

Microwave Assisted Synthesis and Biological Evaluation of 1, 2, 4-Triazolo [1, 5-A] Pyrimidines

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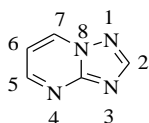
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Abstract: A microwave assisted synthesis of three new series of 1, 2, 4-triazolo [1, 5-a] pyrimidines (PK-101 to PK-110) has been synthesized by the mixture of 5-(methylthio)-2H-1,2,4-triazol-3-amine (0.01 mol), 4,4,4-trifluoro-1-(4-methoxyphenyl)butane-1,3-dione (0.01 mol) and an appropriate aromatic aldehyde (0.01 mol) in ethanol (5 mL) was irradiated under microwave conditions at 120 °C for 10-15 min. The structures of all the newly synthesized compounds are elucidated by FT-IR, mass spectra, ¹H NMR and elemental analysis. The newly synthesized compounds are subjected to various biological activities viz., antimicrobial, antimycobacterial, anticancer and antiviral.

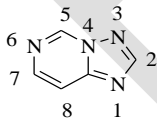
Keywords: Pyrazolo [3, 4-d] pyrimidines, antimicrobial activity, anticancer and antiviral and antituberculosis activity antimycobacterial activity.

I. INTRODUCTION

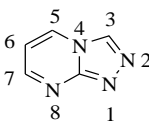
The condensation of a ring of 1, 2, 4-triazole and another one of pyrimidine gives rise to the formation of bicyclic heterocycles known as 1, 2, 4-triazolopyrimidines. Four different possibilities exist for the relative orientation of both rings, so four different isomeric families of compounds are defined: 1,2,4-triazolo[1,5-a]pyrimidine (1), 1,2,4-triazolo[1,5-c]pyrimidine (2), 1,2,4-triazolo[4,3-a]pyrimidine (3) and 1,2,4-triazolo[4,3-c]pyrimidine (4).



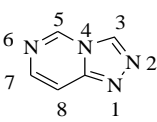
(1) 1,2,4-triazolo[1,5-a]pyrimidine



(2) 1,2,4-triazolo[1,5-c]pyrimidine



(3) 1,2,4-triazolo[4,3-a]pyrimidine



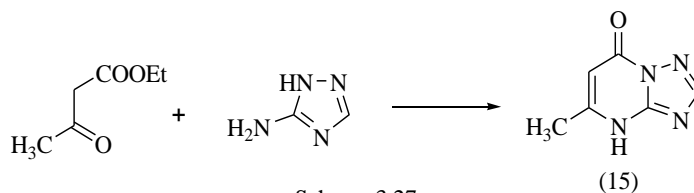
(4) 1,2,4-triazolo[4,3-c]pyrimidine

Among these isomeric families of compounds, 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.

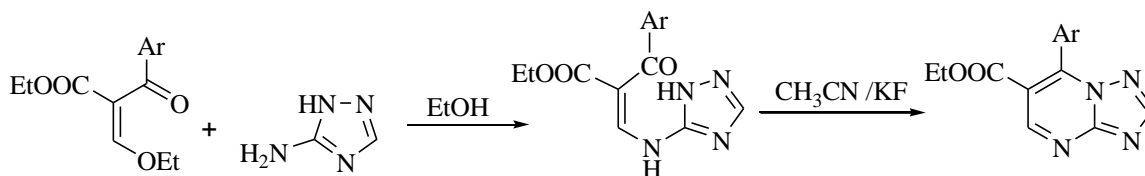
The 1,2,4-triazolo[1,5-a]pyrimidines have aroused increasing attention from the chemical and biological view points, due to their diverse pharmacological activities, such as antitumor potency^[5, 6], inhibition of KDR kinase^[7], antifungal effect^[8] and macrophage activation^[9]. They have proved to be promising anticancer agents with dual mechanisms of tubulin polymerization promotion as well as cyclin dependent kinases 2 inhibition^[10].

By far the most triazolo[1,5-a]pyrimidine synthesis are condensations of dinucleophilic 5-amino-1,2,4-triazoles with 1,3-bifunctional synthons as shown in the formation of triazolo[1,5-a]pyrimidine (15) (Scheme 3.27)^[11-14]. New synthetic conditions recently described involve melting under microwave irradiation, a reaction that is environmental friendly and gives higher yields than conventional heating in solvent^[15]. Furthermore, certain lithium 1,3-diketones have proven to be better synthons than the corresponding diketones^[16].



Scheme 3.27

Previous mechanistic conclusions have been confirmed by isolating stable intermediate 5-amino-1, 2, 4-triazole derivatives such as enamine (16) (Scheme 3.28) on reacting 5-amino-1, 2, 4-triazoles with 3-ketovinyl ethers^[17], 3-ketoenamines^[18], 3-ketoaldehydes^[19], enamine-2-carboxylic esters^[20] or ethoxymethylene malonates^[21].



Scheme 3.28 (16)

II. CURRENT WORK

Microwave assisted organic synthesis has become an important tool to medicinal chemists for rapid organic synthesis. A huge number of research papers have appeared over the last decades on the application of microwave technology in organic synthesis. Some of the major advantages of microwave assisted organic synthesis include spectacular decrease in reaction time, improved conversions, clean product formation and wide scope for the development of new reaction conditions.

The biological importance of 1, 2, 4-triazolo [1, 5-*a*] pyrimidines is well reported. Over the years, various substituted derivatives of these heterocycles have shown utility against a range of biological targets. For example, they

have demonstrated activity against malaria and bronchospasm and shown activity as coronary vasodilators, antihypertensive agents, leishmanicides, antibiotics, adenosine A_{2a} antagonists, immunosuppressants, antitumor agents, fungicides, xanthine oxidase inhibitors, and phosphodiesterase inhibitors.

The microwave assisted synthesis of three new series of 1, 2, 4-triazolo [1, 5-*a*] pyrimidines (PK-101 to PK-110) has been undertaken. The structures of all the newly synthesized compounds are elucidated by FT-IR, mass spectra, ¹H NMR and elemental analysis. The newly synthesized compounds are subjected to various biological activities *viz.*, antimicrobial, antimycobacterial, anticancer and antiviral.

III. REACTION SCHEME

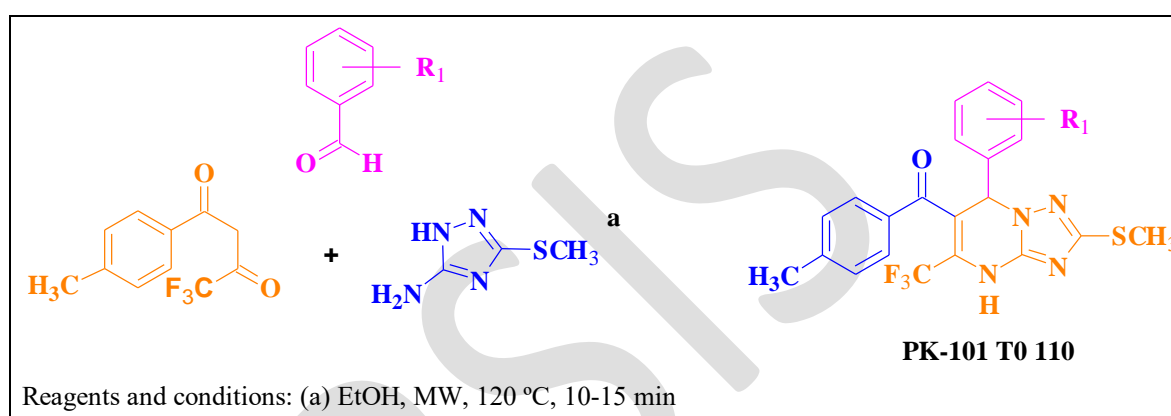


Table-I Physical and analytical data:

Code	R ₁	M.F.	M.W.	M.P. °C	Yield %	R _{f1}	R _{f2}
PK-101	4-OCH ₃	C ₂₂ H ₁₉ F ₃ N ₄ O ₃ S	476	221-227	78	0.55	0.73
PK-102	4-F	C ₂₁ H ₁₆ F ₄ N ₄ O ₂ S	464	228-230	72	0.52	0.70
PK-103	4-CH ₃	C ₂₂ H ₁₉ F ₃ N ₄ O ₂ S	460	198-200	53	0.45	0.66
PK-104	4-NO ₂	C ₂₁ H ₁₆ F ₃ N ₅ O ₄ S	491	235-237	63	0.56	0.65
PK-105	4-Cl	C ₂₁ H ₁₆ ClF ₃ N ₄ O ₂ S	480	240-242	75	0.55	0.75
PK-106	3-Cl	C ₂₁ H ₁₆ ClF ₃ N ₄ O ₂ S	480	188-190	68	0.44	0.71
PK-107	3-NO ₂	C ₂₁ H ₁₆ F ₃ N ₅ O ₄ S	491	256-258	65	0.52	0.72
PK-108	2-Cl	C ₂₁ H ₁₆ ClF ₃ N ₄ O ₂ S	480	260-262	72	0.48	0.65
PK-109	2-NO ₂	C ₂₁ H ₁₆ F ₃ N ₅ O ₄ S	491	255-257	70	0.44	0.60
PK-110	2-OCH ₃	C ₂₂ H ₁₉ F ₃ N ₄ O ₃ S	476	221-223	60	0.45	0.76

TLC Solvent system R_{f1}: Hexane: Ethyl acetate – 5:5;

TLC Solvent system R_{f2}: Chloroform: Methanol - 9:1.

IV. EXPERIMENTAL

Materials and Methods

Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was

routinely checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were located by iodine. Microwave assisted reaction were carried out in QPro-M microwave synthesizer. IR spectra were recorded Shimadzu FT-IR-8400 instrument using KBr pellet method. Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using Direct

Injection Probe technique. ^1H NMR was determined in DMSO- d_6 solution on a Bruker Ac 400 MHz spectrometer. Elemental analysis of the all the synthesized compounds was carried out on Elemental Vario EL III Carlo Erba 1108 model and the results are in agreements with the structures assigned.

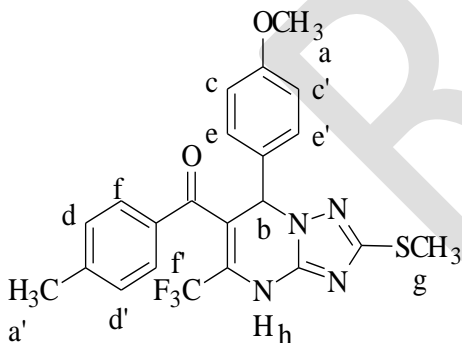
Synthesis of 4, 4, 4-trifluoro-1-(aryl) butane-1, 3-dione

Synthesis of 4, 4, 4-trifluoro-1-(aryl) butane-1, 3-dione was achieved using previously published methods [23].

General procedure for the synthesis of (5-(trifluoromethyl)-4, 7-dihydro-7-(aryl)-2-(methylthio)-[1, 2, 4] triazolo [1, 5-a] pyrimidin-6-yl) (4-methoxyphenyl) methanone (PK- 101 to 110)

A mixture of the 5-(methylthio)-2H-1,2,4-triazol-3-amine (0.01 mol), 4,4,4-trifluoro-1-(4-methoxyphenyl)butane-1,3-dione (0.01 mol) and an appropriate aromatic aldehyde (0.01 mol) in ethanol (5 mL) was irradiated under microwave conditions at 120 °C for 10-15 min. The microwave irradiation was operated in 30-second cycles. The reaction mixture was allowed to stand overnight at room temperature and was then filtered to give the solid triazolopyrazolopyrimidine products PK 101-110, which were washed with ethanol and dried in air. Triazolopyrimidines were obtained in high purity and did not require further purification by recrystallization.

(5-(trifluoromethyl)-4,7-dihydro-7-(4-methoxyphenyl)-2-(methylthio)-[1,2,4]triazolo[1,5-a]pyrimidin-6-yl)(4-methoxyphenyl)methanone (PK-101)



Yield: 78%;

mp 228-230 °C;

IR (cm $^{-1}$): 3265 (N-H stretching of secondary amine), 3022 (C-H stretching of aromatic ring), 2920 (C-H asymmetrical stretching of CH $_3$ group), 2866 (C-H asymmetrical stretching of CH $_3$ group), 1660 (C=O stretching of carbonyl group), 1620 (C=N stretching of triazole ring), 1555 (N-H deformation of pyrimidine ring), 1513, 1481 and 1440 (C=C stretching of aromatic ring), 1414 (C-H asymmetrical deformation of CH $_3$ group), 1328 (C-H symmetrical deformation of CH $_3$ group), 1282 (C-N stretching), 1245 (C-

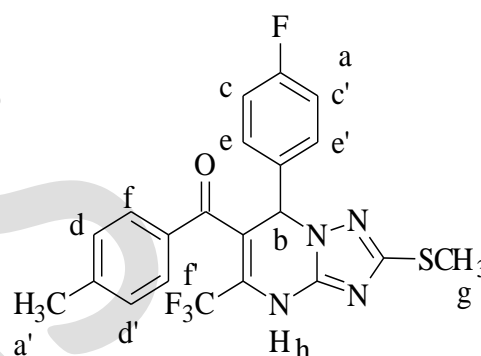
O-C stretching), 1031 (C-H in plane deformation of aromatic ring), 827 (C-H out of plane bending of 1,4-disubstitution);

^1H NMR (DMSO- d_6) δ ppm: 2.47 (3H, SCH $_3$), 3.72 (s, 3H, H $_{aa'}$), 6.56 (s, 1H, H $_b$), 6.75-6.78 (d, 2H, H $_{cc'}$, J = 8.84 Hz), 6.96-7.00 (t, 2H, H $_{dd'}$), 7.32-7.38 (m, 4H, H $_{ee'-ff}$), 7.57 (s, 1H, H $_g$), 11.06 (s, 1H, H $_h$);

MS: m/z 476;

Anal. Calcd. for C $_{22}$ H $_{19}$ F $_3$ N $_4$ O $_3$ S: C, 58.61; H, 3.98; N, 13.02. Found: C, 58.54; H, 3.88; N, 12.95%.

(5-(trifluoromethyl)-7-(4-fluorophenyl)-4,7-dihydro-2-(methylthio)-[1,2,4]triazolo[1,5-a]pyrimidin-6-yl)(4-methoxyphenyl)methanone (PK-102)



Yield: 72%;

mp 226-228 °C;

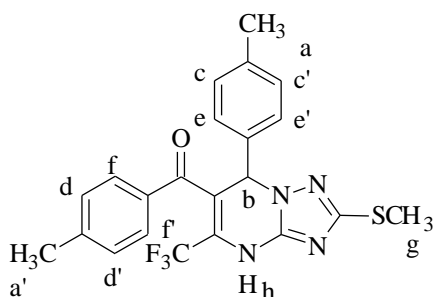
IR (cm $^{-1}$): 3232 (N-H stretching of secondary amine), 3116 (C-H symmetrical stretching of CH $_3$ group), 2935 (C-H asymmetrical stretching of CH $_3$ group), 1715 (C=O stretching of carbonyl group), 1645 (C=N stretching of triazole ring), 1522 and 1481 (C=C stretching of aromatic ring), 1437 (C-H asymmetrical deformation of CH $_3$ group), 1408 (C-N-C stretching of pyrimidine ring), 1340 (C-H symmetrical deformation of CH $_3$ group), 1276 (C-N stretching of pyrimidine ring), 1240 (C-O-C asymmetrical stretching of ether linkage), 1172 (C-H in plane deformation of aromatic ring), 1060 (C-O-C symmetrical stretching of ether linkage), 868 (C-H out of plane deformation of 1,4-disubstitution);

^1H NMR (DMSO- d_6) δ ppm: 2.48 (3H, SCH $_3$), 3.79 (s, 3H, H $_a$), 6.39 (s, 3H, H $_b$), 6.94-6.97 (d, 2H, H $_{cc'}$, J = 8.4 Hz), 7.03-7.09 (m, 4H, H $_{dd'-ee}$), 7.71-7.73 (m, 3H, H $_{ff-g}$), 11.27 (s, 1H, H $_h$);

MS: m/z 464;

Anal. Calcd. for C $_{21}$ H $_{16}$ F $_4$ N $_4$ O $_2$ S: C, 57.42; H, 3.37; N, 13.39. Found: C, 57.30; H, 3.25; N, 13.22%.

(5-(trifluoromethyl)-4,7-dihydro-2-(methylthio)-7-p-tolyl-[1,2,4]triazolo[1,5-a]pyrimidin-6-yl)(4-methoxyphenyl)methanone (PK-103)



Yield: 53%;

mp 198-200 °C;

IR (cm⁻¹): 3261 (N-H stretching of secondary amine), 3035 (C-H stretching of aromatic ring), 2922 (C-H asymmetrical stretching of CH₃ group), 2876 (C-H asymmetrical stretching of CH₃ group), 1670 (C=O stretching of carbonyl group), 1606 (C=N stretching of triazole ring), 1550 (N-H deformation of pyrimidine ring), 1516 and 1482 (C=C stretching of aromatic ring), 1440 (C-H asymmetrical deformation of CH₃ group), 1412 (C-H symmetrical deformation of CH₃ group), 1330 (C-N stretching), 1249 (C-O-C stretching), 1029 (C-H in plane deformation of aromatic ring), 822 (C-H out of plane bending of 1,4-disubstitution);

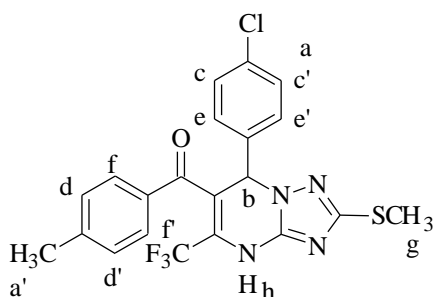
¹H NMR (DMSO-*d*₆) δ ppm: 2.15 (s, 3H, H_a), 2.45 (3H, SCH₃), 3.81 (s, 3H, H_b), 6.28 (s, 1H, H_c), 6.85-6.87 (d, 2H, H_{dd}, *J* = 8.80 Hz), 6.94-6.97 (d, 2H, H_{cc}, *J* = 9.20 Hz), 7.03-7.05 (d, 2H, H_{ff}, *J* = 8.00 Hz), 7.71-7.74 (d, 3H, H_{gg-h}), 11.26 (s, 1H, H_i);

MS: *m/z* 460;

Anal. Calcd. for C₂₂H₁₉F₃N₄O₂S: C, 60.87; H, 4.14; N, 13.52. Found: C, 60.79; H, 4.02; N, 13.40%.

(7-(4-chlorophenyl)-5-(trifluoromethyl)-4,7-dihydro-2-(methylthio)-[1,2,4]triazolo[1,5-a]pyrimidin-6-yl)(4-methoxyphenyl)methanone

(PK-105)



Yield: 75%;

mp 240-242 °C;

IR (cm⁻¹): 3219 (N-H stretching of secondary amine), 3048 (C-H stretching of aromatic ring), 2965 (C-H asymmetrical stretching of CH₃ group), 2874 (C-H asymmetrical stretching

of CH₃ group), 1666 (C=O stretching of carbonyl group), 1595 (C=N stretching of triazole ring), 1516 (N-H deformation of pyrimidine ring), 1440, 1400 (C=C stretching of aromatic ring), 1411 (C-H asymmetrical deformation of CH₃ group), 1344 (C-H symmetrical deformation of CH₃ group), 1280 (C-N stretching), 1248 (C-O-C stretching), 1033 (C-H in plane deformation of aromatic ring), 815 (C-H out of plane bending of 1,4-disubstitution);

¹H NMR (DMSO-*d*₆) δ ppm: 2.48(3H, SCH₃), 3.81 (s, 3H, H_a), 6.39 (s, 3H, H_b), 6.95-6.97 (d, 2H, H_{cc}, *J* = 8.40 Hz), 7.07-7.09 (d, 2H, H_{dd}, *J* = 8.00 Hz), 7.30-7.32 (d, 2H, H_{cc}, *J* = 8.00 Hz), 7.74-7.80 (m, 3H, H_{ff-g}), 11.36 (s, 1H, H_h);

MS: *m/z* 480;

Anal. Calcd. for C₂₁H₁₆ClF₃N₄O₂S: C, 55.25; H, 3.25; N, 12.89. Found: C, 55.10; H, 3.12; N, 12.76%.

V. IR SPECTRAL STUDY

IR spectra were recorded on Shimadzu FT-IR-8400 model using KBr pellet method. Various functional groups present in molecule were identified by characteristic frequency obtained for them. For triazolopyrimidines PK-101 to 110, confirmatory bands for secondary amine and carbonyl groups were observed at 3159-3279 cm⁻¹ and 1658-1712 cm⁻¹ respectively. Another characteristic C=N stretching band of triazole ring was observed at 1521-1641 cm⁻¹, which suggested formation of desired products VP-101 to 110.

VI. ¹H NMR SPECTRAL STUDY

¹H NMR spectra were recorded in DMSO-*d*₆ solution on a Bruker Ac 400 MHz spectrometer using TMS as an internal standard. Number of protons and their chemical shifts were found to support the structure of the synthesized compounds.

¹H NMR spectra confirmed the structures of triazolopyrimidines PK-101 to 110 on the basis of following signals: a singlet for the methine proton of pyrimidine ring at 6.28-6.72 δ ppm, a singlet for the methine proton of triazole ring at 7.27-7.87 δ ppm and singlet for secondary amine group of pyrimidine proton at 11.05-11.46 δ ppm, respectively.

VII. BIOLOGICAL EVALUATION

Antimicrobial evaluation

All of the synthesized compounds (PK-101 to 110) were tested for their antibacterial and antifungal activity (MIC) *in vitro* by broth dilution method [133-135] with two Gram-positive bacteria *Staphylococcus aureus* MTCC-96, *Streptococcus pyogenes* MTCC 443, two Gram-negative bacteria *Escherichia coli* MTCC 442, *Pseudomonas aeruginosa* MTCC 441 and three fungal strains *Candida albicans* MTCC 227, *Aspergillus Niger* MTCC 282, *Aspergillus clavatus* MTCC 1323 taking gentamycin, ampicillin, chloramphenicol, ciprofloxacin, norfloxacin, nystatin and greseofulvin as standard drugs. The standard strains were procured from the Microbial Type Culture

Collection (MTCC), Institute of Microbial Technology, Chandigarh, India.

The minimal inhibitory concentration (MIC) values for all the newly synthesized compounds, defined as the

lowest concentration of the compound preventing the visible growth, were determined by using micro dilution broth method according to NCCLS standards ^[22].

Table 1. Antibacterial and antifungal activity of synthesized compounds PK-101 to 110

Code	Minimum inhibition concentration ($\mu\text{g mL}^{-1}$)						
	Gram-positive		Gram-negative		Fungal species		
	<i>S.a.</i>	<i>S.p.</i>	<i>E.c.</i>	<i>P.a.</i>	<i>C. a.</i>	<i>A. n.</i>	<i>A.c.</i>
PK-101	500	500	1000	>1000	1000	500	125
PK-102	125	100	50	250	500	1000	500
PK-103	100	1000	250	1000	1000	500	1000
PK-104	50	500	250	250	>1000	1000	>1000
PK-105	500	1000	500	1000	500	500	100
PK-106	125	25	100	100	500	>1000	500
PK-107	250	1000	1000	250	500	1000	1000
PK-108	100	250	100	500	500	1000	1000
PK-109	250	125	250	250	100	1000	250
PK-110	62.5	100	100	250	250	500	250
Ampicillin	250	100	100	100	-	-	-
Chloramphenicol	50	50	50	50	-	-	-
Ciprofloxacin	50	50	25	25	-	-	-
Norfloxacin	10	10	10	10	-	-	-
Nystatin	-	-	-	-	100	100	100
Griseofulvin	-	-	-	-	500	100	100

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