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Research Paper

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KEYWORDS : 1,2,4-triazole,pyrimidine ,1,2,4-triazolo [1,5-a]pyrimidines ,Antibacterial and antifungalactivity

Microwave assisted synthesis of 1,2,4-triazolo [1,5-a]pyrimidines and biological evaluation

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ABSTRACT

1,2,4-triazolo [1,5-a]pyrimidines and their derivatives are having very good antibacterial and antifungal properties. All the synthesized compounds arecharacterized by (MIC) in vitro by broth dilution method [129, 130] with two Gram-positive bacteria Staphylococcus aureusMTCC-96, Streptococcus pyogenesMTCC 443, two Gram-negative bacteria Escherichia coli MTCC 442, Pseudomonas aeruginosaMTCC 441 and three fungal strains Candida albicansMTCC 227, Aspergillus Niger MTCC 282, AspergillusclavatusMTCC 1323

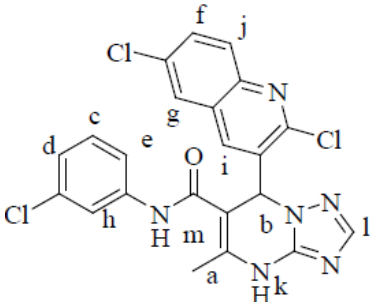
INTRODUCTION:

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). 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.

2. EXPERIMENTAL:

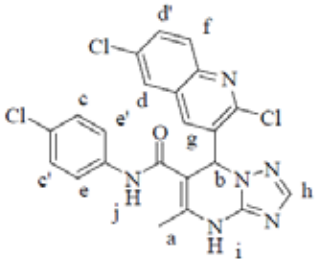
2.1 7-(2,6-dichloroquinoline-3yl)-4,7-dihydro-5-methyl–N-(3-chlorophenyl)–[1,2,4] triazolo[1,5-a]pyrimidine–6–carboxamide.

(AP-02) Yield: 85%; mp 198-202ºC; IR (cm-1): 3259 (N-H stretching of secondary amine), 3032 (C-H stretching of aromatic ring), 2920 (C-H asymmetrical stretching of CH3 group), 2875 (C-H asymmetrical stretching of CH3 group), 1668 (C=O stretching of amide), 1606 (C=N stretching of triazole ring), 1589(N- H deformation of pyrimidine ring), 1514 and 1492 (C=C stretching of aromatic ring), 1440 (C-H asymmetrical deformation of CH3 group), 1410 (C-H symmetrical deformation of CH3 group), 1330 (C-N stretching), 1037 (C-H in plane deformation of aromatic ring), 829 (C-H out of plane bending of 1,4-disubstituion); 1H NMR (DMSO-d6) δ ppm: 2.2935 (s, 3H, Ha), 5.6278 (s, 1H, Hb), 6.9701-6.9730 (s, 2H, Hc), 6.9505-6.9532 (d, 2H, Hd, J = 1.08 Hz), 7.0478-7.0974 (d, 2H, He,), 7.5514-7.5466 (d, 2H, Hf, J = 1.76 Hz), 7.5976-7.5682 (d, 2H, Hg, J = 11.76 Hz), 7.9895 (s, 1H, Hh), 7.9675 (s, 1H, Hi), 8.0 (s, 1H, Hj), 9.5235 (d, 1H, Hk), 7.6574 (d, 1H, Hl), 10.3687 (d, 1H, Hm);MS: m/z 486; Anal Calcd. for C22H15Cl3N6O: C,67.40; H, 5.61; N, 18.60; Found: C, 67.10; H, 5.54; N, 18.49%.



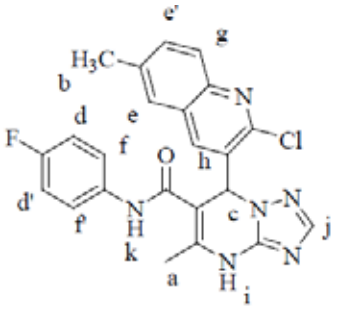
2.7.4.3 7-(2,6-dichloroquinoline-3yl)-4,7-dihydro-5-methyl–N-(4chlorophenyl)[1,2,4]triazolon [1,5-a] pyrimidine–6–carboxamide

(AP-03) Yield: 58%; mp 202-205 ºC; IR (cm-1): 3269 (N-H stretching of secondary amine), 3097 (C-H stretching of aromatic ring), 2922 (C-H asymmetrical stretching of CH3 group), 2868 (C-H asymmetrical stretching of CH3 group), 1651 (C=O stretching of amide), 1600 (C=N stretching of triazole ring), 1539 (N-H deformation of pyrimidine ring), 1510, 1492 and 1454 (C=C stretching of aromatic ring), 1413 (C-H asymmetrical deformation of CH3 group), 1338 (C-H symmetrical deformation of CH3 group), 1280 (C-N stretching), 1074 (C-H in plane deformation of aromatic ring), 825 (C-H out of plane bending of 1,4-disubstituion); 1H NMR (DMSO-d6) δ ppm: 2.2795 (s, 3H, Ha), 5.9180 (s, 1H, Hb), 7.1582-7.1802 (s, 2H, Hcc', J =8.8 Hz), 7.4717-7.4821, (d, 2H, Hdd', J = 4.16 Hz), 7.5506-7.5560, 7.5044-7.5094 (t, 2H, Hee', J = 2.16 Hz ,2.00Hz), 7.9661-7.9732 (m, 1H, Hf, J = 2.84), 7.9955-8.0840 (s, 1H, Hg,, J =2.84), 7.5665 (s, 1H, Hh), 9.3135 (s, 1H, Hi), 10.4128(m, 1H, Hj); MS: m/z 486; Anal. Calcd.for C22H15Cl3N6O: C, 64.01; H, 4.81; N, 18.60; Found: C, 64.00; H, 4.78; N, 18.46%.



2.7.5.8 7-(2-chloro,6-methylquinoline-3yl)-4,7-dihydro-5-methyl–N-(4-flourophenyl)–[1,2,4] triazolo[1,5-a]pyrimidine–6-carboxamide

(AP-18) Yield: 56%; mp 248-250ºC; IR (cm-1): 3246 (N-H stretching of secondary amine), 3045 (C-H stretching of aromatic ring), 2926 (C-H asymmetrical stretching of CH3 group), 2837 (C-H asymmetrical stretching of CH3 group), 1662 (C=O stretching of amide), 1602 (C=N stretching of triazole ring), 1560 (N-H deformation of pyrimidine ring), 1516 and 1479 (C=C stretching of aromatic ring), 1450 (C-H asymmetrical deformation of CH3 group), 1332 (C-H symmetrical deformation of CH3 group), 1282 (C-N stretching), 1031 (C-H in plane deformation of aromatic ring), 831 (C-H out of plane bending of 1,4-disubstituion); 1H NMR (DMSO-d6) δ ppm: 1.8140 (s, 2×3H, Hab), 5.6278 (s, 1H, Hc), 7.3646 (d, 2H, Hdd', J = 9.14 Hz), 7.6850-7.6896,7.7066-7.7112 (d, 2H, Hee', J = 1.84 Hz ,2.16 Hz), 7.8135,7.8590-7.8813 (d, 2H, Hff', J = 8.92 Hz), 7.9028 (d, 1H, Hg, J = 8.96 Hz), 8.0698 (s, 1H, Hh), 9.4011 (s, 1H, Hi), 7.5650 (s, 1H, Hj), 10.4192 (m, 1H, Hk);MS: m/z 449; Anal. Calcd.for C22H18ClFN6O: C, 61.54; H, 4.04; N, 18.72; Found: C, 61.04; H, 4.00; N, 18.23%.



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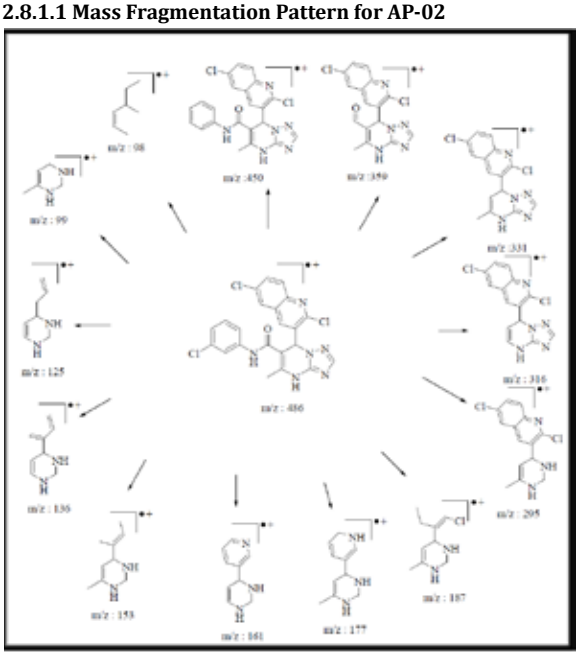
2.2 Spectral discussion

2.2.1 Mass spectral study

Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using Direct Injection Probe technique. Systematic fragmentation pattern was observed in mass spectral analysis. Molecular ion peak was observed in agreement with molecular weight of respective compound. Mass fragmentation pattern for a representative compound of each series is depicted below.

2.8.1.1 Mass Fragmentation Pattern for AP-02



2.2.2 IR spectral study

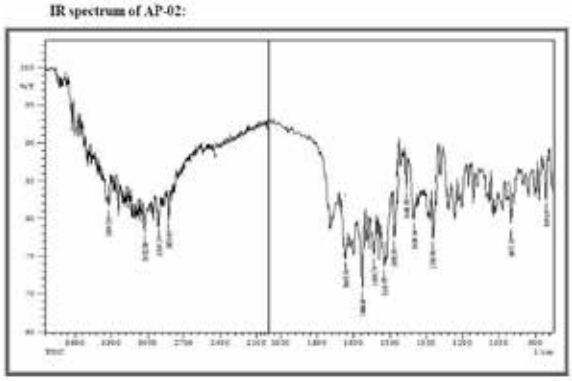
IR spectra were recorded on Shimadzu FT-IR-8400 model using KBr pellet method. Various functional groups present in molecule were identified by characteristic frequency obtained for them. For 1,2,4-triazolo[1,5-a]pyrimidines (AP-1 to AP-40), confirmatory bands for secondary amine (NH) and nitrile (C=N) stretching band were observed at 3190-3500 cm-1 and 1590-1650 cm-1 respectively. Another characteristic band for N-H deformation and C-N stretching were observed at 1600-1680 cm-1 and 1290-1350 cm-1 respectively, which suggested the formation of pyrimidine ring.

2.2.3 1H NMR spectral study

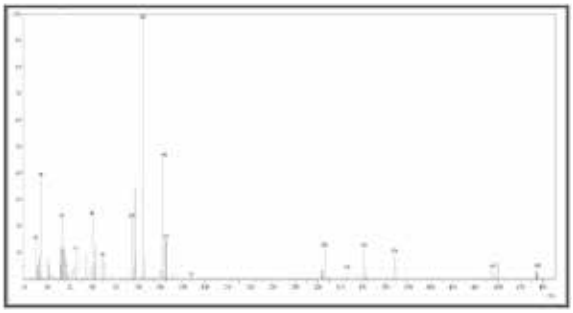
1H NMR spectra were recorded in DMSO-d6 solution on a Bruker Ac 400 MHz spectrometer using TMS as an internal standard. Number of protons and their chemical shifts were found to support the structure of the synthesized compounds.

1H NMR spectra confirmed the structures of triazolopyrimidines AP-01 to AP-40 on the basis of following signals: a singlet for the methine proton of pyrimidine ring at 5.5-6.9 δ ppm, a singlet for the methine proton of triazole ring at 7.50-8.0 δ ppm and singlets

IR spectrum of AP-02:

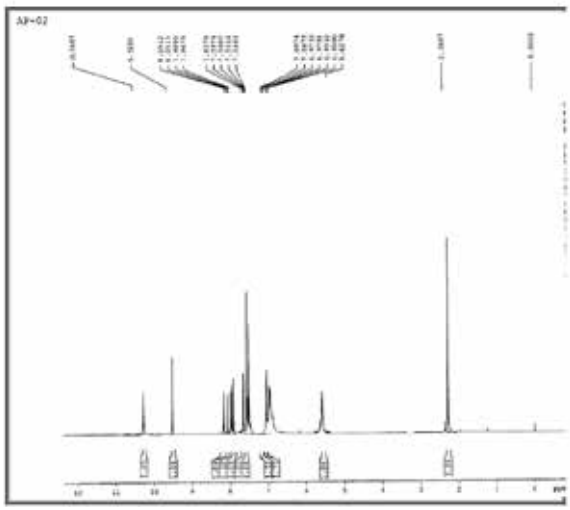


Mass spectrum of AP-02:



for amino and amide group protons at 9.2-9.6 δppm and 10.2-10.6 δ ppm, respectively. The aromatic ring protons and J value were found to be in accordance with substitution pattern on phenyl ring.

^{1}H NMR spectrum of AP-02:



2.3 Biological evaluation

2.3.1 Antimicrobial evaluation

All the synthesized compounds (AP-01 to AP-40) were tested for their antibacterial and antifungal activity (MIC) in vitro by broth dilution method [129, 130] with two Gram-positive bacteria Staphylococcus aureusMTCC-96, Streptococcus pyogenesMTCC 443, two Gram-negative bacteria Escherichia coli MTCC 442, Pseudomonas aeruginosaMTCC 441 and three fungal strains Candida albicansMTCC 227, Aspergillus Niger MTCC 282, AspergillusclavatusMTCC 1323 taking ampicillin, chloramphenicol, ciprofloxacin, norfloxacin, nystatin, and greseofulvin as standard drugs. The standard strains were procured from the Microbial Type Culture Collection (MTCC) and Gene Bank, 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 microdilution broth method according to NCCLS standards [129]. Serial dilutions of the test compounds and reference drugs were prepared in Muellere-Hinton agar. Drugs (10 mg) were dissolved in dimethylsulfoxide (DMSO, 1 mL). Further progressive dilutions with melted Muellere-Hinton agar were performed to obtain the required concentrations. In primary screening 1000 µg mL-1, 500 µg mL-1and 250 µg mL-1 concentrations of the synthesized drugs were taken. The active synthesized drugs found in this primary screening were further tested in a second set of dilution at 200 µg mL-1, 100 µg mL-1, 50 µg mL-1, 25 µg mL-1, 12.5 µg mL-1, and 6.25 µg mL-1 concentration against all microorganisms. The tubes were inoculated with 108 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 had no effect on the

bacterial growth, a control was performed 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.

Table 1. Antibacterial and antifungal activity of synthesized compounds AP-1 to AP-18

Code	Minimum inhibition concentration (μg mL^{-1})						
	Gram-positive		Gram-negative		Fungal species		
	S.a.	*S. p.*	*E.c.*	*P.a.*	*C. a.*	*A. n.*	*A.c.*
AP-01	500	1000	500	100	1000	500	500
AP-02	1000	500	1000	1000	500	500	1000
AP-03	500	500	250	500	>1000	1000	500
AP-04	250	62.5	125	250	1000	500	250
AP-05	125	100	1000	500	100	1000	500
AP-06	500	1000	250	1000	500	500	>10
AP-07	1000	250	500	500	500	100	250
AP-08	100	125	100	62.5	>1000	1000	1000
AP-09	100	>1000	500	1000	>1000	500	1000
AP-10	25	500	250	100	500	1000	>10
AP-11	1000	100	100	500	250	100	250
AP-12	125	100	100	500	500	250	1000
AP-13	500	500	1000	>1000	1000	500	125
AP-14	125	100	50	250	500	1000	500
AP-15	100	1000	250	1000	1000	500	1000
AP-16	50	500	250	250	>1000	1000	>10
AP-17	500	1000	500	1000	500	500	100
AP-18	125	25	100	100	500	>1000	500

REFERENCE

[1] Fischer, G. Adv. Heterocycl. Chem. 1993, 57, 81. | [2] Shaban, M.A.E.; Morgan, A.E.A. Adv. Heterocycl. Chem. 2000, 77, 345. | [3] Shaban, M.A.E.; Morgan, A.E.A. Adv. Heterocycl. Chem. 2000, 73, 131. | [4] Shaban, M.A.E.; Morgan, A.E.A. Adv. Heterocycl. Chem. 2000, 75, 243. | [5] Zhang, N.; Ayral-Kaloustian, S.; Nguyen, T.; Afragola, J.; Hernandez, R.; Lucas, J.; Gibbons, J.; Beyer, C. J. Med. Chem. 2007, 50, 319. | [6] Havlicek, L.; Fuksova, K.; Krystof, V.; Orsag, M.; Vojtesek, B.; Strnad, M. Bioorg. Med. Chem. 2005, 13, 5399. | [7] Fraley M. E., Hoffman W. F., Rubino R. S. Bioorg. Med. Chem. Lett. 2002, 12, 2767.