DETERMINATION OF BIOLOGICAL ACTIVITY, ANTIOXIDANT ACTIVITY AND SYNTHESIS OF TRIAZOLOPYRIMIDINES

V. P. Gilava¹*, P. K. Patel¹, H. K. Ram² and J. H. Chauhan²

¹Department of Chemistry, Smt. J. A. Patel Mahila College, Morbi-363641, (Gujarat), India
²Department of Chemistry, Tolani College of Arts & Science, Adipur-370205, (Gujarat) India

*E-mail: ram.haresh2007@gmail.com

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-(substituted phenyl)-3-oxobutanamide, 2H-1,2,4-triazol-3-amine and N,N'-Dimethylformamide.

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 widespread 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 Grimiaux 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 amidines⁵,⁶. 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 trichloroacetate 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 multifunctionalized 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.

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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. 188ºC; Yield: 56% 
IR (KBr) υ, 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) : δH 2.48 (singlet, 3H, methyl), 3.64 (singlet, 3H, methoxy), 4.99 (singlet, 2H, -CH₂-O), 6.47 (singlet, 1H, -CH- of pyrimidine ring), 6.81-7.63 (multiplet, 13H, Aromatic –H), 9.83 (singlet, 1H, NH-CO), 10.22 (singlet, 1H, -NH- Aromatic-NH); 
MS: m/z 501. Anal.found: C, 64.60; H, 4.82; Cl, 7.06; N, 13.95. C₂₇H₂₄ClN₅O₃ 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. 180ºC; Yield: 60%; IR(KBr) υ cm⁻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) : δH 2.51 (singlet, 3H, methyl), 3.61 (singlet, 3H, methoxy), 5.10 (singlet, 2H, -CH₂-O), 6.41 (singlet, 1H, -CH- of pyrimidine ring), 6.77-7.76 (multiplet, 13H, Aromatic–H), 9.74 (singlet, 1H, -NH-CO-), 10.10 (singlet, 1H, -NH- Aromatic-NH); MS: m/z 501. Anal.found: C, 64.60; H, 4.82; Cl, 7.06; N, 13.95. C₂₇H₂₄ClN₅O₃ 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. 171ºC; Yield: 57%; IR(KBr) υ 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) : δH 2.55 (singlet, 3H, CH₃), 3.49 (singlet, 3H, OCH₃), 4.97 (singlet, 2H, -CH₂-O), 6.38 (singlet, 1H, -CH- of pyrimidine ring), 7.18-7.92 (multiplet, 13H, Aromatic–H), 9.35 (singlet, 1H, -NH-CO-), 9.97 (singlet, 1H, -NH- Aromatic-NH-); MS: m/z 501. Anal.found: C, 64.60; H, 4.82; 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. 169ºC; Yield: 65%; IR(KBr) υ cm⁻¹: 3173, 2868 (C-H), 3356 (N-H), 1678 (C=O), 1556, 1523 (Aromatic skeletons), 1229 (C-O-C), 689 (C-Cl); 
1H NMR (400 MHz, DMSO) : δH 2.51 (singlet, 3H, methyl), 3.40 (singlet, 3H, methoxy), 4.84 (singlet, 2H, -CH₂-O), 6.45 (singlet, 1H, -CH- of pyrimidine ring), 7.73-8.35 (multiplet, 13H, Aromatic–H), 9.42 (singlet, 1H, -NH-CO-), 9.90 (singlet, 1H, -NH- Aromatic-NH-); MS: m/z 485. Anal.found: C, 66.79; H, 4.98; F, 3.91; N, 14.42. C₂₇H₂₄FN₅O₃ requires: C, 66.70; H, 4.91; F, 4.06; 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. 164ºC; Yield: 51%; IR(KBr) υ cm⁻¹: 3133, 2852 (C-H), 3278 (N-H), 1678 (C=O), 1556, 1523 (Aromatic skeletons), 1229 (C-O-C), 689 (C-Cl); 
1H NMR (400 MHz, DMSO) : δH 2.43 (singlet, 3H, methyl), 3.64 (singlet, 3H, methoxy), 4.44 (singlet, 2H, -CH₂-O), 6.64 (singlet, 1H, -CH- of pyrimidine ring), 7.73-8.35 (multiplet, 13H, Aromatic–H), 9.42 (singlet, 1H, -NH-CO-), 9.90 (singlet, 1H, -NH- Aromatic-NH-); MS: m/z 485. Anal.found: C, 64.60; H, 4.82; Cl, 7.06; N, 13.95. C₂₇H₂₄FN₅O₃ requires: C, 64.61; H, 4.80; Cl, 7.07; N, 13.88 %.

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. 170ºC; Yield: 53%; IR(KBr) υ cm⁻¹: 3109, 2862 (C-H), 3313 (N-H), 1671 (C=O), 1562, 1524 (Aromatic skeletons), 1219 (C-O-C), 1043 (C-F); 
1H NMR (400 MHz, DMSO) : δH 2.47 (singlet, 3H, methyl), 3.64 (singlet, 3H, methoxy), 4.44 (singlet, 2H, -CH₂-O), 6.49 (singlet, 1H, -CH- of pyrimidine ring), 7.46-8.10 (multiplet, 13H, Aromatic–H), 9.46 (singlet, 1H, -NH-CO-), 9.79 (singlet, 1H, -NH- Aromatic-NH-); MS: m/z 485. Anal.found: C, 64.60; H, 4.82; Cl, 7.06; N, 13.95. C₂₇H₂₄FN₅O₃ requires: C, 64.39; H, 4.02; F, 8.11; N, 13.40%.
7-(4-(benzyl氧)-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) υ 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) : δH 2.34 (singlet, 3H, methyl), 3.49 (singlet, 3H, methoxy), 4.41 (singlet, 2H, -CH₂-O), 6.12 (singlet, 1H, -CH- of pyrimidine ring), 7.33-8.69 (multiplet, 13H, Aromatic –H-), 9.69 (singlet, 1H, -NH-Aromatic -NH-); MS: m/z 546. Anal. found: C, 59.35; H, 4.43; Br, 14.62; N, 12.82. C₂₇H₂₄BrN₅O₃ requires: C, 59.13; H, 4.69; Br, 14.23; N, 12.74%.

7-(4-(benzyl氧)-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) υ 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) : δH 2.29 (singlet s, 3H, methyl), 3.57 (singlet, 3H, methoxy), 4.36 (singlet s, 2H, -CH₂-O), 6.48 (singlet, 1H, -CH- of pyrimidine ring), 7.41-8.47 (multiplet, 13H, Aromatic -H-), 9.71 (singlet s, 1H, -NHCO-), 10.04 (singlet, 1H, -NH-Aromatic -NH-); MS: m/z 546. Anal. found: C, 59.35; H, 4.43; Br, 14.62; N, 12.82. C₂₇H₂₄BrN₅O₃ requires: C, 59.11; H, 4.64; Br, 14.27; N, 12.71%.

7-(4-(benzyl氧)-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) υ 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) : δH 2.46 (singlet, 3H, methyl), 3.38 (singlet, 3H, methoxy), 4.52 (singlet, 2H, -CH₂-O), 6.78 (singlet, 1H, -CH- of pyrimidine ring), 7.61-8.51 (multiplet, 13H, Aromatic -H-), 9.72 (singlet, 1H, -NH-Aromatic -NH-); MS: m/z 546. Anal. found: C, 59.35; H, 4.43; Br, 14.62; N, 12.82. C₂₇H₂₄BrN₅O₃ requires: C, 59.21; H, 4.59; Br, 14.21; N, 12.61%.

7-(4-(benzyl氧)-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) υ 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) : δH 2.13 (singlet, 3H, methyl), 2.43 (singlet, 3H, methyl), 3.46 (singlet, 3H, methoxy), 4.49 (singlet, 2H, -CH₂-O), 6.64 (singlet, 1H, -CH- of pyrimidine), 7.54-8.13 (multiplet, 13H, Aromatic -H-), 9.84 (singlet, 1H, -NH-Aromatic -NH-); MS: m/z 481. Anal. found: C, 69.84; H, 5.65; N, 14.54. C₂₈H₂₇N₅O₃ 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 μg mL⁻¹, 500 μg mL⁻¹, and 250 μg mL⁻¹ concentrations of the
The active synthesized drugs found in this primary screening were further tested in the second set of dilution at 125 μg mL⁻¹, 62.5 μg mL⁻¹, 50 μg mL⁻¹, 25 μg mL⁻¹, 12.5 μg mL⁻¹, and 6.250 μg mL⁻¹ concentration against all microorganisms. The tubes were inoculated with 10⁶ cfu mL⁻¹ (colony-forming unit/mL) and incubated at 37 ºC 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.

![Chemical structure of V1 to V10](image)

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.

| Compound | 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 | 125 |
| Ampicillin | 250 | 100 | 100 | 100 |
| Chloramphenicol | 50 | 50 | 50 | 50 |
| Norfloxacin | 10 | 10 | 10 | 10 |
The biological screening revealed that compound V5 exhibited maximum antibacterial activity against gram-positive bacteria and E. coli at 125 μg 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: \textit{In vitro} Anti-Fungal Activity Screening Results for (V1-V10)

| Compound | Minimal Inhibition Concentration (µg 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   |
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 | Different Concentration(µg 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 |

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.

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