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Synthesis, Characterization & Biological evaluation of sulfonamide analogue of Tetrazolo[1,5-a]pyrimidines

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ABSTRACT

A novel series of (4-(4-fluorobenzyl)-7-(4-methoxyphenyl)-5-Methyl-4,7-dihydrotetrazolo[1,5-a]pyrimidin-6-yl)(4-(Aryl/heteroaryl sulfonyl)piperazin-1-yl)methanones have been synthesized for evaluation of their antimicrobial activity. The structure of this novel compounds have been delineated by using IR, ^{1}H NMR, Mass spectrometric technique. All the synthesized compounds were screened for their in vitro antimicrobial activity.

Keywords: Dihydropyrimidine, tetrazolo[1,5-a]pyrimidine.

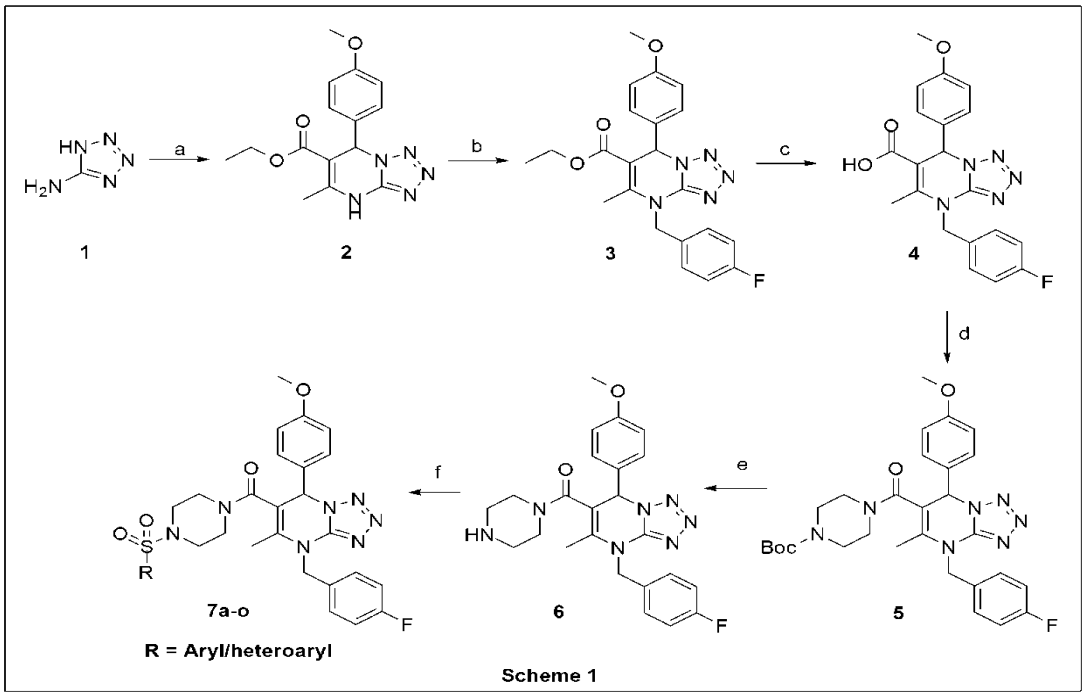
INTRODUCTION

Functionalized nitrogen heterocycles play a prominent role in medicinal chemistry and therefore they have been intensively used as scaffolds for drug development [1]. In context dihydropyrimidine derivative are particular interested of their pharmacological profile [2]. Dihydropyrimidine core unit were found to show interesting Biological activities such as antiproliferative, antiviral, antitumor, anti-inflammatory, antibacterial, antifungal, and antitubercular activity [3]. Furthermore apart from synthetic dihydro pyrimidine derivatives, several marine natural products with interesting biological activities containing the dihydropyrimidine core have been isolated [4]. Compound containing dihydrotetrazolo pyrimidine scaffold have been reported to be used in the treatment of obesity, diabetes, atherosclerosis, hypertension, coronary heart disease, hypercholesterolemia, hyperlipidemia, thyroid cancer, hypothyroidism, depression, glaucoma, and congestive heart failure [5]. Encouraged by all these fact, we aimed the synthesis of a series of novel (4-(4-fluorobenzyl)-7-(4-methoxyphenyl)-5-Methyl-4,7-dihydrotetrazolo[1,5-a]pyrimidin-6-yl)(4-(Aryl/heteroaryl sulfonyl) piperazin-1-yl)methanone derivatives.

MATERIALS AND METHODS

All chemicals were purchased from commercial suppliers and used without further purification. The progress of the reaction was monitored by analytical 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 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. Oxalyl chloride, DCM; ii. Boc-piperazine, TEA, DCM. (e) TFA, DCM, RT; (f) RSO_2Cl, TEA, DCM.

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), 1H-tetrazol-5-amine hydrate **1** (12 g, 0.11mol) and 4-methoxybenzaldehyde (14.2 mL, 0.11mol) in ethanol at RT. The reaction mixture was heated at 80 °C for 7 h. Cool the reaction mixture at 0 °C. The solid product, so formed, was collected by filtration and recrystallized by ethanol as off white solid. 26.3 g (80%). M.P: 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): 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 7 h. 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. 10.19g(73%). M.P: 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): Sodium hydroxide (4.72 g, 0.118 mol) was added to a solution of the ester compound **3** (10.0 g, 0.023 mol) in methanol (50 mL) and water (14 mL). This mixture was heated to reflux for 6 h, then cooled and evaporated under reduced pressure. The residue was diluted with water (100 mL) and acidified with an aqueous solution of hydrochloric acid (2M). The solid product, so formed, was collected by filtration give a white solid. 7.8 g (83%). M.P: 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): 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, 0.018 mol) in DCM and the mixture is cooled at 10-15 °C. The acid chloride is added drop wise to the reaction mixture at 10-15°C. Upon completion of the addition of the acid chloride solution, the mixture is stirred at RT for 1h. The mixture was evaporated under reduced pressure. The residue was dissolve in ethyl acetate and washed with saturated $NaHCO_3$, and water. Organic layer were dried over sodium sulphate and evaporated under reduced pressure to gave yellow solid. 7.2 g (66%). M.P: 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). A solution of compound **5** (7.1 g, 0.012 mol) and TFA(35 ml) in DCM (70 ml) was stirred at 10 °C for 2.5 h. Reaction mixture was charged into saturated $NaHCO_3$ solution and 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%). M.P: 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 preparation of (4-(4-fluorobenzyl)-7-(4-methoxyphenyl)-5-Methyl-4,7-dihydrotetrazolo[1,5-a]pyrimidin-6-yl)(4-(Aryl sulfonyl)piperazin-1-yl)methanone(7a-7o): To a stirred and cooled solution of compound **6** (0.54 mmol), dimethylaminopyridine (0.054 mmol) and TEA (1.08 mmol) in DCM (15 ml) , aryl/heteroaryl sulfonyl chloride (0.59 mmol) was added and the reaction mixture was allowed to warm at RT and stirred at RT for 4-6 h. Reaction mixture was evaporated under reduced pressure to give residue. The residue was dissolved in ethyl acetate and washed with 5% aqueous HCl, saturated aqueous $NaHCO_3$ and water. Ethyl acetate layer was dried and evaporated under reduced pressure to gave solid residue which was subjected to column purification to yield **7a-7o**. The yield, reaction time and physical properties are reported in **table-1.**

(4-(4-fluorobenzyl)-7-(4-methoxyphenyl)-5-methyl)-4,7-dihydrotetrazolo[1,5-a]pyrimidine-6-yl)(4-(m-tolylsulfonyl)piperazin-1-yl)methanone(7a). IR: -SO_2- 1334, 1159 cm^{-1}. ^{1}H NMR (DMSO-d6): δ 1.25 (s, 1H), 1.94 (s, 3H), 2.50 (s, 3H), 2.55 (s, 1H), 2.85 (m, 2H), 3.35 (m, 2H), 3.76 (s, 3H), 4.23 (m, 2H), 5.16 (s, 2H), 6.53 (s, 1H), 6.76-6.95 (m, 4H), 7.10-7.26 (m, 4H), 7.5 (d, 1H), 7.47 (m 1H), 7.26 (d, 1H), 7.75 (s, 1H). MS: m/z 618 (M+1). Anal. Calcd. For $C_{31}H_{32}FN_7O_4S$: C, 60.28; H, 5.22; F, 3.08; N, 15.87; O, 10.36; S, 5.19. Found: C, 60.18; H, 5.15; F, 3.18; N, 15.85; O, 10.35; S, 5.15.

Table 1: Characteristics physical data of amide derivatives 4a-j.

Sr. No.	Compound Code	R	M.P. (°C)	Yield (%)
7a	m-tolyl	white solid	170-172	84
7b	2,4-difluoro phenyl	white solid	181-183	89
7c	4-methanesulfonyl phenyl	Off white solid	120-122	84
7d	3-methoxy phenyl	Pale yellow solid	94-98	86
7e	2-chloro-5-trifluromethyl phenyl	Pale yellow solid	104-106	84
7f	3,5-dimethoxy phenyl	Off white solid	96-99	83
7g	3,5-dimethyl isooxazole	Pale yellow solid	169-72	77
7h	5-chloro thiophene-2-sulfonyl	Pale yellow solid	101-103	76
7i	3-chloro-4-acetamido phenyl	Off white solid	128-130	88
7j	2-methyl-4-nitro phenyl	Pale yellow solid	117-119	72
7k	4-trifluoromethoxy phenyl	Off white solid	218-220	62
7l	3,4-difluro phenyl	Off white solid	108-110	83
7m	3-trifluoromethyl phenyl	white solid	106-108	75
7n	Methyl thiophene 2-carboxylate-3-sulfonyl	Off white solid	101-103	84
7o	Densyl	white solid	106-108	75

(4-(2,4-difluorophenylsulfonyl)piperazin-1-yl)(4-(4-fluorobenzyl)-7-(4-methoxyphenyl)-5-methyl-4,7-dihydrotetrazolo[1,5-a]pyrimidin-6-yl)methanone (7b): IR: -SO_2- 1332, 1160 cm^{-1}. ^{1}H NMR (DMSO-d6): δ 1.25 (s, 1H), 1.95 (s, 3H), 2.27 (s, 1H), 2.98 (m, 2H), 3.32 (m, 2H), 3.46 (s, 1H), 3.82 (s, 3H), 3.85 (s, 1H), 4.31 (m, 2H), 5.17 (s, 2H), 6.51 (s, 1H), 6.76-6.95 (m, 4H), 7.10-7.26 (m, 4H), 6.92 (s, 1H), 7.26 (d, 1H), 7.76 (d, 1H). MS: m/z 640 (M+1). Anal. Calcd. For $C_{30}H_{28}F_3N_7O_4S$: C, 56.33; H, 4.41; F, 8.91; N, 15.33; O, 10.01; S, 5.01. Found: C, 56.25; H, 4.39; F, 8.85; N, 15.25; O, 10.09; S, 5.1.

(4-(4-fluorobenzyl)-7-(4-methoxyphenyl)-5-methyl-4,7-dihydrotetrazolo[1,5-a]pyrimidin-6-yl)(4-(4-(methylsulfonyl)phenylsulfonyl)piperazin-1-yl)methanone(7c): IR: -SO_2- 1330, 1158 cm^{-1}. ^{1}H NMR (DMSO-d6): δ 1.27 (s, 1H), 1.94 (s, 3H), 2.25 (s, 1H), 2.95 (m, 2H), 3.47 (m, 1H), 3.16 (s, 3H), 3.80 (s, 3H), 3.81 (m, 1H), 4.35 (m, 2H), 5.21 (s, 2H), 6.55 (s, 1H), 6.75-6.93 (m, 4H), 7.11-7.25 (m, 4H), 7.92 (d, 2H), 7.98 (d, 2H). MS: m/z 682 (M+1). Anal. Calcd. For $C_{31}H_{32}FN_7O_6S_2$: C, 54.61; H, 4.73; F, 2.79; N, 14.38; O, 14.08; S, 9.41. Found: C, 54.58; H, 4.76; F, 2.75; N, 14.45; O, 14.04; S, 9.43.

(4-(4-fluorobenzyl)-7-(4-methoxyphenyl)-5-methyl-4,7-dihydrotetrazolo[1,5-a]pyrimidin-6-yl)(4-(3-methoxy phenyl sulfonyl)piperazin-1-yl)methanone(7d): IR: -SO_2- 1334, 1161 cm^{-1}. ^{1}H NMR (DMSO-d6): δ 1.21 (s, 1H), 1.94 (s, 3H), 2.26 (s, 1H), 3.01 (m, 2H), 3.41 (m, 1H), 3.79 (s, 3H), 3.81 (s, 3H), 3.92 (m, 1H), 4.38 (m, 2H), 5.20 (s, 2H), 6.55 (s, 1H), 6.75-6.93 (m, 4H), 7.11- 7.25 (m, 4H), 7.42 (d, 1H), 7.62 (m, 1H), 6.99 (d, 1H), 7.56 (s, 1H). MS: m/z 634 (M+1). Anal. Calcd. For $C_{31}H_{32}FN_7O_5S$: C, 58.76; H, 5.09; F, 3.00; N, 15.47; O, 12.62; S, 5.06. Found: C, 58.69; H, 5.01; F, 3.05; N, 15.41; O, 12.67; S, 5.01.

(4-(2-chloro-5-(trifluoromethyl)phenylsulfonyl)piperazin-1-yl)(4-(4-fluorobenzyl)-7-(4-methoxy phenyl)-5-methyl-4,7-dihydrotetrazolo[1,5-a] pyrimidin-6-yl)methanone(7e): IR: -SO_2- 1339, 1167 cm^{-1}. ^{1}H NMR (DMSO-d6): δ 1.26 (s, 1H), 1.95 (s, 3H), 2.23 (s, 1H), 2.92 (m, 2H), 3.45 (m, 1H), 3.81 (s, 3H), 3.86 (m, 1H), 4.32 (m, 2H), 5.19 (s, 2H), 6.52 (s, 1H), 6.73-6.94 (m, 4H), 7.14-7.23 (m, 4H), 7.56 (d, 1H), 7.70 (d, 1H), 7.89 (s, 1H). MS: m/z 707 (M+1). Anal. Calcd. For $C_{31}H_{28}ClF_4N_7O_4S$: C, 52.73; H,

4.00; Cl, 5.02; F, 10.76; N, 13.89; O, 9.06; S, 4.54. Found: C, 52.71; H, 4.05; Cl, 5.02; F, 10.73; N, 13.86; O, 9.03; S, 4.51.

(4-(3,5-dimethoxyphenylsulfonyl)piperazin-1-yl)(4-(4-fluorobenzyl)-7-(4-methoxyphenyl)-5-methyl-4,7-dihydrotetrazolo[1,5-a]pyrimidin-6-yl) methanone(7f): IR: -SO_2- 1330, 1169 cm^{-1}. ^1H NMR (DMSO-d6): δ 1.31 (s, 1H), 1.94 (s, 3H), 2.45 (s, 1H), 2.91 (m, 2H), 3.36 (m, 1H), 3.76 (s, 3H), 3.79 (m, 1H), 3.83 (s, 6H), 4.05 (m, 2H), 5.20 (s, 2H), 6.55 (s, 1H), 6.75-6.93 (m, 4H), 7.11-7.25 (m, 4H), 7.12 (s, 2H), 7.02 (s, 1H). MS: m/z 664 (M+1). Anal. Calcd. For $C_{32}H_{34}FN_7O_6S$: C, 57.91; H, 5.16; F, 2.86; N, 14.77; O, 14.46; S, 4.83. Found: C, 57.89; H, 5.09; F, 2.83; N, 14.71; O, 14.41; S, 4.73.

(4-(3,5-dimethylisoxazol-4-ylsulfonyl)piperazin-1-yl)(4-(4-fluorobenzyl)-7-(4-methoxyphenyl)-5-methyl -4,7-dihydrotetrazolo[1,5-a]pyrimidin-6-yl)methanone(7g). IR: -SO_2- 1345, 1165 cm^{-1}. ^1H NMR (DMSO-d6): δ 1.29 (s, 1H), 1.98 (s, 3H), 2.29 (s, 1H), 2.35 (s, 3H), 2.48 (s, 3H), 3.15 (m, 2H), 3.47 (m, 1H), 3.79 (s, 3H), 3.81 (m, 1H), 4.29 (m, 2H), 5.18 (s, 2H), 6.63 (s, 1H), 6.74- 6.97 (m, 4H), 7.13- 7.27 (m, 4H). MS: m/z 623 (M+1). Anal. Calcd. For $C_{29}H_{31}FN_8O_5S$: C, 55.94; H, 5.02; F, 3.05; N, 18.00; O, 12.85; S, 5.15. Found: C, 55.91; H, 5.07; F, 3.05; N, 18.08; O, 12.82; S, 5.14.

(4-(5-chlorothiophen-2-ylsulfonyl)piperazin-1-yl)(4-(4-fluorobenzyl)-7-(4-methoxyphenyl)-5-methyl-4,7-dihydrotetrazolo[1,5-a]pyrimidin-6-yl) methanone(7h): IR: -SO_2- 1332, 1160 cm^{-1}. ^1H NMR (DMSO-d6): δ 1.28 (s, 1H), 1.98 (s, 3H), 2.19 (s, 1H), 2.85 (m, 2H), 3.37 (m, 1H), 3.71 (m, 1H), 3.84 (s, 3H), 4.05 (m, 2H), 5.19 (s, 2H), 6.61 (s, 1H), 6.72-6.95 (m, 4H), 7.15-7.24 (m, 4H), 6.82 (d, 2H). MS: m/z 645 (M+1). Anal. Calcd. For $C_{28}H_{27}ClFN_7O_4S_2$: C, 52.21; H, 4.22; Cl, 5.50; F, 2.95; N, 15.22; O, 9.94; S, 9.96. Found: C, 52.19; H, 4.24; Cl, 5.50; F, 2.98; N, 15.22; O, 9.91; S, 9.95.

N-(2-chloro-4-(4-(4-(4-fluorobenzyl)-7-(4-methoxyphenyl)-5-methyl-4,7-dihydrotetrazolo[1,5-a] pyrimidine-6-carbonyl)piperazin-1-ylsulfonyl) phenyl) acetamide(7i): IR: -SO_2- 1336, 1171 cm^{-1}. ^1H NMR (DMSO-d6): δ 1.15 (s, 1H), 1.97 (s, 3H), 2.05 (s, 1H), 2.12 (s, 3H), 2.92 (m, 2H), 3.37 (m, 1H), 3.75 (m, 1H), 3.76 (s, 3H), 4.15 (m, 2H), 5.16 (s, 2H), 6.63 (s, 1H), 6.72-6.95 (m, 4H), 7.12-7.26 (m, 4H), 7.65 (d, 1H), 7.79 (d, 1H), 7.85 (s,1H), 8.84 (s, 1H). MS: m/z 696 (M+1). Anal. Calcd. For $C_{32}H_{32}ClFN_8O_5S$: C, 55.29; H, 4.64; Cl, 5.10; F, 2.73; N, 16.12; O, 11.51; S, 4.61. Found: C, 55.32; H, 4.64; Cl, 5.12; F, 2.74; N, 16.11; O, 11.49; S, 4.63.

(4-(4-fluorobenzyl)-7-(4-methoxyphenyl)-5-methyl-4,7-dihydrotetrazolo[1,5-a]pyrimidin-6-yl)(4-(2-methyl-4-nitro phenyl sulfonyl)piperazin-1-yl)methanone(7j): IR: -SO_2- 1345, 1171 cm^{-1}. ^1H NMR (DMSO-d6): δ 1.17 (s, 1H), 1.96 (s, 3H), 2.21 (s, 1H), 2.68 (s, 3H), 2.87 (m, 2H), 3.38 (m, 1H), 3.81 (s, 3H), 3.82 (m, 1H), 4.23 (m, 2H), 5.23 (s, 2H), 6.59 (s, 1H), 6.73-6.91 (m, 4H), 7.11-7.26 (m, 4H), 6.98 (s, 1H), 7.81(d, 1H), 7.85 (d, 1H). MS: m/z 663 (M+1). Anal. Calcd. For $C_{31}H_{31}FN_8O_6S$: C, 56.18; H, 4.72; F, 2.87; N, 16.91; O, 14.49; S, 4.84. Found: C, 56.19; H, 4.77; F, 2.89; N, 16.89; O, 14.43; S, 4.81.

(4-(4-fluorobenzyl)-7-(4-methoxyphenyl)-5-methyl-4,7-dihydrotetrazolo[1,5-a]pyrimidin-6-yl)(4-(4-(trifluoromethoxy) phenylsulfonyl)piperazin-1-yl) methanone (7k): IR: -SO_2- 1332, 1160 cm^{-1}. ^1H NMR (DMSO-d6): δ 1.04 (s, 1H), 1.87 (s, 3H), 1.88 (s, 1H), 3.03 (m, 2H), 3.32 (m, 3H), 3.70 (s, 3H), 4.02 (m, 1H), 5.12 (s, 2H), 6.38 (s, 1H), 6.70- 6.95 (m, 4H), 7.10-7.26 (m, 4H), 7.65 (d, 2H), 7.72 (d, 2H). MS: m/z 688 (M+1). Anal. Calcd. For $C_{31}H_{29}F_4N_7O_5S$: C, 54.14; H, 4.25; F, 11.05; N, 14.26; O, 11.63; S, 4.66. Found: C, 54.12; H, 4.25; F, 11.03; N, 14.21; O, 11.64; S, 4.65.

(4-(3,4-difluorophenylsulfonyl)piperazin-1-yl)(4-(4-fluorobenzyl)-7-(4-methoxyphenyl)-5-methyl-4,7-dihydrotetrazolo[1,5-a]pyrimidin-6-yl)methanone(7l). IR: -SO_2- 1336, 1163 cm^{-1}. ^1H NMR (DMSO-d6): δ 1.16 (s, 1H), 1.87 (s, 3H), 1.95 (s, 1H), 2.97 (m, 2H), 3.41 (m, 1H), 3.78 (s, 3H), 3.82 (m, 1H), 4.29 (m, 2H), 5.21 (s, 2H), 6.57 (s, 1H), 6.69-6.95 (m, 4H), 7.11-7.29 (m, 4H), 7.39 (d, 1H), 7.46 (d, 1H), 7.76

(s, 1H). MS: m/z 640 (M+1). Anal. Calcd. For $C_{30}H_{28}F_3N_7O_4S$: C, 56.33; H, 4.41; F, 8.91; N, 15.33; O, 10.01; S, 5.01. Found: C, 56.31; H, 4.39; F, 8.95; N, 15.31; O, 10.04; S, 5.02.

(4-(4-fluorobenzyl)-7-(4-methoxyphenyl)-5-methyl-4,7-dihydrotetrazolo[1,5-a]pyrimidin-6-yl)(4-(3-(trifluoromethyl)phenylsulfonyl)piperazin-1-yl)methanone (7m): IR: -SO_2- 1352, 1167 cm^{-1}. ^1H NMR (DMSO-d6): δ 1.08 (s, 1H), 1.87 (s, 3H), 1.89 (s, 1H), 3.03 (m, 2H), 3.30 (m, 2H), 3.70 (s, 3H), 4.15 (m, 2H), 5.29 (s, 2H), 6.53 (s, 1H), 6.74-6.97 (m, 4H), 7.14-7.27 (m, 4H), 7.59 (d, 1H), 7.64 (m, 1H), 7.81 (d, 1H), 7.85 (s, 1H). MS: m/z 672 (M+1). Anal. Calcd. For $C_{31}H_{29}F_4N_7O_4S$: C, 55.43; H, 4.35; F, 11.31; N, 14.60; O, 9.53; S, 4.77. Found: C, 55.46; H, 4.31; F, 11.35; N, 14.58; O, 9.51; S, 4.78.

Methyl 3-(4-(4-(4-fluorobenzyl)-7-(4-methoxyphenyl)-5-methyl-4,7-dihydro tetrazolo[1,5-a] pyrimidine -6-carbonyl) piperazin-1-ylsulfonyl)thiophene-2-carboxylate(7n): IR: -SO_2- 1345, 1164 cm^{-1}. ^1H NMR (DMSO-d6): δ 1.09 (s, 1H), 1.98 (s, 3H), 1.95 (s, 1H), 2.98 (m, 2H), 3.29 (m, 1H), 3.77 (m, 1H), 3.78 (s, 3H), 3.83 (s, 3H), 4.21 (m, 2H), 5.27 (s, 2H), 6.51 (s, 1H), 6.77-6.96 (m, 4H), 7.15-7.31 (m, 4H), 7.04 (d, 1H), 7.78 (d, 1H). MS: m/z 668 (M+1). Anal. Calcd. For $C_{30}H_{30}FN_7O_6S_2$: C, 53.96; H, 4.53; F, 2.85; N, 14.68; O, 14.38; S, 9.60. Found: C, 53.91; H, 4.58; F, 2.84; N, 14.64; O, 14.36; S, 9.61.

(4-(5-(dimethylamino)naphthalen-1-ylsulfonyl)piperazin-1-yl)(4-(4-fluorobenzyl)-7-(4-methoxy phenyl)-5-methyl-4,7-dihydrotetrazolo[1,5-a]pyrimidin-6-yl)methanone(7o): IR: -SO_2- 1342, 1171 cm^{-1}. ^1H NMR (DMSO-d6): δ 1.12 (s, 1H), 1.83 (s, 1H), 1.84 (s, 3H), 3.06 (s, 6H), 3.08 (m, 2H), 3.39 (m, 1H), 3.73 (m, 1H), 3.81 (s, 3H), 4.11 (m, 2H), 5.24 (s, 2H), 6.63 (s, 1H), 6.72-6.93 (m, 4H), 7.08-7.28 (m, 4H), 7.01 (d, 1H), 7.32 (m, 1H), 7.48 (m, 1H),7.67 (d, 1H), 7.81 (d, 1H),7.85 (d,1H). MS: m/z 697 (M+1). Anal. Calcd. For $C_{36}H_{37}FN_8O_4S$: C, 62.05; H, 5.35; F, 2.73; N, 16.08; O, 9.18; S, 4.60. Found: C, 62.04; H, 5.41; F, 2.77; N, 16.04; O, 9.18; S, 4.55.

RESULTS AND DISCUSSION

Chemistry: As delineated in synthetic Scheme 1, the Ethyl-7-(4-methoxyphenyl)-5-methyl-4,7-dihydrotetrazolo[1,5-a] Pyrimidine-6-carboxylate **2** obtained by Biginelli like multi component reaction of 5-amino tetrazole **1** with ethylacetoacetate and 4-methoxybenzaldehyde in refluxing ethanol . The formation of **2** was evident by ^1H NMR spectra and mass spectrometry.

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**. In the ^1H NMR spectra of **3**, two proton of benzyl group was observed at 5.18 δ ppm as singlet and in addition molecular ion peak in mass spectrum. Hydrolysis of N-benzyl derivative **3** with aqueous sodium hydroxide in methanol gave acid derivative **4**. The acid **4** converted into corresponding acid chloride using oxalyl chloride. Tert-butyl-4-(4-(4-fluorobenzyl)-7-(4-methoxyphenyl)-5-methyl-4,7-dihydrotetrazolo[1,5-a]Pyrimidine-6-carbonyl) piperazine-1-carboxylate **5** were prepared by reaction of boc-piperazine and acid chloride of **4**. Removal of Boc group by TFA furnished secondary amine derivative **6**, which was supported by molecular ion peak in mass spectrum. The target compounds (4-(4-fluorobenzyl)-7-(4-methoxyphenyl)-5-Methyl-4,7-dihydrotetrazolo[1,5-a]pyrimidin-6-yl)(4-(Aryl/ heteroarylsulfonyl) piperazin-1-yl)methanone **(7a-7o)** were obtained with 62% to 93% yield by reacting secondary amine **6** with various aryl sulfonyl chloride in presence of TEA as base.

The structure of all newly synthesized compound **7a-o** was established on the basis of elemental analysis and spectral analysis like IR, ^1H NMR, and mass data. The physical characterization data are listed in table 1.

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 megaterium and antifungal activity against aspergillus niger and Aspergillus flavus by micro broth dilution method [8-10]. 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^{-1}. In primary screening, 500 µg mL^{-1}, 250 µg mL^{-1}, and 125 µg mL^{-1} 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^{-1}, 50 µg mL^{-1}, and 25 µg mL^{-1}, 12.5 µg mL^{-1}, and 6.25 µg mL^{-1} 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 some of the newly synthesized compounds showed moderate to good inhibition. Compounds 7a, 7b and 7j exhibited good activities against bacterial strains. The MIC values of antifungal activity revealed that compound 7c exhibited good activity against A. niger fungal strain. Rest of all compounds did not exhibit comparable activity against both the fungal strains.

Table 2: Antibacterial and antifungal activity of amide derivatives 7a-o.

	Antibacterial MIC (µg/mL)				Antifungal MIC (µg/mL)	
Compounds	**E. coli**	**P. aeruginosa**	**B. cereus**	**B. megaterium**	**A. niger**	**A. flavus**
Streptomycin	50	50	-	-	-	-
Ampicillin	-	-	100	100	-	-
Nystatin	-	-	-	-	100	100
7a	125	125	1000	125	500	500
7b	250	125	500	125	500	500
7c	250	125	500	250	250	1000
7d	1000	500	1000	500	1000	1000
7e	250	125	250	500	1000	1000
7f	250	125	250	500	500	1000
7g	250	250	250	500	500	1000
7h	250	250	250	250	1000	1000
7i	250	500	250	250	1000	1000
7j	125	500	500	125	1000	1000
7k	125	500	500	250	500	1000
7l	250	250	500	250	500	1000
7m	500	500	500	500	500	500
7n	500	500	500	500	1000	1000
7o	1000	500	1000	500	1000	1000

APPLICATIONS

In the present study the derivatives which we have synthesized were screened for their antimicrobial activity, which are promising as active pharmacophore. Further studies are undergoing to explore the scope of the various biological activities.

CONCLUSIONS

An efficient method for preparing sulfonamide Derivatives of teterazolo[1,5-a]pyrimidine was described and the structure of synthesized compounds was determine by IR, ^{1}H NMR, and LC-Mass spectroscopic analysis and evaluated for their in vitro antimicrobial activity by broth dilution method.

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