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Synthesis and antimicrobal activities of some novel triazolo[1,5-a]pyrimidine derivatives

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ABSTRACT

A convenient synthesis of substituted 1,2,4-triazolo[1,5-a]pyrimidine was carried out by the reaction of various ketene dithioacetals with 5-amino 1,2,4-triazole in methanol in presence of sodium methoxide. The newly synthesized compound were characterized by 1H NMR, ^{13}C NMR, IR, MS, elemental analysis and screened for their antimicrobial activity against various strains of bacteria and fungi.

***Keywords*:** 5-amino-1,2,4-triazole; ketene dithioacetal; antimicrobial activity; triazolopyrimidine

1. INTRODUCTION

Various fused pyrimidines like triazolopyrimidines, pyrazolopyrimidines, pyrimidoazepines, pteridines, pyridopyrimidines, purines, quinazolines, furopyrimidines, and pyrrolopyrimidines were studied in the past decade and were found to possess remarkable pharmacological properties. Triazolopyrimidines are new class of hybrid heterocycles of pyrimidine ring fused with triazole and possessing improved activity. The condensation of pyrimidine with triazole ring gives bicyclic heterocycles known as 1,2,4-triazolopyrimidines, which exist in four isomeric structure.[1-4] Among these four structural isomers, 1,2,4-triazolo[1,5-a]pyrimidine derivatives are thermodynamically more stable and most studied ones.

Fused hetero aromatic systems are often of much greater interest in biological activity than the constituent monocyclic compounds. 1,2,4-triazolo[1,5-a]pyrimidines have diverse pharmacological activities, such as antitumor potency[5-8], antimalarial[9], antimicrobial[10-13], anti-inflammatory[14] inhibition of KDR kinase[15], antifungal[16] and macrophage activation[17]. They have proved to be promising anticancer[18] agents with dual mechanisms of tubulin polymerization promotion[5,6] as well as cyclin dependent kinases 2 inhibition[19].

We have synthesize 1,2,4-triazolo[1,5-a]pyrimidine derivatives by refluxing various ketene dithioacetals with 5-amino-1,2,4-triazole in the presence of sodium methoxide in methanol[20-22]. The newly synthesized compounds were characterized by IR, Mass, 1H NMR, ^{13}C NMR spectroscopy and elemental analysis. All the synthesized compounds were evaluated for their antimicrobial activity.

2. 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. ^{1}H (400 MHz), ^{13}C (100 MHz) NMR spectra were recorded on a Bruker AVANCE II spectrometer in $CDCl_3$ and 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.

General synthesis of 4-methyl-3-oxo-N-arylpentanamide (Int 1a-t); A mixture of aromatic amine (10 mmol), methyl 4-methyl-3-oxopentanoate (10 mmol) and catalytic amount of sodium or potassium hydroxide lie (10 %) in toluene (50 ml) was refluxed at 110 °C for 12-15 h. The reaction was monitored by TLC. After completion of reaction, the solvent was removed under reduce pressure and washed with water to afford pure product.

General synthesis of ketene dithioacetals (Int 2a-t); To a well stirred suspension of 4-methyl-3-oxo-N-arylpentanamide (10 mmol) and potassium carbonate (20 mmol) in DMF (20 mL) at 0-5 °C was added CS_2 (10 mmol) over a period of 30 min. After completion of the addition, the reaction mixture was stirred at 0-5 °C for 1 h. Appearance of reddish solid in the reaction medium indicated the formation of dipotassium salt. To this reaction, a solution of methyl iodide (20 mmol) was added drop wise within 15 min at 0-5 °C. The mixture was allowed to warm at room temperature and stirred for 15 h, and then poured onto crushed ice under stirring. The separated solid was washed with water and collected by filtration.

General synthesis of triazolopyrimidine (3a-t); To a solution of sodium methoxide in methanol (10 mL) ketene dithioacetal (5 mmol) and 5-amino-1,2,4-triazole were added at 0-5 °C. The reaction mixture then refluxed for 2-4 hours. The reaction was monitored by TLC. After the completion of reaction mixture was poured into cold water and the separated solid was dried and purified by column chromatography using ethylacetate and hexane.

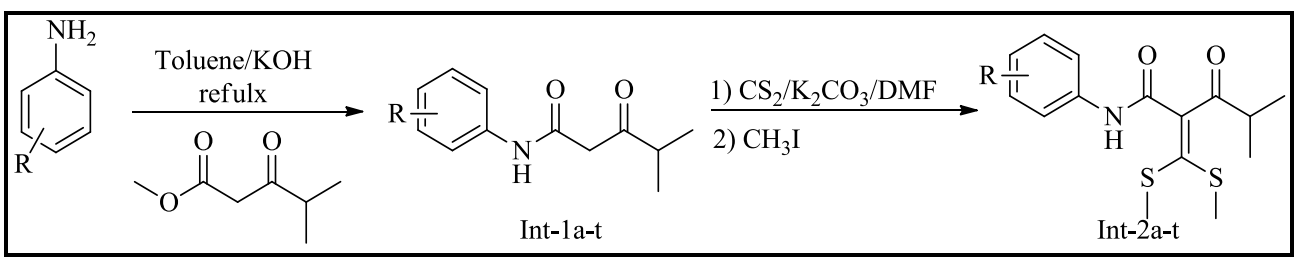

Figure 1. Reaction Scheme for 2a-t.

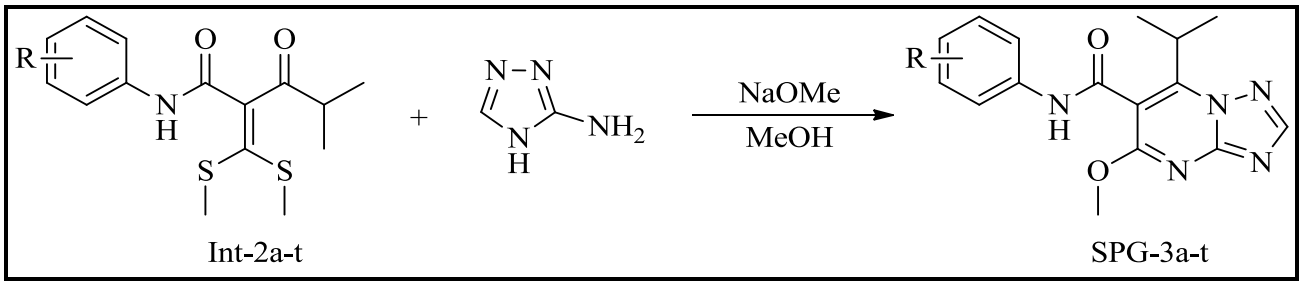

Figure 2. Reaction Scheme for 3a-t.

N-(3-chloro-4-fluorophenyl)-7-isopropyl-5-methoxy-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (SPG-3h): White solid; mp 256-258 °C; Rf 0.45 (4:6 hexane-EtOAc); IR (KBr): 3244, 3198, 3124, 3055, 2933, 2868, 1680, 1622, 1539, 1394, 1336, 1271, 1205, 1124, 1020, 958, 827, 756, 665, 567 cm-1; 1H NMR: δ 1.480-1.1.497 (d, 6H, $(CH_3)_2$) , *j* = 6.8 Hz), 3.506-3.540 (m, 1H, -CH), 4.041 (s, 3H, -OCH3), 7.434-7.479 (t, 1H, Ar-H), 7.522 (s, 1H, Ar-H), 8.010-8.022 (d, 1H, Ar-H, *j* = 4.8 Hz), 8.575 (s, 1H, -CH triazole ring), 10.951 (s, 1H, -CONH); 13C NMR (100 MHz, DMSO): 18.07, 30.56, 55.06, 110.19, 117.14, 119.33, 119.56, 120.61, 135.57, 152.35, 152.95, 153.90, 154.78, 160.84, 162.52; MS (m/z): 363 (M+); Anal. Calcd for $C_{16}H_{15}ClFN_5O_2$: C, 52.83; H, 4.16; N, 19.25; Found: C, 52.78; H, 4.21; N, 19.18.

N-(3,4-dimethylphenyl)-7-isopropyl-5-methoxy-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (SPG-3k): Pale yellow solid; mp 224-226 °C; R_f 0.44 (4:6 hexane-EtOAc); IR (KBr): 3245, 3196, 3117, 3059, 2924, 2858, 1730, 1678, 1616, 1535, 1456, 1396, 1278, 1205, 1124, 1020, 958, 827, 756, 665, 567 cm^{-1}; ^{1}H NMR: *δ* 1.476-1.1.492 (d, 6H, $(CH_3)_2$), *j* = 6.4 *Hz*), 2.198 (s, 3H, $-CH_3$), 2.221 (s, 3H, $-CH_3$), 3.498-3.532 (m, 1H, -CH), 4.029 (s, 3H, $-OCH_3$), 7.111-7.130 (d, 1H, Ar-H, *j* = 7.6 *Hz*), 7.368-7.387 (d, 1H, Ar-H, *j* = 7.6 *Hz*), 7.453 (s, 1H, Ar-H), 8.552 (s, 1H, -CH triazole ring), 10.504 (s, 1H, -CONH); ^{13}C NMR (100 MHz, DMSO): 18.04, 18.77, 19.51, 30.51, 54.94, 110.86, 116.64, 120.24, 128.57, 129.66, 131.50, 136.22, 152.52, 153.83, 154.85, 160.24, 162.68, 166.89; MS (*m/z*): 339 (M^+); Anal. Calcd for $C_{18}H_{21}N_5O_2$: C, 63.70; H, 6.24; N, 20.64; Found: C, 63.68; H, 6.31; N, 20.59.

7-isopropyl-5-methoxy-N-(p-tolyl)-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (SPG-3m): White solid; mp 278-280 °C; R_f 0.49 (4:6 hexane-EtOAc); IR (KBr): 3292, 3115, 3045, 2931, 1633, 1550, 1512, 1465, 1404, 1290, 1199, 989, 812, 759, 659, 505 cm^{-1}; ^{1}H NMR: *δ* 1.476-1.1.494 (d, 6H, $(CH_3)_2$), *j* = 7.2 *Hz*), 2.286 (s, 3H, $-CH_3$), 3.480-3.550 (m, 1H, -CH), 4.031 (s, 3H, $-OCH_3$), 7.171-7.192 (d, 2H, Ar-H, *j* = 8.4 *Hz*), 7.541-7.562 (d, 2H, Ar-H, *j* = 8.4 *Hz*), 8.556 (s, 1H, -CH triazole ring), 10.589 (s, 1H, -CONH); ^{13}C NMR (100 MHz, DMSO): 18.03, 20.40, 30.51, 54.96, 110.77, 119.12, 129.24, 133.13, 135.95, 152.56, 153.82, 154.86, 160.29, 162.65; MS (*m/z*): 325 (M^+); Anal. Calcd for $C_{17}H_{19}N_5O_2$: C, 62.75; H, 5.89; N, 21.52; Found: C, 62.69; H, 5.93; N, 21.48.

Table 1. Physical Data of Compound SPG 3a-t.

Compound	R	M.F.	M.W.	M.P. (^{o}C)	Yield (%)
SPG-**3a**	H	$C_{16}H_{17}N_5O_2$	311	236-238	40

SPG-**3b**	4-Br	$C_{16}H_{16}BrN_5O_2$	390	246-248	36
SPG-**3c**	4-F	$C_{16}H_{16}FN_5O_2$	329	224-226	35
SPG-**3d**	4-OCH_3	$C_{17}H_{19}N_5O_3$	341	238-240	42
SPG-**3e**	4-NO_2	$C_{16}H_{16}N_6O_4$	341	250-252	25
SPG-**3f**	3-CH_3	$C_{17}H_{19}N_5O_2$	325	260-262	35
SPG-**3g**	3,4-di-Cl	$C_{16}H_{15}Cl_2N_5O_2$	380	244-246	30
SPG-**3h**	3-Cl, 4-F	$C_{16}H_{15}ClFN_5O_2$	363	256-258	32
SPG-**3i**	4-Cl	$C_{16}H_{16}ClN_5O_2$	345	258-260	35
SPG-**3j**	2,4-di-Cl	$C_{16}H_{15}Cl_2N_5O_2$	380	282-284	32
SPG-**3k**	3,4-di-CH_3	$C_{18}H_{21}N_5O_2$	339	224-226	40
SPG-**3l**	2-Cl	$C_{16}H_{16}ClN_5O_2$	345	276-278	30
SPG-**3m**	4-CH_3	$C_{17}H_{19}N_5O_2$	325	278-280	35
SPG-**3n**	2,3-di-CH_3	$C_{18}H_{21}N_5O_2$	339	268-270	38
SPG-**3o**	2-F	$C_{16}H_{16}FN_5O_2$	329	232-234	33
SPG-**3p**	2-NO_2	$C_{16}H_{16}N_6O_4$	356	286-288	26
SPG-**3q**	3-Cl	$C_{16}H_{16}ClN_5O_2$	345	264-266	35
SPG-**3r**	4-Cl, 2-NO_2	$C_{16}H_{15}ClN_6O_4$	390	274-276	22
SPG-**3s**	4-Cl, 3-NO_2	$C_{16}H_{15}ClN_6O_4$	390	266-268	25
SPG-**3t**	2-CF_3	$C_{17}H_{16}F_3N_5O_2$	379	234-236	35

Table 2. Antibacterial activity of compound SPG 3a-t.

Sr. No.	**Code**	**MIC (µg/mL)**			
		E.coli	***P.aeruginosa***	***S.aureus***	***S.pyogenus***
1	**SPG-5a**	500	500	500	500
2	**SPG-5b**	250	250	200	250
3	**SPG-5c**	500	500	250	500
4	**SPG-5d**	100	500	250	500
5	**SPG-5e**	500	500	500	500

6	**SPG-5f**	500	100	100	250
7	**SPG-5g**	500	200	250	500
8	**SPG-5h**	250	250	200	250
9	**SPG-5i**	200	250	500	250
10	**SPG-5j**	200	250	200	500
11	**SPG-5k**	500	250	250	250
12	**SPG-5l**	500	250	250	250
13	**SPG-5m**	100	200	500	125
14	**SPG-5n**	250	200	500	250
15	**SPG-5o**	500	500	250	500
16	**SPG-5p**	250	200	500	200
17	**SPG-5q**	250	500	500	200
18	**SPG-5r**	500	250	250	250
19	**SPG-5s**	250	200	250	500
20	**SPG-5t**	500	500	250	500
Gentamycin		0.05	1	0.25	0.5
Ampicilin		100	100	250	100
Chloramphenicol		50	50	50	50
Ciprofloxacin		25	25	50	50
Norfloxacin		10	10	10	10

Table 3. Antifungal activity of Compound SPG 3a-t.

Sr. No.	**Code**	**MIC (µg/mL)**		
		C.albicans	***A.niger***	***A.clavatus***
1	**SPG-5a**	500	500	>1000
2	**SPG-5b**	500	500	250
3	**SPG-5c**	500	500	1000
4	**SPG-5d**	500	500	>1000

5	**SPG-5e**	500	500	500
6	**SPG-5f**	200	250	250
7	**SPG-5g**	500	1000	500
8	**SPG-5h**	250	500	500
9	**SPG-5i**	100	100	100
10	**SPG-5j**	500	250	500
11	**SPG-5k**	200	500	500
12	**SPG-5l**	500	1000	500
13	**SPG-5m**	500	250	250
14	**SPG-5n**	250	250	500
15	**SPG-5o**	1000	1000	500
16	**SPG-5p**	250	250	500
17	**SPG-5q**	500	250	250
18	**SPG-5r**	250	500	250
19	**SPG-5s**	500	500	1000
20	**SPG-5t**	500	500	500
Nystatin		100	100	100
Greseofulvin		500	100	100

3. RESULT AND DISCUSSION

Various methodologies have been described for the synthesis of 1,2,4-triazolo[1,5-a]pyrimidines. During the course of our ongoing interest on synthesis of various heterocyclic compounds using α-oxo ketene dithioacetals, we observed that α-oxo ketene dithioacetals are versatile intermediate for the synthesis of triazolopyrimidines.

Thus, to synthesized target molecules, the various α-oxo ketene dithioacetals (2a-t) were reacted with 5-amino-1,2,4-triazole (2) in the presence sodium methoxide as a base in methanol at reflux temperature to afford 1,2,4-triazolo[1,5-a]pyrimidines (3a-t) (Table 1). Various α-oxo ketene dithioacetals (2a-t) was synthesized by reported method [20] All the synthesized compounds were screened against varieties of bacterial strains (Table 2) such *E. coli, S. pyogenus, S. aureus, P. aeruginosa* and fungi strains (Table 3) *C. albicans, A. niger, A. clavatus* at minimal inhibitory concentration (MIC). Standard drugs like Ampicillin, Chloramphenicol, Nystatin and Greseofulvin were used for the comparison purpose.

4. CONCLUSION

In summary, we have described the synthesis of substituted triazolopyrimidine derivatives in moderate yield. The reaction of various α-oxo ketene dithioacetals with 5-amino-1,2,4-triazole was afforded the triazolopyrimidine derivatives in moderate to good yield in the presence of base. Sodium methoxide was found as an efficient base. All the synthesized compounds were evaluated for their antimicrobial activity. The investigation of antimicrobial and antifungal screening data revealed that all the tested compounds showed moderate to significant activity.

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