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Synthesis, characterization and biological evaluation of 3,4-dihydropyrimidin-2(*1H*)-thione derivatives

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ABSTRACT

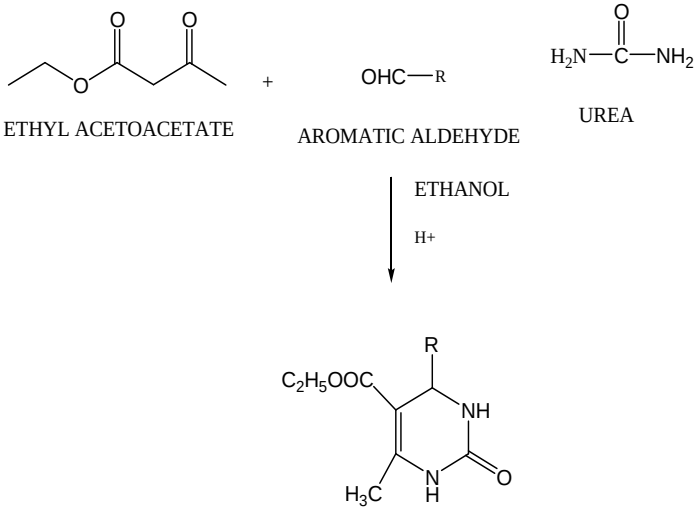
In medicinal chemistry nitrogen containing heterocycles are the most important compounds which show various biological activities. Of these Dihydropyrimidine-2(1H)-thiones are one of the heterocycles reported in 1893 for the first time by P. Biginelli and possess wide spectrum of biological properties such as antiviral, antitumour, calcium channel blocker and antibacterial activity.The present work deals with the synthesis of 3, 4 - Dihydropyrimidin-2(1H) -thione derivatives using phosphorous pentoxide as a catalyst in the reaction . All the synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR and MASS Spectroscopy. The compounds were evaluated for their antibacterial, antifungal and antioxidant activities.4a,4d and 4e had shown good response for Antimicrobial activity.4a,4b and 4d compounds possess good antioxidant activities when compared to standard.

Key words: Dihydropyrimidine-2(1H)-Thiones, phosphorous pentoxide, IR, NMR, Mass.

INTRODUCTION

The practice of medicinal chemistry is devoted to the discovery and development of new agents for treating disease. Most of the activity is directed to new natural or synthetic organic compounds (1). Heterocyclic nucleus imparts an important role in medicinal chemistry and serves as a key template for the development of various therapeutic agents. Significant number of compounds synthesized in industrial sector each year is heterocyclic in nature. In the family of heterocyclic compounds nitrogen containing heterocycles are an important class of compounds in the medicinal chemistry and also contributed to the society from biological and industrial point which helps to understand life processes (2). Pyrimidine is a six membered cyclic compound containing 4 carbon and 2 nitrogen atoms and is pharmacologically inactive but its synthetic derivatives possess an important role in modern medicine. One possible reason for their activity is presence of a pyrimidine base in thymine,cytosine and uracil, which are essential building blocks of nucleic acids, DNA and RNA (3). Moreover, pyrimidines acquired a special place in heterocyclic field because of their diversified activities such as anti-virus, anti-tumor, anti-bacterial agents (4-7) etc.

One prominent MCR that produces an interesting class of nitrogen heterocycles is the Biginelli Dihydropyrimidine synthesis. Its synthesis was first reported by Biginelli in 1893. The original Biginelli condensation (8) involving the reaction of aldehydes, urea, and ethyl acetoacetate under strongly acidic conditions to give 3,4-dihydropyrimidin-2-ones. In recent years, several methods for the synthesis of DHPM's have been developed to improve and modify this reaction by means of microwave irradiation, ultra sound irradiation (9) and ionic liquids(10).



Biginelli reaction, generates compounds with pharmacological activity, including calcium channel modulation, mitotic kinesin Eg5 inhibition, and antiviral and antibacterial activity (11). The intserest focused on Biginelli compounds leading to the development of nitractin (12), that has excellent activity against the virus of trachoma group, the same compounds also exhibit antibacterial activity. 4-Aryl dihydropyrimidines are the important and most studied class as calcium channel modulars. In 1975 their introduction in clinical medicine for the treatment of cardiovascular diseases (13), some of the analogues were screened as antitumor agents.

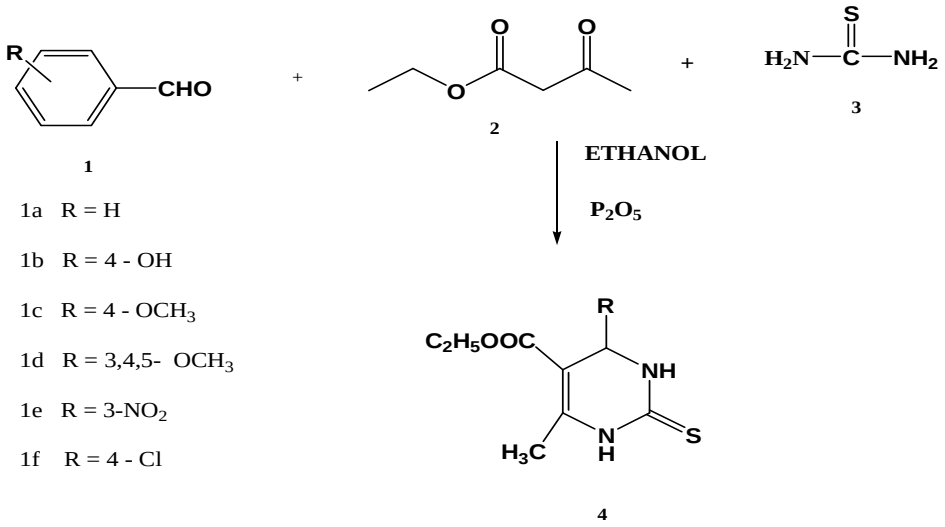
Despite the importance and current interest in dihydropyrimidines of the Biginelli type, the mechanism of the classical three-component Biginelli condensation has not been elucidated with certainty(14)**.** Different mechanisms have been proposed by Folker and Johnson (15), Sweet and Fissekis (16), Atwal and O'Reilly (17) and O.Kappe. Kappe's proposal is currently the accepted mechanism for the Biginelli reaction.

Recently, several methods have been reported for preparing dihydropyrimidines using different Lewis acids such as $BF_3.OEt_2$, $LaCl_3$, $Ca(OTf)_3$, $InCl_3$, $LiClO_4$, $ZrCl_4$, $La(OTf)_3$, $NiCl_2 \cdot 6H_2O$, LiBr, $InBr_3$, $BiCl_3$, $CaCl_2$, CAN, $FeCl_3 \cdot 6H_2O$, TMSCl/NaI and ionic liquids has been employed for this transformations. More recently microwave irradiation clays[19] and I_2 are also reported[18]. However, in spite of their potential utility, some methods suffer from drawbacks like expensive, toxic reagent, longer reaction time and low yields and involve difficult product isolation procedures. Moreover, some of the methods are only practical for aromatic aldehydes. Thus there is still a need for a simple and general procedure for synthesis of dihydropyrimidinones and thiones under simple and mild conditions. This requires the development of a new protocol for high yield and the use of inexpensive reagent.

MATERIALS AND METHODS

All the compounds were synthesized and characterized. The melting point of organic compound was determined by Thiel's melting point tube (Capillary tube method). The IR spectra of the compounds were carried out in FT-IR Bruker α-T model and only characteristics peaks were reported. The ^{1}H NMR and ^{13}C NMR of the compounds were carried out in Bruker AMX 400 MHz NMR with TMS as internal standard. The solvent used was Dueterated Dimethyl sulfoxide. The mass spectra of the compounds were carried out in Agilent 1100 series LC-MSD spectrophotometer. All the reactions were monitored over silica gel-G TLC plates and spots were visualized by iodine vapors or by irradiation with ultraviolet light (254 nm).

General procedure for synthesis of compounds 4a-4f:
The mixture of aromatic aldehyde (0.01 moles), thiourea (0.01 moles) and ethylacetoacetate (0.01moles) and phosphorous pentoxide (0.5g) was taken in a 250ml round bottomed flask and refluxed in ethanol. The progress of the reaction was continuously monitored by TLC. After completion of the reaction the reaction mixture was cooled to room temperature and the reaction mixture was poured on to crushed ice and stirred continuously. The separated solid was filtered, washed with water to remove any unreacted thiourea and recrystallised using ethanol.



1a R = H

1b R = 4 - OH

1c R = 4 - OCH_3

1d R = 3,4,5- OCH_3

1e R = 3-NO_2

1f R = 4 - Cl

RESULTS AND DISCUSSION

All the synthesized compounds were identified and characterized by IR, 1H NMR, ^{13}C NMR and mass spectral studies.Then antimicrobial and antioxidant activities were carried out. The synthesized compounds had shown a good response for both the activities. Compounds containing 3, 4, 5-trimethoxy group substitution (4d) and 3-nitro (4e) and 4a had shown a good response for antimicrobial activity. The entire compound had a good response for anti oxidant activity. Compound 4a and compounds containing 2-OH (4b) substitution and 3, 4 ,5-Trimethoxy substitution (4d) had shown a good anti oxidant activity when compared to standard.

5-Ethoxycarbonyl-4-(Phenyl)-6-methyl-3,4-dihydropyrimidin- 2(1H) – thione (4a):
IR (KBr Pellet, υ in cm^{-1}):3327.47(str, N-H),3175.45(Ar-CH), 3104.05,2981.33 (Aliphatic -CH),1670.92 (str, O-C=O),1574(str, Ar C=C), 1326.88(str, C-O), 1113.81(str, C=S),1025.98 (str, C-N); ^{1}HNMR (DMSO-d6,δ in ppm): 10.346(1H,s,1-NH),9.664(1H,s,3-NH),7.215-7.374(5H,d,Ar-H),5.182(1H,d,CH),3.989-

4.042(2H,q,OCH_2),2.297(3H,s,CH_3)1.107(3H,t,CH_3);
5-Ethoxycarbonyl-4-(4^l-hydroxyphenyl)-6-methyl-3,4-dihydropyrimidin-2(1H) -thione(4b):
IR (KBr Pellet,υ in cm^{-1}): 3502.57(str,O-H),3185.67(str,N-H),3017.20(str,ArC-H), 2601.84 (str, AliphaticC-H),1686.79(str,O-C=O),1579.87(str,ArC=C),1312.80(str,C-O), 1252.05 (str,C-N), 1199.87(str,C=S);^{1}HNMR (DMSO-d6,δinppm): 10.251(1H,s,1-NH), 9.561(1H,s,3-NH),9.426 (1H,s,OH),6.722-7.021(4H,d,Ar-H),5.0655.057 (1H,d,CH),4.0293.976(2H,q,OCH_2),2.279 (3H,s,CH_3),1.105 (3H, t,CH_3).

5-Ethoxycarbonyl-4-(4$^{'}$–methoxyphenyl)-6-methyl-3,4-dihydropyrimdin-2(1H) -thione (4c):
IR(KBrPellet,υincm^{-1}):3307.17(str,N-H),3162.30(str,ArC-H),2973.07(str,AliphaticC-H),1660.32(str,O-C=O),1569.48(str,ArC=C),1328.14(str,C-O),1255.50(str,C-N), 1111.56 (str,C=S), 1021.21(str,Ar-OCH_3)^{1}H NMR (DMSO-d6,δ in ppm): 10.299(1H,s,1-NH),9.606(1H,s,3-NH),7.144-6.91(4H,d,Ar-H), 5.114-5.122(1H,d,CH), 4.035-3.982(2H,q,OCH_2),3.729(3H,s,Ar-OCH_3), 2.291(3H,s,CH_3), 1.105(3H,t,CH_3).

5-Ethoxycarbonyl-4-(3',4$^{'}$,5'–trimethoxyphenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-thione (4d):
IR(KBrPellet,υ in cm^{-1}): 3294.38(str,N-H),3169.43(str,ArC-H),2838.25(str,Aliphatic C-H), 1657.23(str,O-C=O),1574.21(str,ArC=C),1327.33(str,C-O),1251.78(str,C-N),1119.30(str,C=S), 1036.23(str,Ar-OCH_3); ^{1}HNMR (DMSO-d6, δ in ppm):10.355(1H,s,1-NH),9.635(1H,s,3-NH),6.517(2H,d,Ar-H),5.154-5.162(1H,d,CH),4.127-4.029(2H,q,OCH_2),3.733-3.643(9H,s,Ar-OCH_3), 2.295 (3H,s, CH_3),1.136-1.171(3H,t,CH_3).

5-Ethoxycarbonyl-4-(3 ^{l}-nitrophenyl)-6-methyl-3,4- dihydropyrimidin-2(1H)-thione(4e):
IR (KBr Pellet, υ in cm^{-1}): 3179.76(str,N-H),2984.14(str,AliphaticC-H),1706.76(str,O-C=O), 1594.96(str, ArC=C),1526.11(Asymstr,N=O),1343.34Sym (str, N=O),1283.16(str,C-N), 1132.98 (str, C=S);^{1}H NMR (DMSO-d6,δ in ppm):10.156(1H,s,1-NH), 9.784(1H,s,3-NH),7.697-8.129 (4H,d,Ar-H), 5.337-5.346 (1H,d,CH),4.304-4.251(2H,q,OCH_2), 2.323(3H,s,CH_3),1.122-1.140 (3H.t,CH_3).

5-Ethoxycarbonyl-4-(4[l]–chlorophenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-thione(4f):
IR (KBr Pellet, υ in cm^{-1}): 3323.37(str,N-H),3166.57(str,ArC-H),2982.06(str, AliphaticC-H), 1666.74(str,O-C=O),1570.28(str,C=Cstr),1327.81(str,C-O), 1279.19(str,C-N), 1112.43(str,C=S), 744.07(str,C-Cl);^{1}H NMR (DMSO-d6, δ in ppm): 10.387(1H, s,1-NH),9.671(1H,s,3-NH),7.245-7.442(4H,d,Ar-H), 5.170-5.178 (1H,d,CH), 3.990-4.042(2H,q,OCH_2), 2.299(3H,s,CH_3), 1.089-1.124(3H,t,CH_3).

Tab. i : Physical properties of the compounds (4a - 4f)

Compounds	Molecular Formula	Molecular Weight (g/mole)	Melting Point (^{0}C)	R_f*
4a	$C_{14}H_{16}N_2O_2S$	276.35	206-208	0.33
4b	$C_{14}H_{16}N_2O_3S$	292.35	200-203	0.05
4c	$C_{15}H_{18}N_2O_3S$	306.38	147-149	0.22
4d	$C_{17}H_{22}N_2O_5S$	366.43	201-203	0.04
4e	$C_{14}H_{15}N_3O_4S$	321.35	195-200	0.26
4f	$C_{14}H_{15}ClN_2O_2S$	310.80	209-212	0.45

** Mobile Phase: n-Hexane: Ethyl acetate (4:1)*

Anti microbial activity
Antimicrobial activity was examined by the agar -diffusion method. The *in vitro* antimicrobial activity of the synthesized compounds was investigated against *Staphylococcus aureus* (Gram-positive), *Escherichia coli* (Gram-negative), *Aspergillus niger* and *Penicillium notatum* (fungi).Media used were nutrient agar and potato dextrose agar[20] . Non sterile powder of the tested compounds were dissolved in DMSO to yield 250µg/ml and 500µg/ml. Streptomycin and fluconazole were used as reference standards.

All bacterial were incubated overnight at 37 °C and all fungal cultures were incubated at 37° ± 2°C for 48 hrs. After the incubation period, the petri plates were observed for zone of inhibition by using vernier scale. The results evaluated by comparing the zone of inhibition shown by the derivatives with standard drug.

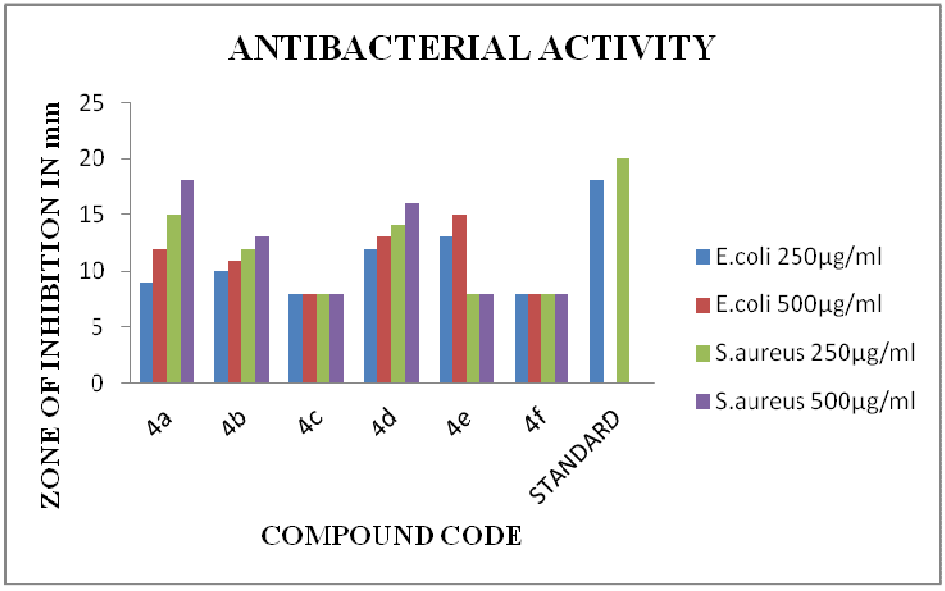


Fig. i: Antibacterial Activity of Synthesized Compounds

Tab. ii: Antibacterial Activity of Synthesized Compound

S.NO	Compound code	Zone of Inhibition (mm)			
		Gram $^{-ve}$		Gram $^{+ve}$	
		E.coli		*S.aureus*	
		250µg/ml	500 µg/ml	250 µg/ml	500 µg/ml
1	4a	9	12	15*	18*
2	4b	10	11	12	13
3	4c	8	8	8	8
4	4d	12*	13*	14*	16*
5	4e	13	15	8	8
6	4f	8	8	8	8
Control	**DMSO**	8	8	8	8
Standard	**Streptomycin**(250µg/ml)	18		20	

***** *Significant zone of Inhibition*

Tab.iii: Antifungal Activity of Synthesized Compounds

S.NO	Compound code	Zone of Inhibition (mm)			
		Aspergillus niger		*Penicillium notatum*	
		250µg/ml	500 µg/ml	250 µg/ml	500 µg/ml
1	4a	12	13	22*	24*
2	4b	10	11	12	15
3	4c	8	8	8	8
4	4d	13*	15*	18*	23*
5	4e	8	8	13	15
6	4f	8	8	8	8
Control	**DMSO**	8	8	8	8
Standard	**Fluconazole** (250µg/ml)	21		24	

***** *Significant zone of Inhibition*

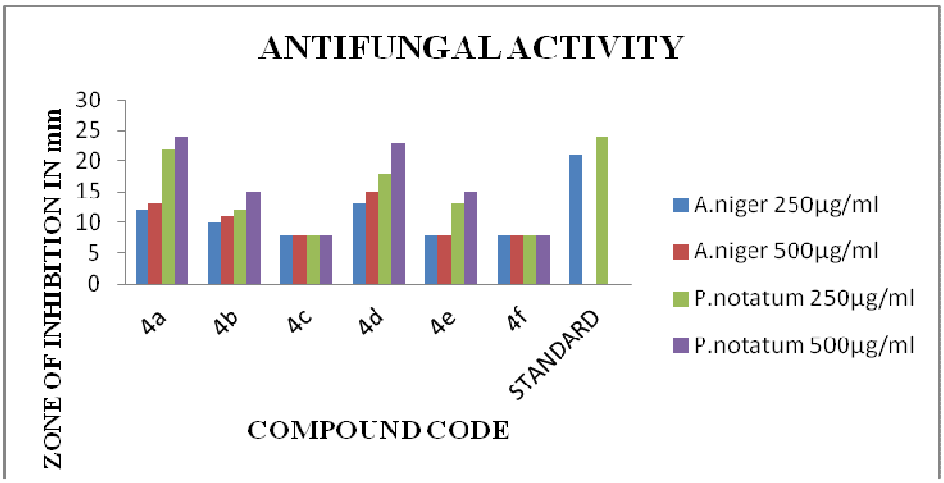


Fig. ii: Antifungal Activity of Synthesized Compounds

Anti oxidant activity

In vitro antioxidant activity of the synthesized compounds was performed using Hydrogen Peroxide Scavenging Assay Method. Ascorbic acid was used as a standard.

100µl DMSO solutions of the test compounds or standards at the concentrations of 125, 250 and 500µg/ml were separately added to 2 ml of the prepared hydrogen peroxide solution(40mM) and the absorbance was measured at 230 nm after 10 min against a blank solution. The blank solution was composed of phosphate buffer. Solution containing 100µl of DMSO and 2ml of hydrogen peroxide solution was used as a control. The hydrogen peroxide scavenging activity for compounds and standards was calculated using the following equation

H_2O_2 scavenging activity (%) = [(Ac-At) / Ac] × 100

Where
Ac = Absorbance of the control and
At = Absorbance of the tested compound or standards

Tab.iv: % Scavenging Activity of Synthesized Compounds

CODE	H_2O_2 SCAVENGING ACTIVITY (%)		
	125µg/ml	250 µg/ml	500µg/ml
4a	70.18	83.33	93.10
4b	72.41	81.81	90.02
4c	67.69	73.42	80.45
4d	67.39	73.51	84.29
4e	64.58	71.37	80.44
4f	54.86	62.34	77.89
ASCORBIC ACID	77.90	88.18	94.00

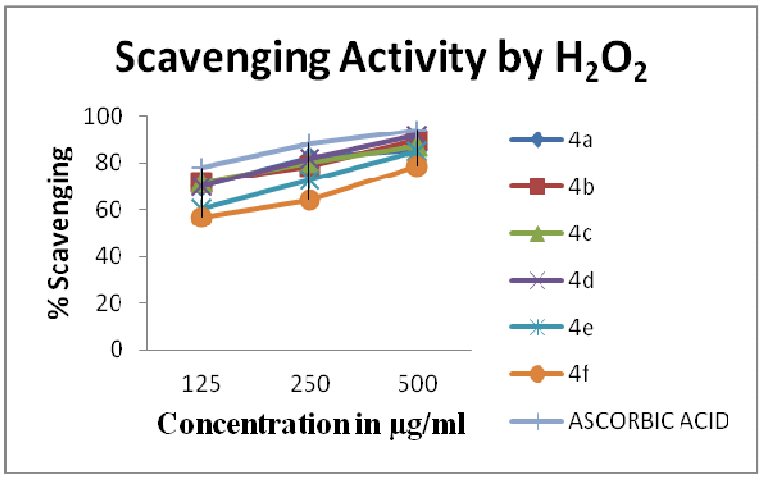


Fig. iii : % Scavenging Activity of Synthesized Compounds

CONCLUSION

All the Synthesized derivatives of 3,4-Dihydropyrimidin-2-thiones were evaluated with Physical, analytical Characterization and Biological methods.All the compounds were subjected to Antimicrobial activity i.e., Anti bacterial and Anti fungal activities.In Antibacterial activity 4d and B4 shows significant activity against both *E.coli* and *S.aureus* when compared to standard.4a and 4e are effective against *E.coli*. 4a,4b,4d showed significant activity against *A.niger* and *P.notatum*. All the compounds iwere subjected to Antioxidant activity by H_2O_2 scavenging assay.4a, 4b and 4d compounds shows significant % Scavenging compared to standard. 4b shows maximum activity of 93.10% at 500 µg/ml when compared to the standard Ascorbic acid.

REFERENCES

[1] John H.Block, John.M.Beale.Wilson and Giswold's-*Textbook of Organic Chemistry Medicinal and Pharmaceutical Chemistry*, 10th ed. Lippincott and Williams publishers, Philadelphia,**1998**, 1.
[2] L. C. C. Heda, Rashmi Sharma, C. PareekP. B. Chaudhari , *E-journal of chemistry*,**2009** ,6(3), 770-774.
[3] Calvin, M.; Jorgenson, M. *Journal of Bio-Organic Chemistry*,**1968**, 78.
[4] Singh K, *Journal of Medicinal Chemistry*,**1992** ,35, 4751-4763.
[5] Singh K, Swanson B, Moreland , *Journal of Medicinal Chemistry*,**1991**, 34, 806-811.
[6] Rovnyak G, Kimbal D, Beyer B, Hedberg D, *Journal of Medicinal Chemistry* ,**1995**, 38 ,119-129.
[7] Lanjewar K, Rahatgaonkar A, Chorghade M, Saraf , *Indian Journal of Chemistry*, **2009** 48 B , 1732-1737.
[8] M. Syamala, *Recent Progress in Three-Component Reactions-An Update*, Organic Preparations and Procedures International,**2009** ,41,2-7.
[9] Li J.T, Han J.F, Yang J.H, Li T.S, *Ultrason Sonochem*, **2003,** 10, 119.

[10] Peng J.J, Deng Y.Q, *Tetrahedron Letters*,**2001,** 42, 5917.
[11] Kappe, C. O, *Eur. J. Med. Chem*, **2000,** 35, 1043-1052.
[12] Matsuda T and Hirao J, Nippon Kagaku Zasshi, **1965** ,86, 1195-1197.
[13] Birgit Jauk, Tetiana Pernat and Kappe C O, *Molecules*,**2000** ,5, 227-239.
[14] Kappe, C. O, *Tetrahedron* ,**1993,** 49, 6937.
[15] Folkers, K.Johnson, *T. B. Journal of American Chemical Society*,**1933** ,55, 3784.
[16] Sweet, F. Fissekis, J. D , *Journal of American Chemical. Society* ,**1973,** 95, 8741-8749.
[17] Atwal, K. S., Rovnyak, G. C., O'Reilly, B. C., Schwartz, *Journal of Organic Chemistry*,**1989**, 54, 5898.
[18] Shubha Jain, *Arch. Appl. Sci. Res.*,**2012**, 4 (2), 988-993
[19] Anil Kumar Jogula, *Der Pharma Chemica*, **2011**, 3 (4), 292-297.
[20] Dr.Dushyanth "*Text Book of Pharmaceutical Microbiology*" , 6th ed. B.S Shah Prakashan Publications, Ahamedabad, India., **2004**, 106-132.