DETERMINATION OF BIOLOGICAL ACTIVITY, ANTIOXIDANT ACTIVITY AND SYNTHESIS OF TRIAZOLOPYRIMIDINES

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ABSTRACT

Potent Synthesis of an uncommon series of 7-(4-(benzyloxy)-3-methoxyphenyl)-N-(4-chlorophenyl)-4,7-dihydro-5-methyl-[1,2,4]triazolo[1,5-*a*]pyrimidine-6-carboxamide (**V1-V10**) was accomplished using Biginelli protocol from 4-(benzyloxy)-3-methoxybenzaldehyde, N-(substituted phenyl)-3-oxobutanamide and 2H-1,2,4-triazol-3-amine and few drop of N,N-Dimethylformamide. All the synthesized compounds were characterized by IR, NMR, mass spectroscopic techniques and elemental analyses. All the synthesized compounds were evaluated for their antimicrobial activity and antioxidant activity.

Keywords: 4-(benzyloxy)-3-methoxybenzaldehyde, N-(substitutedphenyl)-3-oxobutanamide, 2H-1,2,4-triazol-3-amine and N,N'-Dimethylformamide.

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INTRODUCTION

Pyrimidine is a heteroatom-containing compound like pyridine.¹ Pyrimidine is a six-member heterocyclic ring with the nitrogen atom at 1 and 3 positions.² This ring system has wide-spread occurrence and it is found in many biological complexes like nucleotides, vitamin B1 and alloxane etc.³ Uric acid and Alloxane which are derivative of Pyrimidine were identified in the year 1818. Until 1876, the synthesis of pyrimidine was not reported in the literature.⁴ Grimaux first reported the work of preparation of barbituric acid from diaminomethanal and propanedioic acid with POCl₃. Further, Pinner started a systematic study of pyrimidine by synthesizing pyrimidine derivatives by condensation of acetoacetate and amidines^{5,6}. Pinner used the name 'pyrimidine' for the first time in the year 1885.^{7,8} Biginelli reaction is a multi-component one-pot protocol developed by Pietro Biginelli.⁹⁻¹¹ The reaction involves the use of catalysts like Bronsted acid or Lewis acids such as copper trichloro acetate hydrate and boron trifluoride (BF₃) and many others.¹² Dihydropyrimidines and 1,2,3,4-tetrahydro- derivatives are reported to possess a wide range of biological activity.¹³⁻¹⁴ They are widely useful in various branches of Chemistry like Bioinorganic Chemistry, Medicinal Chemistry due to their biological activities. anti-microbial, antiviral, anti-carcinogenic activities etc.¹⁵⁻¹⁸ Some derivatives are also reported as antihypertensive agents, calcium channel blockers and alpha-antagonists. Literature reports various modifications of Biginelli reaction to get diversely substituted pyrimidine derivatives.¹⁹⁻²³ The present work reports the preparation of multi-functionalized triazolo[1,5-*a*]pyrimidines using Biginelli protocol and their antimicrobial and antioxidant activity evaluation.

EXPERIMENTAL

General Procedure for the Synthesis of Triazolo[1,5-*a*]pyrimidines (**V1-V10**)

To a mixture of 2H-1,2,4-triazol-3-amine (0.01 mol), 4-(benzyloxy)-3-methoxybenzaldehyde (0.01 mol), N-(substituted phenyl)-3-oxobutanamide (0.01 mol) in the solvent 1-2 mL of DMF and few drops of con. HCl was added and the resulting mixture was heated for 5 hrs in refluxing condition. The reaction mixture was kept at room temperature for 20 hrs. The thus separated crystalline product was filtered and washed with methanol.

7-(4-(benzyloxy)-3-methoxyphenyl)-N-(4-chlorophenyl)-4,7-dihydro-5-methyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (V1)

M.P. 188°C; Yield: 56% IR (KBr) ν , cm^{-1} : 3149, 2872 (C-H), 3412 (N-H), 1676 (C=O), 1514 (Aromatic skeletons), 1236 (C-O-C), 773 (C-Cl); ^1H NMR (400 MHz, DMSO) : δ_{H} 2.48 (singlet, 3H, methyl), 3.64 (singlet, 3H, methoxy), 4.99 (singlet, 2H, $-\text{CH}_2-\text{O}$), 6.47 (singlet, 1H, $-\text{CH}-$ of pyrimidine ring), 6.81-7.63 (multiplet, 13H, Aromatic -H), 9.83 (singlet, 1H, $-\text{NH}-\text{CO}$), 10.22 (singlet, 1H, $-\text{NH}-$ Aromatic-NH); MS: m/z 501. Anal. found: C, 64.60; H, 4.82; Cl, 7.06; N, 13.95. $\text{C}_{27}\text{H}_{24}\text{ClN}_5\text{O}_3$ requires: C, 64.65; H, 4.82; Cl, 7.01; N, 13.92 %.

7-(4-(benzyloxy)-3-methoxyphenyl)-N-(3-chlorophenyl)-4,7-dihydro-5-methyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (V2)

M.P. 180°C; Yield: 60%; IR(KBr) νcm^{-1} : 3133, 2870 (C-H), 3423 (N-H), 1673 (C=O), 1501 (Aromatic skeletons), 1229 (C-O-C), 782 (C-Cl); ^1H NMR (400 MHz, DMSO) : δ_{H} 2.51 (singlet, 3H, methyl), 3.61 (singlet, 3H, methoxy), 5.10 (singlet, 2H, $-\text{CH}_2-\text{O}$), 6.41 (singlet, 1H, $-\text{CH}-$ of pyrimidine ring), 6.77-7.76 (multiplet, 13H, Aromatic -H) 9.74 (singlet, 1H, $-\text{NH}-\text{CO}-$), 10.10 (singlet, 1H, $-\text{NH}-$ Aromatic-NH); MS: m/z 501. Anal. found: C, 64.60; H, 4.82; Cl, 7.06; N, 13.95. $\text{C}_{27}\text{H}_{24}\text{ClN}_5\text{O}_3$ requires: C, 64.61; H, 4.80; Cl, 7.07; N, 13.88 %.

7-(4-(benzyloxy)-3-methoxyphenyl)-N-(2-chlorophenyl)-4,7-dihydro-5-methyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (V3)

M.P. 171°C; Yield: 57%; IR(KBr) ν cm^{-1} : 3173, 2876 (C-H), 3349 (N-H), 1670 (C=O), 1539, 1514 (Aromatic skeletons), 1229 (C-O-C), 689 (C-Cl); ^1H NMR (400 MHz, DMSO) : δ_{H} 2.55 (singlet, 3H, CH_3), 3.49 (singlet, 3H, OCH_3), 4.97 (singlet, 2H, $-\text{CH}_2-\text{O}$), 6.38 (singlet, 1H, $-\text{CH}-$ of pyrimidine ring), 7.18-7.92 (multiplet, 13H, Aromatic-H), 9.35 (singlet, 1H, $-\text{NH}-\text{CO}-$), 9.97 (singlet, 1H, $-\text{NH}-$ Aromatic-NH-); MS: m/z 501. Anal. found: C, 64.60; H, 4.82; Cl, 7.06; N, 13.95. $\text{C}_{27}\text{H}_{24}\text{ClN}_5\text{O}_3$ requires: C, 64.58; H, 4.71; Cl, 7.14; N, 13.75%.

7-(4-(benzyloxy)-3-methoxyphenyl)-N-(4-fluorophenyl)-4,7-dihydro-5-methyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (V4)

M.P. 169°C; Yield: 65%; IR(KBr) ν cm^{-1} : 3173, 2868 (C-H), 3356 (N-H), 1678 (C=O), 1556, 1523 (Aromatic skeletons), 1223 (C-O-C), 1043 (C-F); ^1H NMR (400 MHz, DMSO) : δ_{H} 2.51 (singlet, 3H, methyl), 3.40 (singlet, 3H, methoxy), 4.84 (singlet, 2H, $-\text{CH}_2-\text{O}$), 6.45 (singlet, 1H, $-\text{CH}-$ of pyrimidine), 7.73-8.35 (multiplet, 13H, Aromatic-H), 9.42 (singlet, 1H, $-\text{NH}-\text{CO}-$), 9.90 (singlet, 1H, $-\text{NH}-$ Aromatic-NH); MS: m/z 485. Anal. found: C, 66.79; H, 4.98; F, 3.91; N, 14.42. $\text{C}_{27}\text{H}_{24}\text{FN}_5\text{O}_3$ requires: C, 66.70; H, 4.91; F, 4.05; N, 14.25 %.

7-(4-(benzyloxy)-3-methoxyphenyl)-N-(3-fluorophenyl)-4,7-dihydro-5-methyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (V5)

M.P. 164°C; Yield: 51%; IR(KBr) ν cm^{-1} : 3133, 2852 (C-H), 3278 (N-H), 1678 (C=O), 1563, 1513 (Aromatic skeletons), 1219 (C-O-C), 1049 (C-F); ^1H NMR (400 MHz, DMSO) : δ_{H} 2.43 (singlet, 3H, methyl), 3.64 (singlet, 3H, methoxy), 4.44 (singlet, 2H, $-\text{CH}_2-\text{O}$), 6.64 (singlet, 1H, $-\text{CH}-$ of pyrimidine), 7.70-8.31 (multiplet, 13H, Aromatic-H), 9.51 (singlet, 1H, $-\text{NHCO}-$), 9.89 (singlet, 1H, $-\text{NH}-$ Aromatic-NH-); MS: m/z 485. Anal. found: C, 64.60; H, 4.82; Cl, 7.08; N, 13.91. $\text{C}_{27}\text{H}_{24}\text{FN}_5\text{O}_3$ requires: C, 64.37; H, 4.06; F, 8.14; N, 13.45 %.

7-(4-(benzyloxy)-3-methoxyphenyl)-N-(2-fluorophenyl)-4,7-dihydro-5-methyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (V6)

M.P. 170°C; Yield: 53%; IR(KBr) ν cm^{-1} : 3109, 2862 (C-H), 3313 (N-H), 1671 (C=O), 1562, 1524 (Aromatic skeletons), 1213 (C-O-C), 1060 (C-F); ^1H NMR (400 MHz, DMSO) : δ_{H} 2.47 (singlet, 3H, methyl), 3.54 (singlet, 3H, methoxy), 4.40 (singlet, 2H, $-\text{CH}_2-\text{O}$), 6.49 (singlet, 1H, $-\text{CH}-$ of pyrimidine ring), 7.46-8.10 (multiplet, 13H, Aromatic-H) 9.46 (singlet, 1H, $-\text{NH}-\text{CO}-$), 9.79 (singlet, 1H, $-\text{NH}-$ Aromatic-NH-); MS: m/z 485. Anal. found: C, 64.60; H, 4.82; Cl, 7.06; N, 13.95. $\text{C}_{27}\text{H}_{24}\text{FN}_5\text{O}_3$ requires: C, 64.39; H, 4.02; F, 8.11; N, 13.40%.

7-(4-(benzyloxy)-3-methoxyphenyl)-N-(4-bromophenyl)-4,7-dihydro-5-methyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (V7)

M.P. 176°C; Yield: 57%; IR(KBr) ν cm^{-1} : 3115, 2876 (C-H), 3334 (N-H), 1668 (C=O), 1577, 1517 (Aromatic skeletons), 1209 (C-O-C), 650 (C-Br); ^1H NMR (400 MHz, DMSO) : δ_{H} 2.34 (singlet, 3H, methyl), 3.49 (singlet, 3H, methoxy), 4.41 (singlet, 2H, $-\text{CH}_2\text{-O}$), 6.12 (singlet, 1H, $-\text{CH-}$ of pyrimidine ring), 7.33-8.69 (multiplet, 13H, Aromatic $-\text{H-}$), 9.69 (singlet, 1H, $-\text{NHCO-}$), 10.19 (singlet, 1H, $-\text{NH-}$ Aromatic $-\text{NH-}$); MS: m/z 546. Anal. found: C, 59.35; H, 4.43; Br, 14.62; N, 12.82. $\text{C}_{27}\text{H}_{24}\text{BrN}_5\text{O}_3$ requires: C, 59.13; H, 4.69; Br, 14.23; N, 12.74%.

7-(4-(benzyloxy)-3-methoxyphenyl)-N-(3-bromophenyl)-4,7-dihydro-5-methyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (V8)

M.P. 172°C; Yield: 58%; IR(KBr) ν cm^{-1} : 3109, 2870 (C-H), 3319 (N-H), 1659 (C=O), 1564, 1509 (Aromatic skeletons), 1218 (C-O-C), 642 (C-Br); ^1H NMR (400 MHz, DMSO) : δ_{H} 2.29 (singlet s, 3H, methyl), 3.57 (singlet, 3H, methoxy), 4.36 (singlet s, 2H, $-\text{CH}_2\text{-O}$), 6.48 (singlet, 1H, $-\text{CH-}$ of pyrimidine ring), 7.41-8.47 (multiplet, 13H, Aromatic $-\text{H-}$), 9.71 (singlet s, 1H, $-\text{NHCO-}$), 10.04 (singlet, 1H, $-\text{NH-}$ Aromatic $-\text{NH-}$); MS: m/z 546. Anal. found: C, 59.35; H, 4.43; Br, 14.62; N, 12.82. $\text{C}_{27}\text{H}_{24}\text{BrN}_5\text{O}_3$ requires: C, 59.11; H, 4.64; Br, 14.27; N, 12.71 %.

7-(4-(benzyloxy)-3-methoxyphenyl)-N-(2-bromophenyl)-4,7-dihydro-5-methyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (V9)

M.P. 163°C; Yield: 52%; IR(KBr) ν cm^{-1} : 3170, 2861 (C-H), 3324 (N-H), 1645 (C=O), 1565, 1507 (Aromatic skeletons), 1208 (C-O-C), 634 (C-Br); ^1H NMR (400 MHz, DMSO) : δ_{H} 2.46 (singlet, 3H, methyl), 3.38 (singlet, 3H, methoxy), 4.52 (singlet, 2H, $-\text{CH}_2\text{-O}$), 6.78 (singlet, 1H, $-\text{CH-}$ of pyrimidine ring), 7.61-8.51 (multiplet, 13H, Aromatic $-\text{H-}$), 9.72 (singlet, 1H, $-\text{NHCO-}$), 10.17 (singlet, 1H, $-\text{NH-}$ Aromatic $-\text{NH-}$); MS: m/z 546. Anal. found: C, 59.35; H, 4.43; Br, 14.62; N, 12.82. $\text{C}_{27}\text{H}_{24}\text{BrN}_5\text{O}_3$ requires: C, 59.21; H, 4.59; Br, 14.21; N, 12.61%.

7-(4-(benzyloxy)-3-methoxyphenyl)-N-(4-methylphenyl)-4,7-dihydro-5-methyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (V10)

M.P. 158°C; Yield: 56%; IR(KBr) ν cm^{-1} : 3163, 2853 (C-H), 3300 (N-H), 1657 (C=O), 1571, 1511 (Aromatic skeletons), 1213 (C-O-C); ^1H NMR (400 MHz, DMSO) : δ_{H} 2.13 (singlet, 3H, methyl), 2.43 (singlet, 3H, methyl), 3.46 (singlet, 3H, methoxy), 4.49 (singlet, 2H, $-\text{CH}_2\text{-O}$), 6.64 (singlet, 1H, $-\text{CH-}$ of pyrimidine), 7.54-8.13 (multiplet, 13H, Aromatic $-\text{H-}$), 9.84 (singlet, 1H, $-\text{NHCO-}$), 10.10 (singlet, 1H, $-\text{NH-}$ Aromatic $-\text{NH-}$); MS: m/z 481. Anal. found: C, 69.84; H, 5.65; N, 14.54. $\text{C}_{28}\text{H}_{27}\text{N}_5\text{O}_3$ requires: C, 69.81; H, 5.67; N, 14.51 %.

Antimicrobial Activity

The '*in vitro*' antimicrobial evaluation was carried out against standard strains of Gram +ve and Gram -ve bacteria and fungi. The standard strains were procured from the Microbial Type Culture Collection (MTCC), Gene Bank, Institute of Microbial Technology, Chandigarh, India. The determination of antimicrobial activity was done by the broth dilution method. Determination of antimicrobial activity was done against two-gram positive bacterial strains, (*Staphylococcus aureus* MTCC-96, *Streptococcus pyogenes* MTCC 443), two Gram-negative bacterial strains (*Escherichia coli* MTCC 442, *Pseudomonas aeruginosa* MTCC 441) and three fungal strains (*Candida albicans* MTCC 227, *Aspergillus Niger* MTCC 282, *Aspergillus clavatus* MTCC 1323) by minimum inhibitory concentration (MIC) method. Here Ampicillin, Nystatin Chloramphenicol, Griseofulvin and Norfloxacin were used as standard drugs. The minimal inhibitory concentration (MIC) values determined *in vitro* by broth dilution method for all the compounds **V1-V10**, specified as the minimum concentration of the compound to stop the detectable growth of specific microorganism. Serial dilutions of the test compounds and reference drugs were prepared in Mueller-Hinton agar. Drugs (10 mg) were dissolved in dimethyl sulfoxide (DMSO, 1 mL). Further progressive dilutions with melted Mueller-Hinton agar were prepared to obtain the desired concentrations. In primary screening 1000 $\mu\text{g mL}^{-1}$, 500 $\mu\text{g mL}^{-1}$, and 250 $\mu\text{g mL}^{-1}$ concentrations of the

synthesized drugs were taken. The active synthesized drugs found in this primary screening were further tested in the second set of dilution at $125 \mu\text{g mL}^{-1}$, $62.5 \mu\text{g mL}^{-1}$, $50 \mu\text{g mL}^{-1}$, $25 \mu\text{g mL}^{-1}$, $12.5 \mu\text{g mL}^{-1}$, and $6.250 \mu\text{g mL}^{-1}$ concentration against all microorganisms. The tubes were inoculated with 10^8cfu mL^{-1} (colony-forming unit/mL) and incubated at 37°C for 24 h. The MIC was the lowest concentration of the tested compound that yields no visible growth (turbidity) on the plate. To ensure that the solvent is inert on the bacterial growth, a control was tested with the test medium supplemented with DMSO at the same dilutions as used in the experiments and it was observed that DMSO had no effect on the microorganisms in the concentrations studied. The results obtained from antimicrobial susceptibility testing are depicted in Table-1 and Table-2.

Antioxidant Activity

Antioxidant activity of the triazolopyrimidines (V1-V10) was determined by ABTS (2,2-diphenyl 1-picrylhydrazyl) free radical assay based on the scavenging effect of them. The results are depicted in Table-3.

RESULTS AND DISCUSSION

Our investigation was focused on the preparation of diversely functionalized triazolo[1,5-a]pyrimidines via a suitable and facile method.

Scheme-1: Reagents and Conditions: 2H-1,2,4-triazol-3-amine, 4-(benzyloxy)-3-methoxybenzaldehyde and N-(substitutedphenyl)-3-oxobutanamide, DMF, reflux, 5 hrs.

Antimicrobial Activity

The minimum inhibitory concentration (MIC) values measured in antibacterial and antifungal activity studies of the compounds are given in the following Table-1.

Table-1: *In vitro* Antibacterial Screening Results for (V1-V10)

Compound	Minimal Inhibition Concentration ($\mu\text{g mL}^{-1}$)			
	Gram-positive		Gram-negative	
	S.a	S.p.	E.c.	P.a.
V1	250	125	250	250
V2	500	125	250	500
V3	250	250	500	250
V4	500	250	500	250
V5	125	125	125	500
V6	250	500	125	500
V7	500	250	125	250
V8	500	250	250	125
V9	250	250	250	125
V10	250	250	250	250
Ampicillin	250	100	100	100
Chloramphenicol	50	50	50	50
Norfloxacin	10	10	10	10

Fig.-1: Antibacterial Activity of Triazolopyrimidines V1-V10

The biological screening revealed that compound V5 exhibited maximum antibacterial activity against gram-positive bacteria and *E. coli*. at $125 \mu\text{g mL}^{-1}$. Compounds V2 and V4 showed minimum antibacterial activity. Rest of the compounds were found to be good to moderate inhibitors against tested microbial strains.

Table-2: *In vitro* Anti-Fungal Activity Screening Results for (V1-V10)

Compound	Minimal Inhibition Concentration ($\mu\text{g mL}^{-1}$)		
	Fungal Species		
	C.a.	A.n.	A.c.
V1	250	125	500
V2	250	500	125
V3	350	125	125
V4	250	500	125
V5	500	125	250
V6	250	500	125
V7	500	125	125
V8	250	500	250
V9	250	125	125
V10	500	500	250
Nystatin	100	100	100
Griseofulvin	450	100	100

Fig.-2: Antifungal Activity of Triazolopyrimidines V1-V10

Antifungal Activity

Antifungal screening of these compound results that compound V3, V7 and V9 showed good antifungal activity against *Aspergillus niger* and *Aspergillus clavatus* while remaining all compounds were good to moderate inhibitors.

Antioxidant Activity

The results of the antioxidant activity are depicted in Table-3.

Table-3: Antioxidant Activity Results for (V1-V10)

Compound	Antioxidant Activity of Compounds				
	Different Concentration($\mu\text{g ml}^{-1}$) of Compounds %				
	25%	50%	100%	200%	400%
V1	56.61	61.23	60.19	59.23	53.46
V2	63.31	58.26	61.01	58.23	59.13
V3	74.46	74.29	77.78	79.14	80.39
V4	64.13	55.13	53.98	64.21	64.16
V5	53.12	54.19	46.13	66.55	59.15
V6	55.19	57.64	48.59	66.36	60.18
V7	75.56	79.35	80.11	78.12	81.46
V8	58.25	66.36	66.89	63.25	65.19
V9	62.23	65.25	61.46	62.15	66.29
V10	61.16	66.18	58.49	58.12	59.36
Ascorbic acid	82.73	87.56	93.17	94.22	97.51

Fig.-3: Antioxidant Activity of Triazolopyrimidines V1-V10

Compounds V3 and V7 showed good antioxidant activity while the other triazolopyrimidines showed moderate to low antioxidant activity as compared to the standard Ascorbic acid.

CONCLUSION

This facile one-pot method efficiently produced diversely functionalized triazolo[1,5-*a*]pyrimidine derivatives. The one-pot method is easy and gives the title compounds in good yield and purity. The protocol does not require tedious isolation or purification methods. The newly synthesized triazolo[1,5-*a*]pyrimidines showed promising antibacterial, antifungal and antioxidant activities. Further structure modification and SAR studies will surely assess the biological importance of these molecules in detail.

ACKNOWLEDGEMENT

The authors are thankful to Smt. J. A. Patel Mahila College, Morbi for providing laboratory facilities.

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[RJC-6072/2020]