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# Synthesis, characterization and *in-vitro* biological evaluation of urea analogue of tetrazolo[1,5-a]pyrimidine

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**ABSTRACT**

*A series of N-(substituted aryl)-4-(4-(4-fluorobenzyl)-7-(4-methoxyphenyl)-5-methyl-4,7-dihydrotetrazolo[1,5-a]pyrimidine-6-carbonyl)piperazine-1-carboxamidehave been synthesized. The structures of all synthesized compounds have been delineated by using $^1$H NMR, Mass and IR. All the synthesized compounds were screened for their in-vitro antimicrobial activity.*

**Key words:** Dihydropyrimidine, Tetrazolo[1,5-a]pyrimidine**,** *in-vitro* antimicrobial activity.

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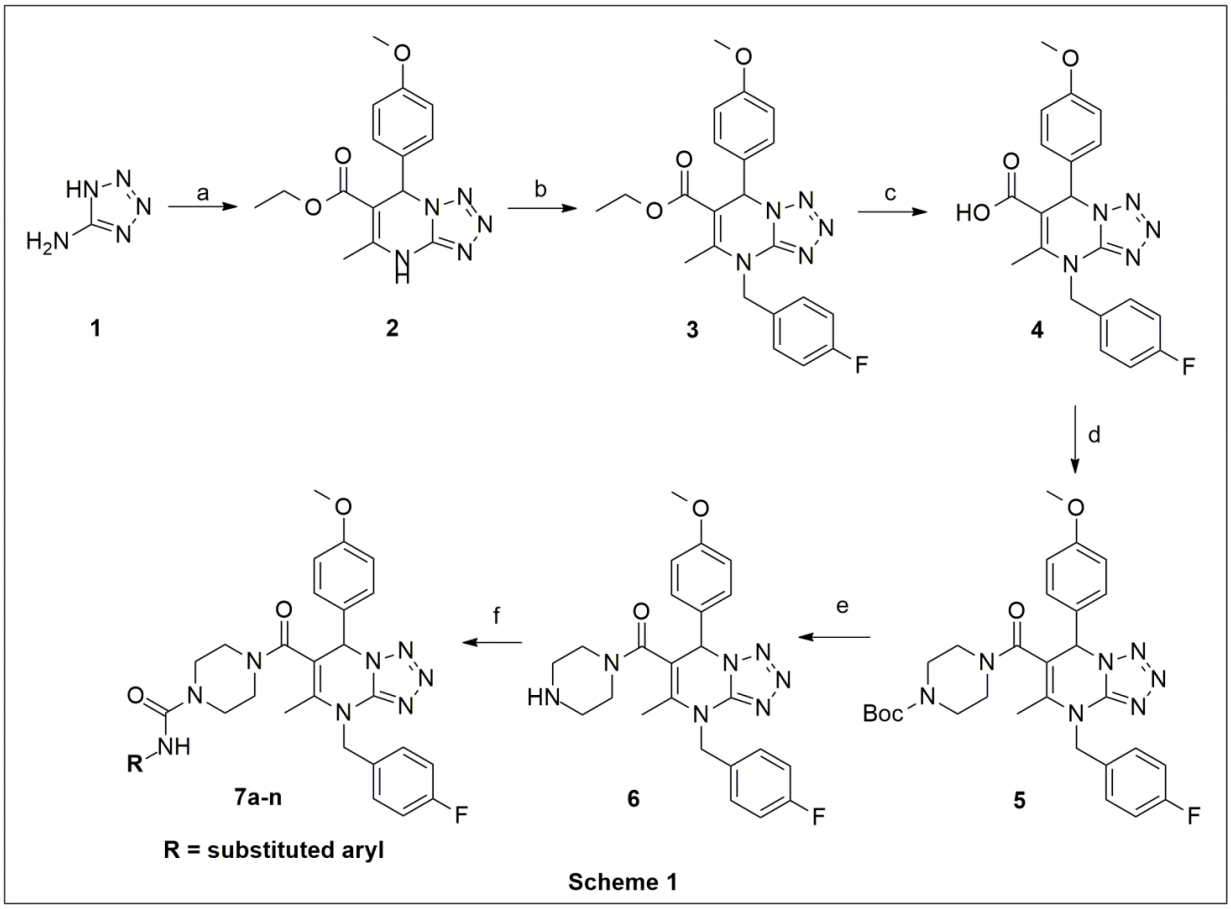
## INTRODUCTION

Organic compounds containing pyrimidine as a core unit are known to exhibit various biological and pharmaceutical activities. [1] The synthesis of these complex heterocyclic scaffolds is assigned as one of the most fertile areas for both organic and medicinal chemistry, in context dihydropyrimidine core unit were found to show interesting biological activities such as antiviral, antitumor, anti-inflammatory and antibacterial. [2] Tetrazolopyrimidines have been reported to be used in the treatment of obesity, diabetes, atherosclerosis, hypertension, coronary heart disease, hypercholesterolemia, hyperlipidemia, thyroid cancer, hypothyroidism, depression, glaucoma, cardiac arrhythmias, and congestive heart failure.[3] Hence the preparation of Tetrazolo[1,5-a]pyrimidine core unit has gained much importance.

In view of these findings and in continuation of our work,[4] we herein report the synthesis, characterization and antimicrobial activity of urea analogue of Tetrazolo[1,5-a]pyrimidine derivatives.

## MATERIALS AND METHODS

All chemicals were LR grade and used without further purification. The progress of the reaction was monitored by TLC on precoated plates (silica gel 60, F254) and visualized with UV light. Melting points were determined using Lab India V10 apparatus and are uncorrected. Flash column chromatography was performed with silica gel 60 (60-120 mesh). NMR spectra ($^1$H at 400 MHz) were recorded using $CDCl_3$/DMSO-$d^6$ as a solvent and chemical shifts are expressed in parts per million (ppm) related to internal TMS. Infrared spectra were determined on a Shimadzu FT-IR. The elemental analysis was carried out by using a Perkin-Elmer2400 series-II elemental analyzer.The specifications of the LC/MS are as follows: electrospray (+) ionization, mass range 100-1500 Da, 20-V cone voltage, and Xterra MS C18 column (2.1 mm x 50 mm x 3.5 µm).




**Scheme 1**

***Reagents:****(a) Ethylacetoacetate, EtOH, reflux; (b) 4-fluorobenzylbromide, $K_2CO_3$, DMF, RT; (c) NaOH, $H_2O$, MeOH, RT; (d) i. Oxalylchloride, DCM; ii. Boc-piperazine, TEA, DCM.(e) TFA, DCM, RT; (f) Aryl isocynate, TEA, DMF.*

**Synthesis of Ethyl 7-(4-methoxyphenyl)-5-methyl-4,7-dihydrotetrazolo[1,5-a]Pyrimidine-6-carboxylate (2).**To a stirred solution of Ethylacetoacetate(14.8 ml, 0.11mol) in ethanol (120 ml) was added 1H-tetrazol-5-amine hydrate **1**(12 gm, 0.11mol) and 4-methoxybenzaldehyde (14.2 ml, 0.11mol) at RT. The reaction mixture was heated at 80 °C for 7 h. The reaction mixture was cooled at 0 °C. The solid product, so formed, was collected by filtration and recrystallized by ethanol as off white solid. 26.3 g (80%). MP: 202-204 °C. $^1$H NMR (DMSO-d6): δ 1.05 (t, 3H), 2.45 (s, 3H), 3.75 (s, 3H), 4.02(m, 2H), 6.53(s, 1H), 7.02-7.26 (m, 4H), 11.25 (s, 1H); MS: m/z 316(M+1). Anal.Calcd. For $C_{15}H_{17}N_5O_3$: C, 57.13; H, 5.43; N, 22.21; O, 15.22.Found: C, 57.11; H, 5.38; N, 22.24; O, 15.27.

**Synthesis of Ethyl 4-(4-fluorobenzyl)-7-(4-methoxyphenyl)-5-methyl-4,7-dihydrotetrazolo[1,5-a]Pyrimidine-6-carboxylate(3).**To a solution of compound **2**(10.4 g, 0.032mol), Potassium carbonate (6.83 g, 0.049mol) and 4-fluoro benzyl bromide (4.11ml, 0.032mol) in DMF (40ml) was stirred at RT for 7h. The reaction mixture was then poured in to ice cold water. The solid product, so formed, was collected by filtration and recrystallized from ethanol as off white solid. 10.19 g (73%). MP: 192-194 °C. $^1$H NMR (DMSO-d6): δ 1.08 (t, 3H), 1.98 (t, 3H), 3.81 (s, 3H), 4.01(m, 2H), 5.18(s, 2H), 6.51(s, 1H), 6.72- 6.99(m, 4H), 7.02- 7.26 (m, 4H); MS: m/z 424 (M+1). Anal.Calcd. For $C_{22}H_{22}FN_5O_3$: C, 62.40; H, 5.24; F, 4.49; N, 16.54; O, 11.34.Found: C, 62.47; H, 5.18; F, 4.52; N, 16.49; O, 11.39.

**Synthesis of 4-(4-fluorobenzyl)-7-(4-methoxyphenyl)-5-methyl-4,7-dihydrotetrazolo[1,5-a]Pyrimidine-6-carboxylic acid (4).**To the solution of ester compound **3**(10.0 g, 0.023 mol) in methanol (50 ml) was added a solution of sodium hydroxide (4.72 g, 0.118 mol) in water (14 ml) at RT. This mixture was heated to reflux for 6 h,then evaporated methanol under reduced pressure. The residue was diluted with cold water (100 ml) and acidified with an2M hydrochloric acid. The solid product, so formed, was collected by filtrationto give a white solid. 7.8 g (83%). MP: 216-217 °C. $^1$H NMR (DMSO-d6): δ 1.98 (t, 3H), 3.81 (s, 3H), 5.29(s, 2H), 6.49(s, 1H), 6.72- 6.99(m, 4H), 7.02- 7.26 (m, 4H), 12.08 (s, 1H); MS: m/z 396 (M+1). Anal.Calcd. For $C_{20}H_{18}FN_5O_3$: C, 60.75; H, 4.59; F, 4.81; N, 17.71; O, 12.14.Found: C, 60.69; H, 4.63; F, 4.77; N, 17.75; O, 12.16.

**Synthesis of tert-butyl 4-(4-(4-fluorobenzyl)-7-(4-methoxyphenyl)-5-methyl-4,7-dihydrotetrazolo[1,5-a]Pyrimidine-6-carbonyl) piperazine-1-carboxylate (5).**To a solution of compound **4** (7.6 g, 0.019 mol) in DCM (40 mL) was added oxalyl chloride (5.0 mL, 0.057 mol) followed by the addition of catalytic amount of DMF under nitrogen atmosphere at RT. The resulting mixture is stirred for 3h at RT and then concentrated. The resulting yellow crude was acid chloride. A solution of triethylamine (7.02 mL, 0.038mol), Boc- piperazine (4.5 g, 0.018mol) in DCM and the mixture is cooled at 10-15°C. A solution of acid chloride was added dropwise to the reaction mixture at 10-15°C. After completion of the addition, the reaction mixture is stirred at RT for 1h. The reaction mixture was evaporated under reduced pressure and the residue was dissolve in ethyl acetate and washed with saturated $NaHCO_3$ solution, and water. The organic layer was dried over sodium sulphate and evaporated under reduced pressureto give yellow solid. 7.2 g (66%). MP: 115-117 °C. 1H NMR (DMSO-d6): δ 1.04(s, 1H), 1.45(s, 9H),1.86(s, 1H), 2.02(s, 3H), 3.03(m, 2H), 3.32 (m, 2H), 3.81(s, 3H), 4.02(m, 2H), 5.16(s, 2H), 6.56(s, 1H), 6.78-6.97(m, 4H), 7.12-7.28 (m, 4H); MS: m/z 564 (M+1). Anal.Calcd. For $C_{29}H_{34}FN_7O_4$: C, 61.80; H, 6.08; F, 3.37; N, 17.40; O, 11.35.Found: C, 61.86; H, 6.11; F, 3.41; N, 17.42; O, 11.36.

**Synthesis of (4-(4-fluorobenzyl)-7-(4-methoxyphenyl)-5-Methyl-4,7-dihydrotetrazolo[1,5-a] pyrimidin-6-yl)(piperazin-1-yl) methanone (6).**To a solution of compound **5**(7.1 g, 0.012 mol) in DCM (70 ml) was added drop wise TFA(35 ml) at 10 °C and stirred at same temperature for 2.5 h. Reaction mixture was charged into saturated $NaHCO_3$solution, DCM layer was separated, aqueous layer were extracted with DCM . Combined organic layers were washed with water, dried over sodium sulphate and evaporated under reduced pressure to yield compound **6** as yellow solid. 5.2 g (88%). MP: 127-129 °C. $^1$H NMR (DMSO-d6): δ 1.03(s, 1H), 1.82(s, 1H), 2.01 (s, 3H), 2.05(m, 1H), 3.01(m, 2H), 3.31(m, 2H), 3.76 (s, 3H), 4.01(m, 2H),5.16(s, 2H), 6.54(s, 1H), 6.76-6.95(m, 4H), 7.10-7.26 (m, 4H); MS: m/z 464 (M+1). Anal.Calcd. For $C_{24}H_{26}FN_7O_2$: C, 62.19; H, 5.65; F, 4.10; N, 21.15; O, 6.90.Found: C, 62.15; H, 5.56; F, 4.07; N, 21.05; O, 6.83.

**General procedure for preperation of 4-(4-(4-fluorobenzyl)-7-(4-methoxyphenyl)-5-methyl-4,7-dihydrotetrazolo[1,5-a]pyrimidine-6-carbonyl)-N-(Aryl)piperazine-1-carboxamide (7a-7n).** To a stirred and cooled solution of compound **5** (0.54 mmol) and triethylamine(1.08 mmol) in DMF (15 ml) , aryl isocyanate (0.59 mmol) was added and the reaction mixture was allowed to warm at RT and stirred at RT for 4-6 h. The progress of reaction was monitored by TLC. Reaction mixture was charged in ice water (100 ml) and stirred for 20 min. Product was separated by filtration and washed with water (20 ml) and dried, gave 7a-7n. The yield, reaction time and physical properties are reported in Table-1

**4-(4-(4-fluorobenzyl)-7-(4-methoxyphenyl)-5-methyl-4,7-dihydrotetrazolo[1,5-a]pyrimidine-6-carbonyl)-N-(4-(trifluoromethyl) phenyl)piperazine-1-carboxamide (7a).**
IR: 3312, 3018, 2885, 2235, 1691, 1090 $cm^{-1}$ ; $^1$H NMR ($CDCl_3$): δ 1.25(s, 1H), 1.81(s, 1H), 1.98(s, 3H), 2.88(s, 1H), 3.10(d, 2H), 3.39(s, 1H), 3.61(s, 1H), 3.76(s, 3H), 4.11(s, 1H), 5.17(s, 2H), 6.44(s, 1H), 6.59(s, 1H), 6.85(d, 2H), 7.07(m, 4H), 7.21(d, 1H), 7.42(d, 2H), 7.51(d, 2H). ms: m/z 651 (M+1). Anal.Calcd. For $C_{32}H_{30}F_4N_8O_3$:C, 59.07; H, 4.65; F, 11.68; N, 17.22; O, 7.38found: C, 59.11; H, 4.59; F, 11.74; N, 17.25; O, 7.33.

**4-(4-(4-fluorobenzyl)-7-(4-methoxyphenyl)-5-methyl-4,7-dihydrotetrazolo[1,5-a]pyrimidine-6-carbonyl)-N-(4-(chloro)-2-(trifluoromethyl) phenyl)piperazine-1-carboxamide (7b).**
IR: 3299, 3026, 2872, 2239, 1683, 1088, 823 $cm^{-1}$ ; $^1$H NMR ($CDCl_3$): δ 1.18(s, 1H), 1.79(s, 1H), 1.97(s, 3H), 2.88(s, 1H), 2.95(d, 2H), 3.32(s, 3H), 3.58(s, 1H), 3.76(s, 3H), 4.15(s, 1H), 5.17(s, 2H), 6.55(s, 1H), 6.59(s, 1H), 6.88(d, 2H), 7.09(m, 3H), 7.11(d,1H), 7.50(d, 1H), 7.94(s, 1H). ms: m/z 686 (M+1). Anal. Calcd. For $C_{32}H_{29}ClF_4N_8O_3$:C, 56.10; H, 4.27; Cl, 5.18; F, 11.09; N, 16.36; O, 7.01found:C, 56.18; H, 4.21; Cl, 5.19; F, 11.23; N, 16.31; O, 7.07.

**4-(4-(4-fluorobenzyl)-7-(4-methoxyphenyl)-5-methyl-4,7-dihydrotetrazolo[1,5-a]pyrimidine-6-carbonyl)-N-(2-(trifluoromethyl) phenyl)piperazine-1-carboxamide (7c).**
IR: 3314, 3035, 2875, 2240, 1685, 1083 $cm^{-1}$ ; $^1$H NMR ($CDCl_3$): δ 1.24(s, 1H), 1.78(s, 1H), 1.97(s, 3H), 2.86(s, 1H), 2.91(d, 2H), 3.32(s, 1H), 3.62(s, 1H), 3.77(s, 3H), 4.13(s, 1H), 5.16(s, 2H), 6.47(s, 1H), 6.58(s, 1H), 6.88(d, 2H), 7.11(m, 4H), 7.17(d, 1H), 7.41(d, 1H), 7.46(d, 1H), 7.53(d, 1H), 7.58(s,1H). ms: m/z 651 (M+1). Anal.Calcd. For $C_{32}H_{30}F_4N_8O_3$:C, 59.07; H, 4.65; F, 11.68; N, 17.22; O, 7.38found: C, 59.03; H, 4.69; F, 11.78; N, 17.10; O, 7.25.

**4-(4-(4-fluorobenzyl)-7-(4-methoxyphenyl)-5-methyl-4,7-dihydrotetrazolo[1,5-a]pyrimidine-6-carbonyl)-N-(3-(trifluoromethyl) phenyl)piperazine-1-carboxamide (7d).**
IR: 3298, 3028, 2871, 2232, 1679, 1072 $cm^{-1}$ ; $^1$H NMR ($CDCl_3$): δ 1.21(s, 1H), 1.77(s, 1H), 1.98(s, 3H), 2.87(s, 1H), 2.98(d, 2H), 3.34(s, 1H), 3.59(s, 1H), 3.76(s, 3H), 4.13(s, 1H), 5.17(s, 2H), 6.49(s, 1H), 6.53(s, 1H), 6.84(d,

2H), 7.13(m, 4H), 7.31(d, 1H), 7.44(d, 2H), 7.57(d, 1H), 7.92(s, 1H),. ms: m/z 651 (M+1). Anal.Calcd. For $C_{32}H_{30}F_4N_8O_3$:C, 59.07; H, 4.65; F, 11.68; N, 17.22; O, 7.38found: C, 59.11; H, 4.61; F, 11.71; N, 17.19; O, 7.33.

**4-(4-(4-fluorobenzyl)-7-(4-methoxyphenyl)-5-methyl-4,7-dihydrotetrazolo[1,5-a]pyrimidine-6-carbonyl)-N-(4-(methylthio) phenyl)piperazine-1-carboxamide (7e).**
IR: 3274, 3033, 2865, 2209, 1688, 1098, 689cm$^{-1}$ ; $^1$H NMR ($CDCl_3$): δ 1.26(s, 1H), 1.80(s, 1H), 2.01(s, 3H), 2.63(s, 3H) 2.85(s, 1H), 3.10(d, 2H), 3.36(s, 1H), 3.68(s, 1H), 3.75(s, 3H), 4.16(s, 1H), 5.19(s, 2H), 6.52(s, 1H), 6.58(s, 1H), 6.91(d, 2H), 7.25(m, 5H), 7.49(d, 1H), 7.82(d, 2H), 7.89(d, 1H). ms: m/z 629 (M+1). Anal.Calcd. For $C_{32}H_{33}FN_8O_3S$:C, 61.13; H, 5.29; F, 3.02; N, 17.82; O, 7.63; S, 5.10found: C, 61.18; H, 5.24; F, 3.07; N, 17.79; O, 7.69; S, 5.18.

**4-(4-(4-fluorobenzyl)-7-(4-methoxyphenyl)-5-methyl-4,7-dihydrotetrazolo[1,5-a]pyrimidine-6-carbonyl)-N-(3-(Fluoro) phenyl)piperazine-1-carboxamide (7f).**
IR: 3304, 2983, 2850, 2245, 1646, 1167, 1045cm$^{-1}$ ; $^1$H NMR ($CDCl_3$): δ 1.23(s, 1H), 1.79(s, 1H), 1.93(s, 3H), 2.88(s, 1H), 3.12(d, 2H), 3.37(s, 1H), 3.63(s, 1H), 3.78(s, 3H), 4.11(s, 1H), 5.21(s, 2H), 6.48(s, 1H), 6.51(s, 1H), 6.63(d, 2H), 7.15(m, 4H), 7.32(d, 2H), 7.48(d, 1H), 7.53(d, 1H), 7.59(s, 1H),. ms: m/z 601 (M+1). Anal.Calcd. For $C_{31}H_{30}F_2N_8O_3$: C, 61.99; H, 5.03; F, 6.33; N, 18.66; O, 7.99found: C, 61.93; H, 5.10; F, 6.28; N, 18.71; O, 7.93.

**4-(4-(4-fluorobenzyl)-7-(4-methoxyphenyl)-5-methyl-4,7-dihydrotetrazolo[1,5-a]pyrimidine-6-carbonyl)-N-(3-(Chloro) phenyl) phenyl)piperazine-1-carboxamide (7g).**
IR: 3318, 3017, 2865, 2235, 1672, 1178, 1078, 823 cm$^{-1}$ ; $^1$H NMR ($CDCl_3$): δ 1.28(s, 1H), 1.73(s, 1H), 1.99(s, 3H), 2.87(s, 1H), 3.19(d, 2H), 3.32(s, 1H), 3.71(s, 1H), 3.77(s, 3H), 4.13(s, 1H), 5.30(s, 2H), 6.55(s, 1H), 6.59(s, 1H), 6.95(d, 2H), 7.29(m, 4H), 7.37(d, 2H), 7.52(d, 1H), 7.63(d, 1H), 7.87(s, 1H),. ms: m/z 618 (M+1). Anal.Calcd. For $C_{31}H_{30}ClFN_8O_3$: C, 60.34; H, 4.90; Cl, 5.75; F, 3.08; N, 18.16; O, 7.78found: C, 60.38; H, 4.95; Cl, 5.79; F, 3.04; N, 18.11; O, 7.84.

**4-(4-(4-fluorobenzyl)-7-(4-methoxyphenyl)-5-methyl-4,7-dihydrotetrazolo[1,5-a]pyrimidine-6-carbonyl)-N-(3,4,5-trimethoxyphenyl)piperazine-1-carboxamide (7h).**
IR: 3288, 3019, 2878, 2238, 1685, 1085cm$^{-1}$ ; $^1$H NMR ($CDCl_3$): δ 1.27(s, 1H), 1.79(s, 1H), 1.98(s, 3H), 2.75(s, 1H), 3.15(d, 2H), 3.38(s, 1H), 3.73(s, 1H), 3.78(s, 3H), 3.83(s, 9H), 4.14(s, 1H), 5.33(s, 2H), 6.49(s, 1H), 6.53(s, 1H), 6.67(d, 2H), 6.84(d, 2H), 7.09(m, 4H), 7.19(d, 1H). ms: m/z 673 (M+1). Anal.Calcd. For $C_{34}H_{37}FN_8O_6$: C, 60.70; H, 5.54; F, 2.82; N, 16.66; O, 14.27found: C, 60.65; H, 5.61; F, 2.74; N, 16.61; O, 14.23.

**4-(4-(4-fluorobenzyl)-7-(4-methoxyphenyl)-5-methyl-4,7-dihydrotetrazolo[1,5-a]pyrimidine-6-carbonyl)-N-(2,4-difluorophenyl)piperazine-1-carboxamide (7i).**
IR: 3289, 2995, 2863, 2248, 1653, 1164, 1038 cm$^{-1}$ ; $^1$H NMR ($CDCl_3$): δ 1.27(s, 1H), 1.81(s, 1H), 1.95(s, 3H), 2.89(s, 1H), 3.17(d, 2H), 3.36(s, 1H), 3.69(s, 1H), 3.79(s, 3H), 4.14(s, 1H), 5.29(s, 2H), 6.44(s, 1H), 6.53(s, 1H), 6.58(d,1H), 6.81(d, 2H), 7.05(d, 2H), 7.09(d, 2H), 7.28(d, 1H), 7.36(d, 1H), 7.39(d, 1H),. ms: m/z 619 (M+1). Anal.Calcd. For $C_{31}H_{29}F_3N_8O_3$: C, 60.19; H, 4.73; F, 9.21; N, 18.11; O, 7.76found:C, 60.11; H, 4.63; F, 9.18; N, 18.16; O, 7.70

**4-(4-(4-fluorobenzyl)-7-(4-methoxyphenyl)-5-methyl-4,7-dihydrotetrazolo[1,5-a]pyrimidine-6-carbonyl)-N-(benzyl)piperazine-1-carboxamide (7j).**
IR: 3182, 3090, 2924, 1720, 1654, 1141, 1099cm$^{-1}$ ; $^1$H NMR ($CDCl_3$): δ 1.26(s, 1H), 1.89(s, 1H), 1.99(s, 3H), 2.81(s, 1H), 3.16(d, 2H), 3.33(s, 1H), 3.64(s, 1H), 3.77(s, 3H), 4.19(s, 1H), 5.19(s, 2H), 5.26(s, 2H), 6.42(s, 1H), 6.53(d, 2H), 6.78(d,1H), 6.81(d, 1H), 7.05(m, 4H), 7.09(m, 4H), 7.28(d, 1H). ms: m/z 597 (M+1). Anal.Calcd. For $C_{32}H_{33}FN_8O_3$:C, 64.42; H, 5.57; F, 3.18; N, 18.78; O, 8.04found: C, 64.38; H, 5.61; F, 3.09; N, 18.72; O, 8.14.

**4-(4-(4-fluorobenzyl)-7-(4-methoxyphenyl)-5-methyl-4,7-dihydrotetrazolo[1,5-a]pyrimidine-6-carbonyl)-N-(3,5-ditrifluoromethyl)piperazine-1-carboxamide (7k).**
IR: 3293, 3034, 2873, 2238, 1671, 1079cm$^{-1}$ ; $^1$H NMR ($CDCl_3$): δ 1.19(s, 1H), 1.87(s, 1H), 1.96(s, 3H), 2.89(s, 1H), 2.93(d, 2H), 3.31(s, 1H), 3.62(s, 1H), 3.79(s, 3H), 4.19(s, 1H), 5.21(s, 2H), 6.45(s, 1H), 6.59(s, 1H), 6.94(d, 2H), 7.19(m, 4H), 7.29(d, 1H),7.85(s, 1H), 7.92(s, 2H),. ms: m/z 719 (M+1). Anal.Calcd. For $C_{33}H_{29}F_7N_8O_3$:C, 55.15; H, 4.07; F, 18.51; N, 15.59; O, 6.68found C, 55.23; H, 4.16; F, 18.45; N, 15.50; O, 6.64.

**4-(4-(4-fluorobenzyl)-7-(4-methoxyphenyl)-5-methyl-4,7-dihydrotetrazolo[1,5-a]pyrimidine-6-carbonyl)-N-(4-fluoro)piperazine-1-carboxamide (7l).**
IR: 3318, 2978, 2868, 2274, 1654, 1178, 1089 cm$^{-1}$ ; $^1$H NMR ($CDCl_3$): δ 1.23(s, 1H), 1.79(s, 1H), 1.93(s, 3H), 2.88(s, 1H), 3.12(d, 2H), 3.37(s, 1H), 3.63(s, 1H), 3.78(s, 3H), 4.11(s, 1H), 5.21(s, 2H), 6.48(s, 1H), 6.57(s, 1H),

6.69(d, 2H), 7.14(m, 4H), 7.18(d,1H), 7.39(d, 2H), 7.48(d, 2H). ms: m/z 601 (M+1). Anal.Calcd. For $C_{31}H_{30}F_2N_8O_3$: C, 61.99; H, 5.03; F, 6.33; N, 18.66; O, 7.99found: C, 62.05; H, 5.05; F, 6.29; N, 18.59; O, 7.95

**4-(4-(4-fluorobenzyl)-7-(4-methoxyphenyl)-5-methyl-4,7-dihydrotetrazolo[1,5-a]pyrimidine-6-carbonyl)-N-(4-trifluoromethoxy)piperazine-1-carboxamide (7m).**
IR: 3319, 3028, 2885, 2234, 1674, 1123 $cm^{-1}$ ; $^1H$ NMR ($CDCl_3$): δ 1.19(s, 1H), 1.76(s, 1H), 1.93(s, 3H), 2.76(s, 1H), 3.15(d, 2H), 3.37(s, 1H), 3.61(s, 1H), 3.79(s, 3H), 4.13(s, 1H), 5.36(s, 2H), 6.51(d, 2H), 6.57(d, 2H), 6.91(d, 2H), 7.19(m, 3H), 7.28(d, 2H), 7.41(d, 2H). ms: m/z 667 (M+1). Anal.Calcd. For $C_{32}H_{30}F_4N_8O_4$: C, 57.65; H, 4.54; F, 11.40; N, 16.81; O, 9.60found:C, 57.61; H, 4.61; F, 11.49; N, 16.73; O, 9.65.

**4-(4-(4-fluorobenzyl)-7-(4-methoxyphenyl)-5-methyl-4,7-dihydrotetrazolo[1,5-a]pyrimidine-6-carbonyl)-N-(3-methoxy)piperazine-1-carboxamide (7n).**
IR: 3319, 3028, 2885, 2234, 1674, 1123 $cm^{-1}$ ; $^1H$ NMR ($CDCl_3$): δ 1.26(s, 1H), 1.81(s, 1H), 1.98(s, 3H), 2.79(s, 1H), 3.12(d, 2H), 3.39(s, 1H), 3.65(s, 1H), 3.76(s, 6H), 4.11(s, 1H), 5.31(s, 2H), 6.44(s, 1H), 6.59(d, 2H), 6.82(d, 2H), 7.04(m, 4H), 7.17(d, 2H), 7.38(d, 2H). ms: m/z 613 (M+1). Anal.Calcd. For $C_{32}H_{33}FN_8O_4$: C, 62.73; H, 5.43; F, 3.10; N, 18.29; O, 10.45found:C, 62.68; H, 5.38; F, 3.19; N, 18.21; O, 10.38.

## RESULT AND DISCUSSION

**Chemistry:**The synthetic route used to synthesize the title compounds 7a–n is illustrated in Scheme 1.the Ethyl-7-(4-methoxyphenyl)-5-methyl-4,7-dihydrotetrazolo[1,5-a]Pyrimidine-6-carboxylate **2**prepared by reaction of 5-amino tetrazole**1**with ethylacetoacetate and 4-methoxybenzaldehyde in refluxing ethanol using BiginelliMCR. The N-benzylation of **2** with 4-fluoro benzyl bromide and potassium carbonate in DMF at RT lead to formation of Ethyl-4-(4-fluorobenzyl)-7-(4-methoxyphenyl)-5-methyl-4,7-dihydrotetrazolo[1,5-a]Pyrimidine-6-carboxylate **3**. Two proton of benzyl group was observed at 5.18 δppm as singlet and M+1 at 424 confirm the formation of compound **3**. Hydrolysis of ester **3**using aqueous sodium hydroxide in methanol gave acid derivative **4**. The acid **4** converted into corresponding acid chloride using oxalylchloride and DCM. Acid chloride **4** react with Bocpiperazine to give Tert-butyl-4-(4-(4-fluorobenzyl)-7-(4-methoxyphenyl)-5-methyl-4,7-dihydrotetrazolo[1,5-a]Pyrimidine-6-carbonyl) piperazine-1-carboxylate **5**. Removal of Boc group by TFA gave secondary amine derivative **6.** Finally compound **6** were treated with aryl isocynate in DMF gave N-(substituted aryl)-4-(4-(4-fluorobenzyl)-7-(4-methoxyphenyl)-5-methyl-4,7-dihydrotetrazolo[1,5-a]pyrimidine-6-carbonyl)piperazine-1-carboxamide**(7a–n)** derivatives.

The structures of all the synthesized compounds were confirmed by 1H NMR, Mass spectrometry,IR and elemental analysis. In $^1H$ NMR spectra, the chiral C–H proton of pyrimidine ring appeared as a singlate around at 6.45-6.55 δ ppm. The mass spectrum of all the synthesized compounds showed a molecular ion peak as M+1. The physical characterization data are listed in table 1.

**Table 1: Characteristics physical data of amide derivatives 7a-n**

| Sr. No. | Compound Code | M.P. (°C) | Yield (%) |
|---|---|---|---|
| 7a | 4-Trifluromethyl | 136-38 | 78 |
| 7b | 4-Chloro 2-Trifluromethyl | 114-16 | 75 |
| 7c | 2-Trifluromethyl | 206-08 | 71 |
| 7d | 3-Trifluromethyl | 120-22 | 65 |
| 7e | 4-thiomethyl | 153-55 | 82 |
| 7f | 3-fluoro | 233-35 | 69 |
| 7g | 3-chloro | 141-43 | 64 |
| 7h | 3,4,5-trimethoxy | 153-55 | 79 |
| 7i | 2,4-difluoro | 115-17 | 82 |
| 7j | benzyl | 102-04 | 87 |
| 7k | 3,5-bis(trifluoromethyl) | 123-25 | 66 |
| 7l | 4-fluoro | 109-11 | 81 |
| 7m | 4-trifluoromethoxy | 106-108 | 75 |
| 7n | 3-methoxy | 101-103 | 84 |

*(7a,7c,7d,7f,7h,7i,7k,7n- Off white solid, 7b,7e,7g,7j,7l- pale yellow solid, 7m-white solid)*

**Biological activities**
**Antibacterial and antifungal activities:** The newly synthesized derivatives were evaluated for their in vitro antibacterial activity against gram negative Escherichia coli and pseudomonas aeruginosa, gram positive Bacillus cereus and Bacillus megateriumand antifungal activity against aspergillusnigerand Aspergillusflavusby micro broth dilution method[5-7]. The standard strains used for screening antibacterial and antifungal activities were procured from institute of microbial technology (IMTECH), Chandigarh, India. The MIC values are given in Table-2. The standard drugs used for antibacterial activity were Streptomycin, ampicillin and nystatin for antifungal activity. Mueller Hinton Broth was used as neutriant medium for bacteria and Sabouraud Dextrose Broth for fungal to grow.

Inoculums size for test strain was adjusted to $10^8$ CFU/mL by comparing the turbidity. The serial dilutions were prepared in primary and secondary screening. The target compounds and standard drugs were dissolved in DMSO-water at a concentration of 2.0 mg/ml. In primary screening, 500 µg/mL, 250 µg/mL, and 125 µg/mL concentrations of the synthesized drugs were taken. Data were not taken for the initial solution because of the high DMSO concentration (10%). The actively synthesized drugs found in this primary screening were further tested in a second set of dilution against all microorganisms. In secondary screening, the drugs found active in primary screening were similarly diluted to obtain 100 µg/mL, 50 µg/mL, and 25 µg/mL, 12.5 µg/mL, and 6.25 µg/mL concentrations. The inoculated wells were incubated overnight at 37ºC in a humid atmosphere overnight. The highest dilution showing at least 99% inhibition zone is taken as MIC.

The MIC values revealed that the synthesized compounds showed moderate to good inhibition. Compounds 7b, 7c, 7e, 7iand7nexhibited good activities against bacterial strains. The MIC values of antifungal activity shown that compound 7c and 7eexhibited good activity against all fungal strain. Compound 7b shows good activity against A. flavus and compound 7i shows good activity against A. niger.

**Table 2:Antibacterial and antifungal activity of amidederivatives 7a-n**

| | Antibacterial MIC (µg mL $^{-1}$) | | | | Antifungal MIC (µg mL $^{-1}$) | |
|---|---|---|---|---|---|---|
| **Compound** | ***E. coli*** | ***P. aeroginosa*** | ***B. megaterium*** | ***B. cereus*** | ***A. niger*** | ***A. flavus*** |
| Streptomycin | 50 | 50 | - | - | - | - |
| Ampicillin | - | - | 100 | 100 | - | - |
| Nystatin | - | - | - | - | 100 | 100 |
| 7a | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| 7b | 1000 | 1000 | 500 | 1000 | 1000 | 500 |
| 7c | 1000 | 500 | 1000 | 500 | 500 | 500 |
| 7d | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| 7e | 1000 | 1000 | 250 | 250 | 250 | 250 |
| 7f | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| 7g | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| 7h | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| 7i | 500 | 500 | 1000 | 1000 | 500 | 1000 |
| 7j | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| 7k | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| 7l | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| 7m | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| 7n | 500 | 500 | 1000 | 1000 | 1000 | 1000 |

## CONCLUSION

A series of Tetrazolo[1,5-a]pyrimidine derivatives have been synthesized in good yield(64% to 87%) and screened for their *in-vitro* biological activity with the aim of discovering innovative structure leads serving as potent antimicrobial agents.

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