Available online at www.joac.info

ISSN: 2278-1862

Journal of Applicable Chemistry

2014, 3 (4): 1510-1516

(International Peer Reviewed Journal)

Synthesis And Antimicrobial Evaluation Of Some Triazole Incorporated Pyrimidine Derivatives

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Accepted on 17th July 2014

ABSTRACT

A new series of triazole incorporated pyrimidine derivatives were synthesized by treating Substituted aldehyde with ethylacetoacetate and urea / thiourea to yield pyrimidine derivatives which on reaction with thiosemicarbazide followed by cyclization to give title compound that is triazole incorporated pyrimidine derivatives. Synthesis was carried out by convectional as well as microwave method. All the intermediates and synthesized compounds were characterized by running TLC, determining M.P. and IR, ^{1}H NMR spectral analysis. All the synthesized compounds were subjected to preliminary in-vitro evaluation for antibacterial activity against various Gram-positive bacterial strains Bacillus Subtillis, Klebsiella Pneumoniae and Gram-nagative bacterial strains E.coli, Pseudomonas aeruginasa and moreover the compounds were also evaluated for their antifungal activity in fungal strains like Candida albicans, Aspergillus fumigates.

Keywords: Synthesis, Antibacterial, Antifungal, Microwave, Triazole, Pyrimidine.

INTRODUCTION

Dihydropirimidinones (DHPM)[1] is an important class of compounds due to their therapeutic significance as antimicrobial[2-5], antiviral, anti-HIV[6-7], antineoplastic[8-9], anticonvulsant, sedative, hypnotic, analgesic and anti-inflammatory[10], calcium channel blockers and antihypertensive agents[11-12]. On the other hand triazole also possess broad spectrum of biological activities like anticonvulsant[13], analgesic[14], anti-inflammatory[15], antidepressant [16], and anti-tubercular and antimicrobial[17]. In the light of these finding we felt it is worth synthesizing a series of compounds in which triazole ring incorporate in the DHPM nucleus. Due to the fast development of microbial resistance towards the existing drug, new chemical entities were synthesized by coupling triazole ring with pyrimidine ring. Therefore present research work was aimed to synthesize derivatives of triazole incorporated pyrimidine ring. The synthesis of the title compounds were affected as outlined in the scheme. Substituted aldehyde was react with ethylacetoacetate and urea / thiourea to yield pyrimidine derivatives which on reaction with thiosemicarbazide followed by cyclization to give title compound that is triazole incorporated pyrimidine derivatives.

All the intermediates and final compounds were characterized by running TLC, determination of melting point and spectral studies. The title compounds were screened for antimicrobial activity using cup plate method .All newly synthesized compounds were found to possess comparable antimicrobial activity to standard drug. Further these compounds can be evaluated for antitumor and anti-HIV activity.

MATERIALS AND METHODS

All chemicals were obtained from Dow chemical Pvt. Ltd., Mumbai. All chemical and solvents used were of analytical grade. All the melting points of synthesized compounds were determined by open capillary tube method and are uncorrected. The synthesis and analytical studies of the compounds were carried out using laboratory grade and analytical grade reagents as the case may be standard procedure or reported methods were followed with or without modification appropriately as and when required. The IR absorption spectra of the compounds were recorded on FTIR Bruker Tensor-27 model. The ^{1}H NMR absorption spectra of the compounds were recorded on Bruker model. Physical and spectral data are presented in tables 1-3.

Step 1: Synthesis of Ethyl 6-methyl- 2-oxo/thio-4-aryl-1, 2, 3, 4-tetrahydropyrimidine-5- carboxylate (I-VI)

Conventional Method*:* Derivatives of benzaldehyde (10 mmol), urea or thiourea (10 mmol), ethyl aceto acetate (10 mmol) and phosphorus pentaoxide (3.54 mmol) in 250 mL round bottom flask were refluxed on water bath. The reaction mixture was cooled and poured into crushed ice. The separated solid was then filtered, washed with water, dried and recrystallized with ethanol. The completion of reaction was monitored by running T.L.C.

Microwave Method*:* Derivatives of benzaldehyde (10 mmol), urea or thiourea (10 mmol), ethyl aceto acetate (10 mmol) and phosphorus pentaoxide (3.54 mmol) were placed in an Erlenmeyer flask and then irradiated in a microwave oven at 120 W for 140 sec. After completion of reaction, the mixture was cooled and poured into crushed ice. The solid thus obtained was filtered, washed with water, dried and recrystallized with ethanol. The completion of reaction was monitored by running T.L.C.

Step 2: Synthesis of 1- (6-Methyl-2-oxo/thio-4-aryl-1, 2, 3, 4-tetrahydropyrimidine - 5-carbonyl) thiosemi carbazide (VII-XII)

Conventional Method*:* Compounds I-VI and thiosemicarbazide (10mmol) were dissolved in sufficient amount of glacial acetic acid in 250mL round bottom flask. The reaction mixture was refluxed on water bath for 21 h. The reaction mixture was cooled and poured into crushed ice. The separated solid was then filtered, washed with water, dried & recrystallized with ethanol.

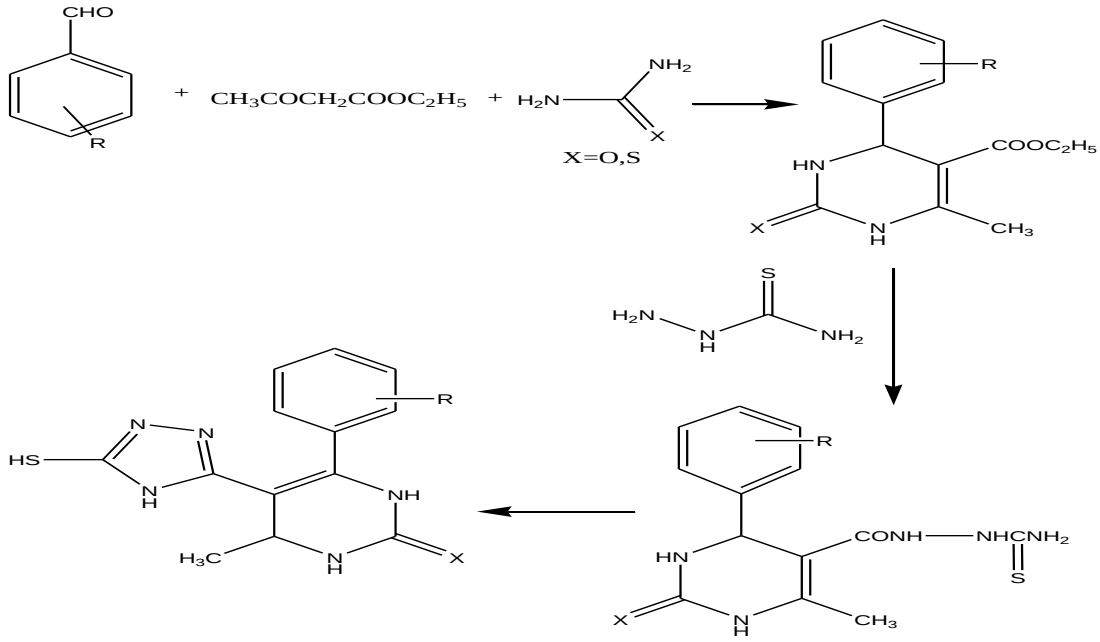
Microwave Method: Compounds I-VI (10mmol) and thiosemicarbazide (10mmol) were placed in an Erlenmeyer flask and then irradiated in a microwave oven at 120 W for 15 minutes. After completion of reaction, the mixture was cooled and poured into crushed ice. The solid thus obtained was filtered, washed with water, dried & recrystallized with ethanol.

Step 3 - Synthesis of 5-(5-Mercapto-4H-1, 2, 4-triazol-3-yl)-6-methyl-4-aryl- 3, 4-dihydropyrimidin-2(1H)-one/thione (F_1-F_6) : Compounds VII-XII (0.005mol) was refluxed with 17.85mL of 4% sodium hydroxide solution for 4 h. The resulting solution was cooled and acidified with 0.1N hydrochloric acid to pH 5-6. The solid thus obtained was filtered, dried and recrystallized from ethanol.

Title compounds:

F_1 : 5-(5-Mercapto-4H-1, 2, 4-triazol-3-yl)-6-methyl-4-phenyl-3,4-dihydropyrimidin- 2(1H)-one
F_2: 5-(5-mercapto-4H-1, 2, 4-triazol-3-yl)-6-methyl-4-phenyl-3,4-dihydropyrimidine- 2(1H)-thione
F_3: 4-(4-chlorophenyl)-5-(5-mercapto-4H-1, 2, 4-triazol-3-yl)-6-methyl-3, 4- dihydropyrimidin-2(1H)-one
F_4:4-(4-chlorophenyl)-5-(5-mercapto-4H-1,2,4-triazol-3-yl)-6-methyl-3,4-dihydropyrimidine-2(1H)-thione

F_5:4-(4-(dimethylamino)phenyl)-5-(5-mercapto-4H-1,2,4-triazol-3-yl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one
F_6:4-(4-(dimethylamino)phenyl)-5-(5-mercapto-4H-1,2,4-triazol-3-yl)-6-methyl-3,4-dihydropyrimidine-2 (1H) -thione



Scheme of Work

Where	R	X
F_1	H	O
F_2	H	S
F_3	Cl	O
F_4	Cl	S
F_5	N $(CH_3)_2$	O
F_6	N $(CH_3)_2$	S

RESULTS AND DISCUSSION

A new series of triazole incorporated pyrimidine ring were synthesized and the synthetic scheme is presented in scheme of work. All the synthesized compounds were in conformity with the structures envisaged. The structures are confirmed on the basis of physical and spectral data viz. IR, ^{1}H-NMR spectroscopy (Tables 1,2,3) . All the compounds were evaluated for antimicrobial screening by using cup-plate method. All the compounds have shown mild to moderate activity against *E.coli* in which compound F_5 shown good activity. In case of *B.subtillis, K.pneumoniae* and *P.aeruginasa* compounds did not show any significant activity in comparison with the standard i.e. Ciprofloxacin. In case of antifungal activity, compound F_2 and F_5 have shown excellent activity against *C.albicans,* whereas other compounds posses moderate activity. All the compounds have shown to posses excellent activity against *A.fumigatus* except compound F_6 in comparison with the standard i.e. Fluconazole.

Table 1. The Physical And Analytical Data of Following Compounds

S. NO.	MOLECULAR FORMULA	M. WT.	R_f (cm)	M.P. (° C)	% Yield
I	$C_{14}H_{16}N_2O_3$ Ethyl 6-methyl-4-phenyl-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate	260.12	0.27	202-204	75%
II	$C_{14}H_{16}N_2O_2S$ Ethyl 6-methyl-4-phenyl-2-thio-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate:	276.35	0.23	204-205	59.2%
III	$C_{14}H_{15}ClN_2O_3$ Ethyl 4-(4-chlorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate	294.73	0.86	220-224	65%
IV	$C_{14}H_{15}ClN_2O_2S$ Ethyl 4-(4-chlorophenyl)-6-methyl-2-thio-1,2,3,4-tetrahydropyrimidine-5-carboxylate	310.80	0.79	186-190	63%
V	$C_{16}H_{21}N_3O_3$ Ethyl 4-(4-(dimethylamino)phenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate	303.36	0.61	246-248	82%
VI	$C_{16}H_{21}N_3O_2S$ Ethyl 4-(4-(dimethylamino)phenyl)-6-methyl-2-thio-1,2,3,4-tetrahydropyrimidine-5-carboxylates	319.36	0.52	214-220	79%
VII	$C_{13}H_{15}N_5O_2S$ 1-(6-Methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydro pyrimidine-5-carbonyl)thiosemicarbazide	305	0.25	242-248	80%
VIII	$C_{13}H_{15}N_5OS_2$ 1-(6-Methyl-4-phenyl-2-thio-1,2,3,4-tetrahydropyrimidine -5-carbonyl) thiosemicarbazide	321.42	0.64	248-250	62%
IX	$C_{13}H_{14}ClN_5O_2S$ 1-(4-(4-Chlorophenyl)-6-methyl-2-oxo-1,2,3,4 tetrahydropyrimidine-5-carbonyl) thiosemicarbazide	339.80	0.55	234-236	73%
X	$C_{13}H_{14}ClN_5OS_2$ 1-(4-(4-Chlorophenyl)-6-methyl-2-thio-1,2,3,4-tetrahydropyrimidine-5-carbonyl) thiosemicarbazide	355.80	0.62	80-82	45%
XI	$C_{15}H_{20}N_6O_2S$ 1-(4-(4-(Dimethylamino)phenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carbonyl) thiosemicarbazide	348.42	0.64	210-215	85%
XII	$C_{15}H_{20}N_6OS_2$ 1-(4-(4-(Dimethylamino)phenyl)-6-methyl-2-thio-1,2,3,4-tetrahydropyrimidine-5-carbonyl) thiosemicarbazide	364.42	0.78	160-165	62%
F_1	$C_{13}H_{13}N_5OS$ 5-(5-Mercapto-4H-1, 2, 4-triazol-3-yl)-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1H)-one	287	0.38	205-206	40%
F_2	$C_{13}H_{13}N_5S_2$ 5-(5-mercapto-4H-1,2,4-triazol-3-yl)-6-methyl-4-phenyl-3,4-dihydropyrimidine-2(1H)-thione	303.41	0.44	175-180	38%
F_3	$C_{13}H_{12}ClN_5OS$ 4-(4-chlorophenyl)-5-(5-mercapto-4H-1,2,4-triazol-3-yl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one	321.79	0.75	198-200	48%
F_4	$C_{13}H_{12}ClN_5S_2$ 4-(4-chlorophenyl)-5-(5-mercapto-4H-1,2,4-triazol-3-yl)-6-methyl-3,4-dihydropyrimidine-2(1H)-thione	337.79	0.50	118-120	33 %
F_5	$C_{15}H_{18}N_6OS$ 4-(4-(dimethylamino)phenyl)-5-(5-mercapto-4H-1,2,4-triazol-3-yl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one	330.41	0.53	228-230	85 %
F_6	$C_{15}H_{18}N_6S_2$ 4-(4-(dimethylamino)phenyl)-5-(5-mercapto-4H-1,2,4-triazol-3-yl)-6-methyl-3,4-dihydropyrimidine-2(1H)-thione	346.41	0.75	140-146	71 %

Table 2. Data of IR Absorption Spectra

Compound No.	Wave Number in cm^{-1}
F_1	-NH (3234.09), -SH (2742.02), -CH_3 (2960.71), -C=N (1639.73), Ar –CH (2988.05) and -C=O (1721.74)
F_2	-NH (3322.92), -SH (2593.44), - CH_3 (2944.67), -C=S (1229.52), -C=N (1651.67) and Ar –CH (2978.4)
F_3	-NH (3326.91), -SH (2590.26), -CH_3 (2944.67), -C=N (1650.81), Ar –CH (2978.4) and -C=O (1696.05)
F_4	-NH (3327.60), -SH (2528.51), - CH_3 (2924.58), -C=S (1176.79), -C=N (1659.72) and Ar –CH (2980.17)
F_5	-NH (3324.07), -SH (2510.23), -CH_3 (2938.34), -C=N (1615.05), Ar –CH (2963.07) , -C=O (1667.35) and –N< (1344.75)
F_6	-NH (3301.03), -SH (2549.24), - CH_3 (2932.34), -C=S (1187.29), -C=N (1594.38), Ar –CH (2978.4) and –N< (1360.11)

Table 3 . Data of ^1H NMR Absorption Spectra

Compound No.	Chemical shift (δ ppm)
F_1	m, 5H, Ar-H (7.23-7.40); s, 1H, NH (8.35); s, 1H, NH (7.68); d, 1H, CH (5.39); s, 3H, CH_3 (1.78); s, 1H, SH (2.30) and m, tr, NH (4-4.2).
F_2	m, 5H, Ar-H (7.23-7.35); s, 1H, NH (7.73); s, 1H, NH (7.13); d, 1H, CH (5.39-5.42); s, 3H, CH_3 (1.59); s, 1H, SH (2.17) and m, tr,NH (4.04-4.16).
F_3	m, 5H, Ar-H (7.23-7.30); s, 1H, NH (7.65); s, 1H, NH (5.65); d, 1H, CH (5.37-5.39); s, 3H, CH_3 (1.60); s, 1H, SH (2.17) and m, tr,NH (4.04-4.14).
F_4	m, 5H, Ar-H (7.20-7.31); s, 1H, NH (8.20); s, 1H, NH (7.70); d, 1H, CH (5.35-5.40); s, 3H, CH_3 (1.20); s, 1H, SH (2.17) and m, tr,NH (4.1).
F_5	m, 5H, Ar-H (7.20); s, 1H, NH (7.30); s, 1H, NH (6.50); d, 1H, CH (5.30); s, 3H, CH_3 (1.60); s, 1H, SH (2.15-2.20); m, tr, NH (4.00-4.10) and s, 6H, N $(CH_3)_2$ (2.30-2.35).
F_6	m, 5H, Ar-H (7.10-7.20); s, 1H, NH (7.70); s, 1H, NH (7.20-7.30); d,1H, CH (5.30); s, 3H, CH_3 (1.20); s, 1H, SH (2.17); m, tr, NH (4.04-4.13) and s, 6H, N $(CH_3)_2$ (2.30-2.40).

APPLICATIONS

Synthesis of newer compounds have been carried out by different methods and these new methodology have been developed to synthesize similar type of molecules. The synthesized molecules have been screened for anti microbial activities.

Biological Evaluation

In vitro Antimicrobial screening [18]: The synthesized compounds were subjected to antimicrobial screening by cup plate method for zone of inhibition.

Microorganisms:
Selected bacterial Strains: Bacillus Subtillis, Klebsiella Pneumoniae (gram positive**),** E.coli, Pseudomonas aeruginasa **(**gram negative**)**
Selected fungal strains: Candida albicans, Aspergillus fumigatus
Standard drug: Ciprofloxacin (Antibacterial), Fluconazole (Antifungal)
Solvent (control): DMF
Culture Media: For bacteria: Nutrient broth
For fungi: Sabourd dextrose broth
Testing: By cup-plate method [19]
The results of antimicrobial studies are presented in tables 4 and 5

Table 4 . *In vitro* antibacterial activity of the synthesized compound F_1- F_6

Compound	***Gram +ve bacteria***						***Gram -ve bacteria***					
	Bacillus Subtillis			***Klebsiella Pneumoniae***			***E.coli***			***Pseudomonas aeruginasa***		
	Z.O.I	% Inhi.	Activity Index	Z.O.I	% Inhi.	Activity Index	Z.O.I	% Inhi.	Activity Index	Z.O.I	% Inhi.	Activity Index
F_1	-	-	-	8	25.80	0.25	13	41.93	0.41	-	-	-
F_2	-	-	-	-	-	-	13	41.93	0.41	-	-	-
F_3	-	-	-	10	32.25	0.32	14	45.16	0.45	-	-	-
F_4	-	-	-	4	12.90	0.12	19	61.29	0.61	-	-	-
F_5	-	-	-	-	-	-	20	64.51	0.64	9	32.14	0.32
F_6	-	-	-	-	-	-	18	58.06	0.58	9	32.14	0.32
Standard *	29	100		31	100	1	31	100	1	28	100	1

*Ciprofloxacin, - Denotes no activity.

Table *5*. *In vitro* antifungal activity of the synthesized compound F_1- F_6

Compound no	***Candida albicans***			***Aspergillus fumigatus***		
	Z.O.I	% Inhi.	Activity Index	Z.O.I	% Inhi.	Activity Index
F_1	21	84.00	0.84	26	96.29	0.96
F_2	24	96.00	0.96	25	92.59	0.92
F_3	14	56.00	0.56	23	85.18	0.85
F_4	17	68.00	0.68	-	-	-l
F_5	23	92.00	0.92	26	96.29	0.96
F_6	19	76.00	0.76	14	51.85	0.51
Standard *	25	100	1	27	100	1

* Fluconazole, - Denotes no activity.

CONCLUSIONS

Based on the compound synthesized it can be concluded that the introduction of dimethyl amino moiety to the aromatic ring system is favourable for both the activities that has been performed, as in the case of compound F_5 and F_6.

The substitution of S in place of O favors good anti bacterial activity against E. coli.

Therefore it was concluded that the incorporation of triazole moiety to substituted pyrimidines enhance the anti-microbial activities of the resultant molecules as compared to the separate entities.

REFERENCES

[1] C.O.Kappe, Synthesis of new biological active dihydropyrimidinones, *Tetrahedron*, **1993**, 49, 6937-6963.

[2] M.M.Shetty,Y.S.Sadanandam, P.V.Diwan, Synthesis of new 3, 4 - dihydro-6-methyl-5-N-methyl carbamoyl-4 (substituted phenyl)-2(1H) pyrimidinones and pyrimidine thiones with antibacterial and antifungal activities. *European J. Med. Chem*., **1992**, 27, 87-92.

[3] B.S.Ashok M, Holla, N.S. Kumari, Convenient one pot synthesis of some novel 4- methyl thiophenyl incorporated thiazolo [2, 3-b]-dihydro pyrimidinone derivatives as potent antibacterial and antifungal agents, **2007,** 42, 380-385.

[4] O.Prakash, R.Kumar, P.Tyagi, R.C.Kuhad, Synthesis of 3, 9-diaryl-bis-1, 2, 4-triazolo [4, 3-a] [4, 3-c] pyrimidines as antibacterial agents. *European J. Med.Chem.* **2007**, 42, 868.

[5] V.N.Ingle, S.T.Kharche , U.G.Upadhyay, Synthesis of some new 4-O-(-ß-D-gluco pyranosyloxy) -4,6-diaryltetrahydropyrimidine-2-thiones as potent antimicrobial agents. *Indian J Chem*. **2004**, 43B, 2027-2031.

[6] Chen F Er, Ji L, Xie B, Clereq D E, Balzarini J ,Pannecouque C. Synthesis of 1-[(alkenyl or alkynyl or alkyloxy) methyl]-5-alkyl-6- (1-naphthoyl)-2, 4-pyrimidinediones as potent novel non-nucleoside HIV-1 reverse transcriptase inhibitors, *European J. Med. Chem*. **2007**, 42,198.

[7] J.Guillemont, C.Mordant, B.Schmitt et. al., Synthesis of novel diaryl pyrimidine analogues of TMC278 and their antiviral activity against HIV-1 wild type & mutant strains. *European J. Med. Chem.* **2007**, 42, 567-579.

[8] H.A.Stefani, C.B.Oliveira, R.B.Almeida. al., Synthesis of Dihydropyrimidin-(2H)-ones by ultrasound irradiation as potent antioxidant agents. *European J. Med. Chem.* **2006**, 41, 513.

[9] H.S.Joshi, M.R.Patel, D.H.Purohit, J.D.Akbari, Synthesis of some new amino-pyrimidine & thio-pyrimidine derivatives as antitubercular and antimicrobial agents. *Indian J Chem.* **2007**, 84, 1169-1173.

[10] S.J.Melo, R.M.Srivastava, M.T.J.A.Catanho, S.C.D.Nascimento. Synthesis of 4-amino-2-aryl-5-cyano-6-{3-and 4-(N-phthalimidophenyl)} pyrimidines as anti-inflammatory agents. *European J. Med. Chem.* **2006**, 41, 276.

[11] J.S.Sandhu, A. Saini, S.Kumar, Synthesis of 5-unsubstituted 3, 4-dihydropyrimidine-2(1H)-ones as calcium channel blockers and antihypertensive agents. *Indian J Chem.* **2007**, 46B, 1690.

[12] P.T.Perumal, K.Sujatha, P.Shanmugam, D.Muralidharan, M.Rajendran, Synthesis & cardiac effects of 3,4-dihydropyrimidine-2-(1H)one-5-carboxylates. *Bioorg. & Med. Chem. Lett*., **2006**, 16, 4893-4897.

[13] J.M.Kane, B.M.Baron, M.W.Dudley, S.M.Sorensen, M.A. Staeger, F.P.Miller, The synthesis of 2, 4-dihydro-3H-1, 2, 4-triazol-3-ones as potent anticonvulsant agents. *J. Med. Chem*.**1990**, 33, 2772-2777.

[14] P.C.Wade, B.R.Vogt, T.P.Kissick, L.M.Simpkins, D.M.Palmer, R.C.Millonig, The synthesis of 1-acyltriazoles as anti-inflammatory agents. *J. Med. Chem*.**1982,** 25, 331-333.

[15] K.Sung, A.R.Lee, The synthesis of [(4, 5-disubstituted-4H-1, 2, 4-triazol-3-yl) thio alkanoic acids & their analogues as possible anti-inflammatory agents. *J. Heterocyclic Chem.***1992**, 29, 1101.

[16] J.M.Kane, M.W.Dudley, S.M.Sorensen, F.P.Miller, Synthesis of 2,4-dihydro-3H-1, 2, 4-triazole-3-thiones as potent antidepressant agents. *J. Med. Chem.* **1988,** 31, 1253.

[17] H.S.Joshi, S.L.Vasoya, P.T.Chovatia, D.J.Paghdar. The synthesis of some new 1, 2, 4-triazoles heterocycles bearing thiophene nucleus as a potent antitubercular & antimicrobial agents. *Indian J. Chem.* **2007,** 84, 709-710.

[18] R.Ananthnarayan, J.Paniker, Text book of microbiology. 5th Edition, Madras: Orient Longman, **1997,** 36-44.

[19] S.K. Kulkarni, Hand book of experimental Pharmacology. III ed. Vallabh Prakashan. **2005,** 131-132.