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

Sagar P. Gami, Kalpesh V. Vilapara, Hasmukh R. Khunt, Jayesh S. Babariya, Yogesh T. Naliapara*

Department of Chemistry, Saurashtra University, Rajkot-36005, Gujarat, India
*E-mail address: naliaparachem@yahoo.co.in

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 IH NMR, 13C 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. 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, antimalarial, antimicrobial, anti-inflammatory, inhibition of KDR kinase, antifungal and macrophage activation. They have proved to be promising anticancer agents with dual mechanisms of tubulin polymerization promotion as well as cyclin dependent kinases 2 inhibition.

We have synthesized 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. The newly synthesized compounds were characterized by IR, Mass, IH NMR, 13C 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.](image-url)
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, (CH₃)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 C₁₆H₁₅ClFN₅O₂: 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; Rf 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; 1H NMR: δ 1.476-1.1.492 (d, 6H, (CH₃)₂, J = 6.4 Hz), 2.198 (s, 3H, -CH₃), 2.211 (s, 3H, -CH₃), 3.498-3.532 (m, 1H, -CH), 4.029 (s, 3H, -OCH₃), 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); 13C 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 C₁₈H₂₁N₅O₂: 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-3n): White solid; mp 278-280 °C; Rf 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; 1H NMR: δ 1.476-1.1.494 (d, 6H, (CH₃)₂, J = 7.2 Hz), 2.286 (s, 3H, -CH₃), 3.480-3.550 (m, 1H, -CH), 4.031 (s, 3H, -OCH₃), 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); 13C NMR (100 MHz, DMSO): 18.03, 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 C₁₇H₁₉N₅O₂: 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. (°C) | Yield (%) |
|----------|---------|-----------------------|------|-----------|-----------|
| SPG-3a   | H       | C₁₆H₁₇N₅O₂            | 311  | 236-238   | 40        |
| SPG-3b   | 4-Br    | C₁₆H₁₆BrN₅O₂          | 390  | 246-248   | 36        |
| SPG-3c   | 4-F     | C₁₆H₁₆FN₅O₂           | 329  | 224-226   | 35        |
| SPG-3d   | 4-OCH₃  | C₁₇H₁₉N₅O₃           | 341  | 238-240   | 42        |
| SPG-3e   | 4-NO₂   | C₁₆H₁₆N₆O₄           | 341  | 250-252   | 25        |
| SPG-3f   | 3-CH₃   | C₁₇H₁₉N₅O₂           | 325  | 260-262   | 35        |
| SPG-3g   | 3,4-di-Cl | C₁₆H₁₅Cl₂N₅O₂       | 380  | 244-246   | 30        |
| SPG-3h   | 3-Cl, 4-F | C₁₆H₁₅ClFN₅O₂       | 363  | 256-258   | 32        |
| SPG-3i   | 4-Cl    | C₁₆H₁₆ClN₅O₂          | 345  | 258-260   | 35        |
| SPG-3j   | 2,4-di-Cl | C₁₆H₁₅Cl₂N₅O₂       | 380  | 282-284   | 32        |
| SPG-3k   | 3,4-di-CH₃ | C₁₈H₂₁N₅O₂           | 339  | 224-226   | 40        |
| SPG-3l   | 2-Cl    | C₁₆H₁₆ClN₅O₂          | 345  | 276-278   | 30        |
| SPG-3m   | 4-CH₃   | C₁₇H₁₉N₅O₂           | 325  | 278-280   | 35        |
| SPG-3n   | 2,3-di-CH₃ | C₁₈H₂₁N₅O₂           | 339  | 268-270   | 38        |
| SPG-3o   | 2-F     | C₁₆H₁₆FN₅O₂           | 329  | 232-234   | 33        |
| SPG-3p   | 2-NO₂   | C₁₆H₁₆N₆O₄           | 356  | 286-288   | 26        |
| SPG-3q   | 3-Cl    | C₁₆H₁₆ClN₅O₂          | 345  | 264-266   | 35        |
| SPG-3r   | 4-Cl, 2-NO₂ | C₁₆H₁₅ClN₆O₄       | 390  | 274-276   | 22        |
| SPG-3s   | 4-Cl, 3-NO₂ | C₁₆H₁₅ClN₆O₄       | 390  | 266-268   | 25        |
| SPG-3t   | 2-CF₃   | C₁₇H₁₆F₃N₅O₂         | 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      | 500        |
| 2       | SPG-5b | 250         | 250    | 200          | 250      | 250        |
| 3       | SPG-5c | 500         | 500    | 250          | 500      | 250        |
| 4       | SPG-5d | 100         | 500    | 250          | 500      | 250        |
| 5       | SPG-5e | 500         | 500    | 500          | 500      | 500        |
| 6       | SPG-5f | 500         | 100    | 100          | 250      | 250        |
| 7       | SPG-5g | 500         | 200    | 250          | 500      | 250        |
| 8       | SPG-5h | 250         | 250    | 200          | 250      | 250        |
| 9       | SPG-5i | 200         | 250    | 500          | 250      | 250        |
| 10      | SPG-5j | 200         | 250    | 200          | 500      | 250        |
| 11      | SPG-5k | 500         | 250    | 250          | 250      | 250        |
| 12      | SPG-5l | 500         | 250    | 250          | 250      | 250        |
| 13      | SPG-5m | 100         | 200    | 500          | 125      | 250        |
| 14      | SPG-5n | 250         | 200    | 500          | 250      | 250        |
| 15      | SPG-5o | 500         | 500    | 250          | 500      | 250        |
| 16      | SPG-5p | 250         | 200    | 500          | 200      | 200        |
| 17      | SPG-5q | 250         | 500    | 500          | 200      | 200        |
| 18      | SPG-5r | 500         | 250    | 250          | 250      | 250        |
| 19      | SPG-5s | 250         | 200    | 250          | 500      | 500        |
| 20      | SPG-5t | 500         | 500    | 250          | 500      | 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 as 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.

ACKNOWLEDGEMENT

We are very much thankful to department of chemistry for providing lab facilities and National Facility For Drug Discovery center, saurashtra university for Spectral analysis.

References

[1] G. Fischer, Adv. Heterocycl. Chem. 57 (1993) 81.
[2] M. Shaban, A. Morgan, Adv. Heterocycl. Chem. 77 (2000) 345.
[3] M. Shaban, A. Morgan, Adv. Heterocycl. Chem., 73 (2000) 131.
[4] M. Shaban, A. Morgan, Adv. Heterocycl. Chem. 75 (2000) 243.
[5] N. Zhang, A. Semiramis, N. Thai, J. Med. Chem. 50 (2007) 319.
[6] L. Havlicek; K. Fuksova; V. Krystof, Bioorg. Med. Chem. 13 (2005) 5399.
[7] X. Zhao; Y. Zhao; S. Guo; H. Song; D. Wang; P. Gong, Molecules 12 (2007) 1136.
[8] L. Iwona, F. Marzena, M. Tadeusz, S. Tadeusz, J. Julia, Dalton Trans. 42 (2013) 6219.
[9] A. Marwaha, J. White, F. El Mazouni, S. Creason, S. Kokkonda, F. Buckner, P. Rathod, J. Med. Chem. 55 (2012) 7425.
[10] L. Yin; Z. Shuai; L. Zhi-Jun; Z. Hai-Liang, *Euro. J. Med. Chem.* 64 (2013) 54.
[11] H. Ashraf, *Abd El-Wahab Pharmaceuticals* 5 (2012) 745.
[12] A. Abdel-Aziem, M. Sayed El-Gendy, A. Abdelhamid, *Euro. J. Chem.* 3 (2012) 455.
[13] M. Khera, I. Cliffe, T. Mathur, O. Prakash, *Bioorg. Med. Chem. Lett.* 21 (2011) 2887.
[14] H. Ashour, O. Shaaban, O. Rizk, I. El-Ashmawy, *Euro. J. Med. Chem.* 62 (2013) 341.
[15] M. Fraley, W. Hoffman, R. Rubino, *Bioorg. Med. Chem. Lett.* 12 (2002) 2767.
[16] Q. Chen, X. Zhu, Z. Liu, *Euro. J. Med. Chem.* 43 (2008) 595.
[17] S. Uryu, S. Tokuhiro, T. Murasugi, *Brain Research* 946 (2002) 298
[18] L. Antonino, A. Ilenia, P. Chiara, M. Annamaria, D. Gaetano, A. Maria, *Euro. J. Med. Chem.* 62 (2013) 416.
[19] B. Fairfield, C. Andrew, J. Allan, WO2004108136, (2004).
[20] C. Tamilselvan, S. John Joseph, G. Mugunthan, S. Syed Musthaq Ahamed, *International Letters of Chemistry, Physics and Astronomy* 9 (2014) 93-102.
[21] R. G. Vaghasiya, H. B. Ghodasara, P. R. Vachharajani, V. H. Shah, *International Letters of Chemistry, Physics and Astronomy* 8 (2014) 30-37.
[22] Piyush B. Vekariya, Jalpa R. Pandya, Vaishali Goswami, Hitendra S. Joshi, *International Letters of Chemistry, Physics and Astronomy* 7 (2014) 45-52.

(Received 05 March 2014; accepted 10 March 2014)