-inflammatory [5], antimalarial [6], antifungal effect [7], macrophage activation [8], antitumor potency [9-12], antimicrobial [13-16,20-24] and inhibition of KDR kinase [17]. They have proven to be promising anticancer [18] agents with dual mechanisms of tubulin polymerization promotion [9-10] as well as cyclin dependent kinases 2 inhibition [19].

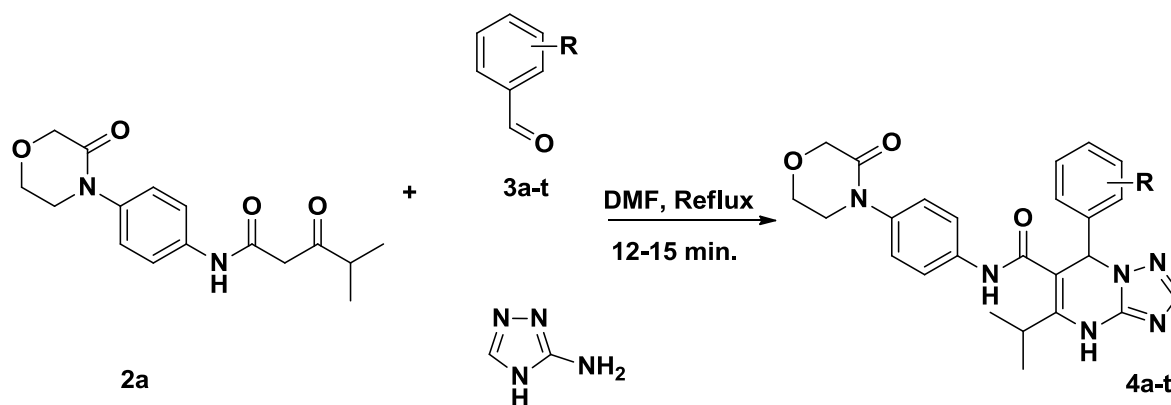
2. RESULTS AND DISCUSSION

Chemistry:

4-methyl-3-oxo-N-(4-(3-oxomorpholino)phenyl)pentanamide (2a) were prepared by reacting 4-(4-aminophenyl)morpholin-3-one (1a) and methyl-4-methyl-3-oxopentanoate in toluene with a catalytic amount of NaOH/KOH (Scheme 1). The reaction mixture was refluxed for 15-20 h.



Scheme 1. Synthesis of 4-methyl-3-oxo-N-(4-(3-oxomorpholino)phenyl)pentanamide.



Scheme 2. Synthesis of 5-isopropyl-N-(4-(3-oxomorpholino)phenyl)-7-phenyl-4,7-dihydro-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide by using 4H-1,2,4-triazol-3-amine.

Reaction of acetoacetamide (2a) derivative with various aromatic aldehydes (3a-t) and 1,2,4-triazol-amine in presence of trace amount of dimethyl formamide at high temperature (fusion) gives the novel triazolo[1,5-a]pyrimidinederivatives (4a-t) with excellent yields (Scheme 2). All the synthesized compounds were characterized by various spectroscopic techniques like ^1H NMR, ^{13}C NMR, IR and Mass.

Biology:

All the synthesized compounds were tested against different bacterial and fungal strains i.e. *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Escherichia Coli*, *Staphylococcus aureus*, *Candida albican* for their in vitro antibacterial activity. Well Diffusion/Agar Cup Method was used and results are listed in Table 2. The investigation of antibacterial and antifungal screening data revealed that all the tested compounds 4a-t showed moderate to potent activity. The compounds 4f, 4g and 4i showed comparatively good activity against all the bacterial strains.

2. 1. Experimental

Thin-layer chromatography was accomplished on 0.2-mm precoated plates of silica gel G60 F₂₅₄ (Merck). Visualization was made with UV light (254 and 365 nm) or with an iodine vapor. IR spectra were recorded on a FTIR-8400 spectrophotometer using DRS prob. ¹H (400 MHz) and ¹³C (400 MHz) NMR spectra were recorded on a Bruker AVANCE II spectrometer in DMSO. Chemical shifts are expressed in δ ppm downfield from TMS as an internal standard. Mass spectra were determined using direct inlet probe on a GCMS-QP 2010 mass spectrometer (Shimadzu). Solvents were evaporated with a BUCHI rotary evaporator. Melting points were measured in open capillaries and are uncorrected.

2. 1. 1. General procedure for the synthesis of 4-methyl-3-oxo-N-(4-(3-oxomorpholino)phenyl)pentanamide (2a)

A mixture of 4-(4-aminophenyl)morpholin-3-one (10 mmol), methyl 4-methyl-3-oxopentanoate (10 mmol) and catalytic amount of sodium or potassium hydroxide (10 %) in toluene (50ml) was refluxed at 110 °C for 12-15 h. The reaction was monitored by TLC. After completion of reaction, the solid was precipitated out, filtered and washed with n-hexane giving 2a.

2. 1. 2. General procedure for the synthesis of substituted triazolopyrimidines (4a-t)

A mixture of the aminoazole (0.01 mol), 4-methyl-3-oxo-N-(4-(3-oxomorpholino)phenyl)pentanamide (0.01 mol) **2a** and an appropriate aromatic aldehyde (0.01 mol) **3a-t** was fused in presence of trace amount of DMF for 12-15 min. After cooling, methanol (~10 mL) was added. The reaction mixture was allowed to stand overnight and then filtered to give the solid triazolopyrimidine products **4a-t**, which were crystallized from ethanol and subsequently dried in air.

2. 1. 3. Spectroscopic data for the compounds (4a-t):

5-isopropyl-N-(4-(3-oxomorpholino)phenyl)-7-phenyl-4,7-dihydro-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (4a): White solid; mp 240-242 °C; R_f 0.53 (4:6 hexane-EtOAc); IR (KBr): 3643, 3375, 3061, 2922, 2858, 1919, 1790, 1635, 1552, 1504, 1458, 1294, 1192, 1076, 1026, 997, 856, 785, 696, 580 cm⁻¹; ¹H NMR: δ 1.185-1.307 (d, 6H, (CH₃)₂, $j = 6.88$ Hz), 3.306-3.396 (m, 1H, -CH), 3.652-3.678 (t, 2H, -CH₂- in morpholinone ring), 3.937-3.962 (t, 2H, -CH₂- in morpholinone ring), 4.174 (s, 2H, -CH₂- in morpholinone ring near ketone), 6.528 (s, 1H, -CH pyrimidine ring), 7.205-7.222 (t, 2H, Ar-H, $j = 4.92$ Hz), 7.239-7.255 (d, 2H, Ar-H, $j = 5.0$ Hz), 7.254-7.281 (t, 1H, Ar-H), 7.296-7.316 (d, 2H, Ar-H ring nearby -NHCO-), 7.520-7.542 (d, 2H, Ar-H ring nearby -NHCO-), 7.593 (s, 1H, -CH triazole ring), 9.926 (s, 1H, -NH pyrimidine ring), 10.002 (s, 1H, -NH amide); ¹³C NMR (400

MHz, DMSO): 19.54, 28.75, 38.88, 39.09, 39.29, 39.50, 39.71, 39.92, 40.13, 49.09, 60.69, 63.47, 67.73, 102.53, 119.83, 125.62, 126.94, 127.97, 128.37, 136.65, 137.32, 140.45, 143.99, 148.32, 149.71, 165.83; MS (*m/z*): 458 (M^+); Anal. Calcd for $C_{25}H_{26}N_6O_3$: C, 65.49; H, 5.72; N, 18.33; Found: C, 65.30; H, 5.70; N, 18.10.

7-(4-chlorophenyl)-5-isopropyl-N-(4-(3-oxomorpholino)phenyl)-4,7-dihydro[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (4b): White solid; mp 245-248 °C; R_f 0.48 (4:6 hexane-EtOAc); IR (KBr): 3742, 3377, 3095, 2974, 2861, 2155, 1919, 1735, 1550, 1461, 1236, 1192, 1001, 923, 833, 783, 636, 588 cm^{-1} ; MS (*m/z*): 492 (M^+); Anal. Calcd for $C_{25}H_{25}ClN_6O_3$: C, 60.91; H, 5.11; N, 17.05; Found: C, 60.85; H, 5.10; N, 17.10.

7-(4-fluorophenyl)-5-isopropyl-N-(4-(3-oxomorpholino)phenyl)-4,7-dihydro[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (4c): White solid; mp 260-265 °C; R_f 0.52 (4:6 hexane-EtOAc); IR (KBr): 3738, 3614, 3383, 3048, 2933, 2864, 1919, 1730, 1651, 1557, 1520, 1454, 1291, 1177, 1072, 999, 895, 792, 646, 559 cm^{-1} ; MS (*m/z*): 476 (M^+); Anal. Calcd for $C_{25}H_{25}FN_6O_3$: C, 63.01; H, 5.29; N, 17.64; Found: C, 63.05; H, 5.35; N, 17.80.

7-(2-chlorophenyl)-5-isopropyl-N-(4-(3-oxomorpholino)phenyl)-4,7-dihydro[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (4d): White solid; mp 235-237 °C; R_f 0.50 (4:6 hexane-EtOAc); IR (KBr): 3631, 3248, 3107, 3052, 2964, 2868, 1784, 1649, 1579, 1533, 1475, 1247, 1193, 1076, 842, 767, 657, 559 cm^{-1} ; 1H NMR: δ 1.193-1.312 (d, 6H, $(CH_3)_2$, $j = 6.92$ Hz), 3.274-3.408 (m, 1H, -CH of isopropyl gp.), 3.666-3.691 (t, 2H, -CH₂- in morpholinone ring), 3.948-3.973 (t, 2H, -CH₂- in morpholinone ring), 4.179 (s, 2H, -CH₂- in morpholinone ring near ketone), 6.528 (s, 1H, -CH pyrimidine ring), 7.187-7.283 (m, 4H, Ar-H, $j = 4.96$ Hz), 7.295-7.313 (d, 2H, Ar-H ring nearby -NHCO-, $j = 1.52$ Hz), 7.332-7.539 (d, 2H, Ar-H ring nearby -NHCO-, $j = 7.96$ Hz), 8.213 (s, 1H, -CH triazole ring), 10.004 (s, 1H, -NH pyrimidine ring), 10.031 (s, 1H, -NH amide); ^{13}C NMR (400 MHz, DMSO): 19.52, 19.76, 28.91, 38.87, 39.08, 39.28, 39.49, 39.70, 39.91, 40.12, 49.08, 60.11, 63.47, 67.73, 78.44, 78.77, 79.10, 101.88, 119.93, 125.63, 126.88, 128.10, 130.21, 133.27, 136.78, 137.14, 142.53, 144.27, 148.28, 149.95, 164.87, 165.83; MS (*m/z*): 492 (M^+); Anal. Calcd for $C_{25}H_{25}ClN_6O_3$: C, 60.91; H, 5.11; N, 17.05; Found: C, 59.76; H, 5.20; N, 17.10.

7-(2,5-dimethoxyphenyl)-5-isopropyl-N-(4-(3-oxomorpholino)phenyl)-4,7-dihydro-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide(4e): White solid; mp 238-240 °C; R_f 0.43 (4:6 hexane-EtOAc); IR (KBr): 3642, 3239, 3095, 2962, 2831, 1789, 1668, 1581, 1543, 1467, 1276, 1184, 1047, 1027, 837, 709, 679, 547 cm^{-1} ; MS (*m/z*): 518 (M^+); Anal. Calcd for $C_{27}H_{30}N_6O_5$: C, 62.54; H, 5.83; N, 16.21; Found: C, 62.00; H, 5.75; N, 16.30.

5-isopropyl-N-(4-(3-oxomorpholino)phenyl)-7-(p-tolyl)-4,7-dihydro-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (4f): White solid; mp 240-245 °C; R_f 0.51 (4:6 hexane-EtOAc); IR (KBr): 3379, 3284, 3099, 2978, 2870, 1658, 1610, 1597, 1408, 1348, 1238, 1192, 1072, 786, 684, 648, 588, 511 cm^{-1} ; 1H NMR: δ 1.177-1.300 (d, 6H, $(CH_3)_2$, $j = 7.04$ Hz), 2.252 (s, 3H, -CH₃gp.), 3.301-3.388 (m, 1H, -CH), 3.659-3.685 (t, 2H, -CH₂- in morpholinone ring), 3.946-3.971 (t, 2H, -CH₂- in morpholinone ring), 4.174 (s, 2H, -CH₂- in morpholinone ring near ketone), 6.482 (s, 1H, -CH pyrimidine ring), 7.095-7.117 (d, 2H, Ar-H, $j = 8.68$ Hz), 7.211-7.218 (d, 2H, Ar-H, $j = 2.6$ Hz), 7.240-7.527 (d, 2H, Ar-H ring nearby -NHCO-), 7.549-7.573 (d, 2H, Ar-H ring nearby -NHCO-), 8.213 (s, 1H, -CH triazole ring), 9.875 (s, 1H, -NH pyrimidine ring), 9.983 (s, 1H, -NH amide); ^{13}C NMR (400 MHz,

DMSO):19.55, 19.78, 20.66, 28.70, 38.88, 39.09, 39.30, 39.51, 39.72, 39.92, 40.13, 49.10, 60.40, 63.47, 67.72, 78.45, 78.78, 79.11, 102.61, 119.77, 125.61, 126.87, 128.94, 136.62, 137.30, 137.36, 137.58, 143.89, 148.23, 149.62, 165.82; MS (*m/z*): 472 (M^+); Anal. Calcd for $C_{26}H_{28}N_6O_3$: C, 66.09; H, 5.97; N, 17.78; Found: C, 66.10; H, 5.91; N, 17.90.

7-(3,4-dimethoxyphenyl)-5-isopropyl-N-(4-(3-oxomorpholino)phenyl)-4,7-dihydro-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (4g): White solid; mp 240-243 °C; R_f 0.42 (4:6 hexane-EtOAc); IR (KBr): 3642, 3232, 3071, 2968, 2876, 1784, 1649, 1591, 1521, 1411, 1261, 1128, 1028, 875, 732, 659, 555 cm^{-1} ; MS (*m/z*): 518 (M^+); Anal. Calcd for $C_{27}H_{30}N_6O_5$: C, 62.54; H, 5.83; N, 16.21; Found: C, 62.70; H, 5.90; N, 16.17.

5-isopropyl-7-(4-methoxyphenyl)-N-(4-(3-oxomorpholino)phenyl)-4,7-dihydro-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (4h): White solid; mp 225-228 °C; R_f 0.43 (4:6 hexane-EtOAc); IR (KBr): 3610, 3244, 3113, 3055, 2933, 2868, 1791, 1622, 1593, 1321, 1271, 1205, 1166, 1028, 827, 794, 659, 565 cm^{-1} ; MS (*m/z*): 488 (M^+); Anal. Calcd for $C_{26}H_{28}N_6O_4$: C, 63.92; H, 5.78; N, 17.20; Found: C, 64.00; H, 5.60; N, 17.10.

5-isopropyl-7-(2-nitrophenyl)-N-(4-(3-oxomorpholino)phenyl)-4,7-dihydro-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (4i): White solid; mp 248-250 °C; R_f 0.48 (4:6 hexane-EtOAc); IR (KBr): 3634, 3527, 3228, 3099, 2958, 2872, 1778, 1664, 1583, 1531, 1473, 1356, 1317, 1253, 1199, 1072, 1020, 995, 844, 731, 684, 547 cm^{-1} ; MS (*m/z*): 503 (M^+); Anal. Calcd for $C_{25}H_{25}N_7O_5$: C, 59.63; H, 5.00; N, 19.47; Found: C, 59.50; H, 5.10; N, 19.55.

7-(2,4-dichlorophenyl)-5-isopropyl-N-(4-(3-oxomorpholino)phenyl)-4,7-dihydro-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (4j): White solid; mp 250-255 °C; R_f 0.40 (4:6 hexane-EtOAc); IR (KBr): 3610, 3236, 3080, 2933, 2891, 1763, 1630, 1570, 1538, 1470, 1276, 1170, 1072, 1041, 800, 870, 736, 667, 589 cm^{-1} ; MS (*m/z*): 527 (M^+); Anal. Calcd for $C_{25}H_{24}Cl_2N_6O_3$: C, 56.93; H, 4.59; N, 15.93; Found: C, 57.00; H, 4.50; N, 16.01.

7-(3,4-dimethylphenyl)-5-isopropyl-N-(4-(3-oxomorpholino)phenyl)-4,7-dihydro-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (4k): Whitesolid; mp 252-255 °C; R_f 0.44 (4:6 hexane-EtOAc); IR (KBr): 3630, 3240, 3090, 2936, 2890, 1763, 1640, 1580, 1542, 1480, 1276, 1170, 1074, 1043, 800, 880, 735, 662, 590 cm^{-1} ; MS (*m/z*): 527 (M^+); Anal. Calcd for $C_{25}H_{24}Cl_2N_6O_3$: C, 56.93; H, 4.59; N, 15.93; Found: C, 57.01; H, 4.50; N, 15.90.

7-(2-chlorophenyl)-5-isopropyl-N-(4-(3-oxomorpholino)phenyl)-4,7-dihydro-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (4l): White solid; mp 236-237 °C; R_f 0.43 (4:6 hexane-EtOAc); IR (KBr): 3233, 3188, 3064, 2918, 2864, 1741, 1680, 1624, 1542, 1449, 1390, 1280, 1213, 1136, 1035, 966, 835, 768, 670, 595 cm^{-1} ; MS (*m/z*): 492 (M^+); Anal. Calcd for $C_{25}H_{25}ClN_6O_3$: C, 60.91; H, 5.11; N, 17.05; Found: C, 60.85; H, 5.20; N, 17.10.

5-isopropyl-N-(4-(3-oxomorpholino)phenyl)-7-(m-tolyl)-4,7-dihydro-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (4m): White solid; mp 242-245 °C; R_f 0.49 (4:6 hexane-EtOAc); IR (KBr): 3290, 3045, 2936, 1730, 1633, 1555, 1512, 1465, 1370, 1290, 1195, 990, 815, 769, 669, 515 cm^{-1} ; MS (*m/z*): 472 (M^+); Anal. Calcd for $C_{26}H_{28}N_6O_3$: C, 66.09; H, 5.97; N, 17.78; Found: C, 66.15; H, 5.93; N, 17.80.

7-(2,3-dimethylphenyl)-5-isopropyl-N-(4-(3-oxomorpholino)phenyl)-4,7-dihydro-[1,2,4] triazolo[1,5-a]pyrimidine-6-carboxamide (4n): White solid; mp 255-258 °C; R_f 0.48 (4:6 hexane-EtOAc); IR (KBr): 3242, 3180, 3054, 2928, 2857, 1740, 1675, 1632, 1437, 1391, 1270, 1225, 1144, 1048, 951, 843, 779, 683, 591 cm^{-1} ; MS (m/z): 487 (M^+); Anal. Calcd for $\text{C}_{27}\text{H}_{30}\text{N}_6\text{O}_3$: C, 66.65; H, 6.21; N, 17.27; Found: C, 66.67; H, 6.31; N, 17.33.

7-(2-fluorophenyl)-5-isopropyl-N-(4-(3-oxomorpholino)phenyl)-4,7-dihydro-[1,2,4] triazolo[1,5-a]pyrimidine-6-carboxamide (4o): White solid; mp 262-265 °C; R_f 0.47 (4:6 hexane-EtOAc); IR (KBr): 3277, 3155, 3058, 2920, 2874, 1670, 1590, 1478, 1327, 1288, 1210, 1192, 1022, 976, 817, 747, 662, 556 cm^{-1} ; MS (m/z): 476 (M^+); Anal. Calcd for $\text{C}_{25}\text{H}_{25}\text{FN}_6\text{O}_3$: C, 63.01; H, 5.29; N, 17.64; Found: C, 63.11; H, 5.35; N, 17.70.

5-isopropyl-7-(2-nitrophenyl)-N-(4-(3-oxomorpholino)phenyl)-4,7-dihydro-[1,2,4] triazolo[1,5-a]pyrimidine-6-carboxamide (4p): Off White solid; mp 250-252 °C; R_f 0.40 (4:6 hexane-EtOAc); IR (KBr): 3290, 3150, 3063, 2929, 2870, 1671, 1550, 1482, 1370, 1278, 1186, 1076, 965, 831, 750, 675, 525 cm^{-1} ; MS (m/z): 503 (M^+); Anal. Calcd for $\text{C}_{25}\text{H}_{25}\text{N}_7\text{O}_5$: C, 59.63; H, 5.00; N, 19.47; Found: C, 59.50; H, 4.92; N, 19.60.

7-(3-chloro-4-fluorophenyl)-5-isopropyl-N-(4-(3-oxomorpholino)phenyl)-4,7-dihydro-[1,2,4] triazolo[1,5-a]pyrimidine-6-carboxamide (4q): White solid; mp 264-266 °C; R_f 0.45 (4:6 hexane-EtOAc); IR (KBr): 3240, 3190, 3068, 2950, 2860, 1690, 1597, 1590, 1490, 1280, 1185, 1085, 1050, 873, 789, 680, 596 cm^{-1} ; MS (m/z): 510 (M^+); Anal. Calcd for $\text{C}_{25}\text{H}_{24}\text{ClFN}_6\text{O}_3$: C, 58.77; H, 4.73; N, 16.45; Found: C, 58.82; H, 4.69; N, 16.50.

7-(4-chloro-2-nitrophenyl)-5-isopropyl-N-(4-(3-oxomorpholino)phenyl)-4,7-dihydro-[1,2,4] triazolo[1,5-a]pyrimidine-6-carboxamide (4r): Off white solid; mp 260-265 °C; R_f 0.39 (4:6 hexane-EtOAc); IR (KBr): 3250, 3189, 3072, 2939, 2879, 1730, 1667, 1550, 1470, 1350, 1310, 1290, 1160, 1073, 884, 778, 677, 592 cm^{-1} ; MS (m/z): 537 (M^+); Anal. Calcd for $\text{C}_{25}\text{H}_{24}\text{ClN}_7\text{O}_5$: C, 55.82; H, 4.50; N, 18.23; Found: C, 55.90; H, 4.61; N, 18.30.

7-(4-chloro-3-nitrophenyl)-5-isopropyl-N-(4-(3-oxomorpholino)phenyl)-4,7-dihydro-[1,2,4] triazolo[1,5-a]pyrimidine-6-carboxamide (4s): White solid; mp 266-268 °C; R_f 0.36 (4:6 hexane-EtOAc); IR (KBr): 3260, 3190, 3072, 2949, 2880, 1750, 1660, 1550, 1470, 1340, 1310, 1280, 1180, 1073, 884, 779, 678, 590 cm^{-1} ; MS (m/z): 537 (M^+); Anal. Calcd for $\text{C}_{25}\text{H}_{24}\text{ClN}_7\text{O}_5$: C, 55.82; H, 4.50; N, 18.23; Found: C, 55.90; H, 4.55; N, 18.35.

5-isopropyl-N-(4-(3-oxomorpholino)phenyl)-7-(2-(trifluoromethyl)phenyl)-4,7-dihydro-[1,2,4] triazolo[1,5-a]pyrimidine-6-carboxamide (4t): White solid; mp 234-236 °C; R_f 0.48 (4:6 hexane-EtOAc); IR (KBr): 3260, 3188, 3067, 2935, 2875, 1710, 1665, 1583, 1539, 1487, 1282, 1154, 1093, 1040, 885, 796, 680, 595 cm^{-1} ; MS (m/z): 526 (M^+); Anal. Calcd for $\text{C}_{26}\text{H}_{25}\text{F}_3\text{N}_6\text{O}_3$: C, 59.31; H, 4.79; N, 15.96; Found: C, 59.39; H, 4.69; N, 16.01.

Table 1. Synthesis of substituted Triazolopyrimidines.

| Entry | Code | R | Yield (%) | Time (min.) |
|-------|------|-------------------------|-----------|-------------|
| 1 | 4a | H | 75 | 12 |
| 2 | 4b | 4-Cl | 78 | 13 |
| 3 | 4c | 4-F | 80 | 13 |
| 4 | 4d | 3-Cl | 68 | 14 |
| 5 | 4e | 2,5-di-CH ₃ | 80 | 15 |
| 6 | 4f | 4-CH ₃ | 78 | 15 |
| 7 | 4g | 3,4-di-OCH ₃ | 69 | 15 |
| 8 | 4h | 4-OCH ₃ | 88 | 12 |
| 9 | 4i | 3-NO ₂ | 84 | 13 |
| 10 | 4j | 2,4-di-Cl | 83 | 16 |
| 11 | 4k | 3,4-di-CH ₃ | 73 | 15 |
| 12 | 4l | 2-Cl | 82 | 13 |
| 13 | 4m | 3-CH ₃ | 76 | 14 |
| 14 | 4n | 2,3-di-CH ₃ | 73 | 14 |
| 15 | 4o | 2-F | 71 | 16 |
| 16 | 4p | 2-NO ₂ | 83 | 18 |
| 17 | 4q | 3-Cl, 4-F | 85 | 17 |
| 18 | 4r | 4-Cl, 2-NO ₂ | 72 | 16 |
| 19 | 4s | 4-Cl, 3-NO ₂ | 70 | 15 |
| 20 | 4t | 2-CF ₃ | 76 | 15 |

Biology:**Antimicrobial Sensitivity Testing
Well Diffusion/Agar Cup Method**

In vitro affectivity of antimicrobial agents can be demonstrated by observing their capacity to inhibit bacterial growth on suitable media. The production of a zone depends on two factors namely bacterial growth and concentration of antimicrobial agent. The hole/well punch method was first used by Bennett. This diffusion method has proved more effective

than many other methods. According to Lt. General Raghunath the well technique is 5-6 times more sensitive than using disk method.

Table 2. Antimicrobial Sensitivity Assay.

| Sr. No. | Code no. | MIC ($\mu\text{g/mL}$) | | | | | | |
|---------|-----------------|--------------------------|---------------------|-----------------|-------------------|---------------------|----------------|-------------------|
| | | antibacterial activity | | | | antifungal activity | | |
| | | <i>E.coli</i> | <i>P.aeruginosa</i> | <i>S.aureus</i> | <i>S.pyogenus</i> | <i>C.albicans</i> | <i>A.niger</i> | <i>A.clavatus</i> |
| 1 | 4a | 250 | 200 | 250 | 100 | 500 | 1000 | 1000 |
| 2 | 4b | 200 | 125 | 100 | 125 | 1000 | >1000 | >1000 |
| 3 | 4c | 100 | 100 | 125 | 250 | 250 | >1000 | >1000 |
| 4 | 4d | 125 | 250 | 250 | 100 | 100 | 500 | 500 |
| 5 | 4e | 200 | 250 | 250 | 250 | 1000 | >1000 | >1000 |
| 6 | 4f | 62.5 | 200 | 100 | 200 | >1000 | 500 | 500 |
| 7 | 4g | 100 | 62.5 | 125 | 100 | >1000 | 250 | 250 |
| 8 | 4h | 250 | 200 | 250 | 250 | 500 | 1000 | 1000 |
| 9 | 4i | 125 | 200 | 50 | 100 | 250 | >1000 | >1000 |
| 10 | 4j | 100 | 100 | 100 | 125 | 250 | 1000 | 1000 |
| 11 | 4k | 50 | 100 | 250 | 250 | 1000 | >1000 | >1000 |
| 12 | 4l | 100 | 250 | 100 | 200 | 1000 | >1000 | >1000 |
| 13 | 4m | 250 | 500 | 500 | 100 | 1000 | 1000 | 1000 |
| 14 | 4n | 250 | 1000 | 250 | 500 | 1000 | 500 | 500 |
| 15 | 4o | 100 | 250 | 200 | 250 | 500 | >1000 | >1000 |
| | Gentamycin | 0.05 | 1 | 0.25 | 0.5 | - | - | - |
| | Ampicilin | 100 | - | 250 | 100 | - | - | - |
| | Chloramphenicol | 50 | 50 | 50 | 50 | - | - | - |
| | Ciprofloxacin | 25 | 25 | 50 | 50 | - | - | - |
| | Norfloxacin | 10 | 10 | 10 | 10 | - | - | - |
| | Nystatin | - | - | - | - | 100 | 100 | 100 |
| | Greseofulvin | - | - | - | - | 500 | 100 | 100 |

3. CONCLUSION

In summary, we have described the synthesis of substituted triazolopyrimidine derivatives in moderate yield. The reaction of various aldehydes with acetoacetamide and 5-amino-1,2,4-triazole was afforded the triazolopyrimidine derivatives in moderate to good yield. All the synthesized compounds were evaluated for their antimicrobial activity. The investigation of antibacterial and antifungal screening data revealed that all the tested compounds 4a-t showed moderate to potent activity. The compounds 4f, 4g and 4i are found comparatively good active against all the bacterial strains.

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References

- [1] Fischer G., *Adv. Heterocyclic Chemistry* 57 (1993) 81.
- [2] Shaban M.A.E., Morgan A.E.A., *Adv. Heterocyclic Chemistry* 77 (2000) 345.
- [3] Shaban, M.A.E., Morgan A.E.A., *Adv. Heterocyclic Chemistry* 73 (2000) 131.
- [4] Shaban, M.A.E.; Morgan A.E.A., *Adv. Heterocyclic Chemistry* 75 (2000) 243.
- [5] Ashour H. M., Shaaban O. G., Rizk O. H., El-Ashmawy I. M., *Euro. J. Med. Chem.* 62 (2013) 341.
- [6] Marwaha A., White J., El Mazoun F., Creason S. A., Kokkond S., Buckner F. S., Rathod P. K., *J. Med. Chem.* 55 (2012) 7425.
- [7] Chen Q., Zhu X. L., Liu, et al., *Euro. J. Med. Chem.* 43 (2008) 595.
- [8] Uryu S., Tokuhiko S., Murasugi T., *Brain Research* 946 (2002) 298.
- [9] Zhang N., Semiramis A. K., Thai N., *J. Med. Chem.* 50 (2007) 319.
- [10] Havlicek L., Fuksova K., Krystof V., *Bioorg. Med. Chem.* 13 (2005) 5399.
- [11] Zhao X., Zhao Y., Guo S., Song H., Wang D., Gong P., *Molecules* 12 (2007) 1136.
- [12] Iwona L., Marzena F., Tadeusz M., Tadeusz S., Julia J., *Dalton Transactions* 42 (2013) 6219.
- [13] Yin L., Shuai Z., Zhi-Jun L., Hai-Liang Z., *Euro. J. Med. Chem.* 64 (2013) 54.
- [14] Ashraf H. F., Abd El-Wahab, *Pharmaceuticals* 5 (2012) 745.
- [15] Abdel-Aziem A., Sayed El-Gendy M., Abdelhamid A. O., *Euro. J. Chem.* 3 (2012) 455.
- [16] Khera M. K., Cliffe I. A., Mathur T., Prakash O., *Bioorg & Med. Chem. Lett.* 21 (2011) 2887.
- [17] Fraley M. E., Hoffman W. F., Rubino R. S., *Bioorg. Med. Chem. Lett.* 12 (2002) 2767.
- [18] Antonino L., Ilenia A., Chiara P., Annamaria M., Gaetano D., Maria A. A., *Euro. J. Med. Chem.* 62 (2013) 416.
- [19] Fairfield B. J., Andrew C., Allan J., WO2004108136, 2004.

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- [20] G. Thirunarayanan, *International Letters of Chemistry, Physics and Astronomy* 5 (2014) 89-98.
- [21] Nirav M. Shah, Hitendra S. Joshi, *International Letters of Chemistry, Physics and Astronomy* 6 (2014) 56-60.
- [22] Piyush B. Vekariya, Jalpa R. Pandya, Vaishali Goswami, Hitendra S. Joshi, *International Letters of Chemistry, Physics and Astronomy* 7 (2014) 45-52.
- [23] Sagar P. Gami, Kalpesh V. Vilapara, Hasmukh R. Khunt, Jayesh S. Babariya, Yogesh T. Naliapara, *International Letters of Chemistry, Physics and Astronomy* 11(2) (2014) 127-134.
- [24] Hasmukh R. Khunt, Piyush P. Pipaliya, Satish M. Ghelani, Jayesh S. Babariya, Yogesh T. Naliapara, *International Letters of Chemistry, Physics and Astronomy* 12 (2014) 20-25.

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