SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF PYRIMIDO [4,5-*d*] [1,3,4] THIADIAZOLO[3,2-*a*] PYRIMIDINEDIONE

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Abstract : A simple, most efficient and green procedure has been developed for the synthesis of pyrimido [4,5-*d*] [1,3,4] thiadiazolo [3,2-*a*] pyrimidinedione derivatives from one-pot three component condensation reactions of barbituric acid, substituted aromatic aldehydes, and 2-amino 5-(4-chlorophenyl)-1,3,4-thiadiazole using 1-Butyl-3-methyl Imidazolium Chloride [BMIM]Cl as a green catalyst in ethanol-water. The newly synthesized compounds evaluated by their spectral techniques and screened for their Antimicrobial activity.

Keywords: Barbituric acid, Aromatic Aldehyde, 2-amino 5-(4-chlorophenyl)-1,3,4-thiadiazole, MCRs, [BMIM]Cl.

I. INTRODUCTION

The fused heterocyclic compounds are indispensable class of organic chemistry such as pharmaceutical and biological properties [1]. In a multicomponent reaction (MCRs) three or more reactant are converted into a higher molecular weight compound in a one pot method. Today' s, MCR protocol has become very popular in the field of Chemistry. The thiadiazoles exhibit a broad range of biological properties and pharmacological properties such as antimicrobial [2], anti-inflammatory [3] and anticancer [4].

Thiadiazolo [3,2-*a*] pyrimidine dione derivatives occupies an vital role in organic chemistry and medicinal field, because of its has diverse application such as antifungal activity [5], antitumor [6], antioxidant [7], anticonvulsant[8], antihypertensive [9], analgesic[10], anti HIV activity[11], and antibiotics[12]. Thiadiazolo [3,2-*a*] pyrimidine derivatives was reported through the various catalyst like NaOH in ethanol [13] , molecular iodine[14] 2-[5-(4-methoxyphenyl) 4-*H*-1,2,4-triazole] acetic acid [15], and SBA-15 [16]. Some of the reported methods were also reported expensive catalysts, strong acidic conditions, higher temperature, require longer reaction time, resulting cumbersome product isolation procedure.

1-Butyl-3-methyl Imidazolium Chloride [BMIM]Cl , act as a phase transfer catalyst (PTC) and it perform much organic transformation under mild condition. Thus new route utilizing a MCR protocol, for the synthesis of pyrimido [4,5-*d*] [1,3,4] thiadiazolo [3,2-*a*] pyrimidinedione derivatives can attract considerable attention in the field of heterocycles. Consequently, we thought that there is scope for further innovation towards milder reaction condition, short reaction time and better yield in choosing 1-Butyl-3-methyl Imidazolium Chloride [BMIM]Cl for this multicomponent reaction (MCRs).

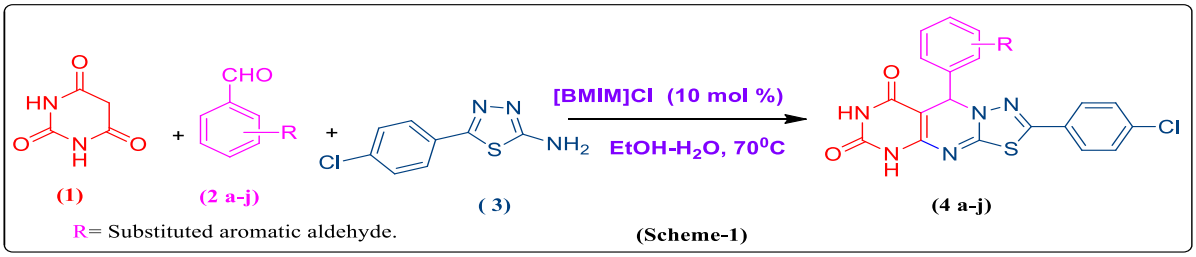
II.EXPERIMENTAL

Open capillary tubes were used for melting points of isolated synthesized compounds and are uncorrected. Perkin-Elmer FTIR spectrophotometer was used for IR (KBr) spectra of compounds. Mass spectral data were recorded on liquid chromatography mass spectrometer (Shimadzu 2010Ev) using ESI probe. The ^{1}H and ^{13}C NMR spectra were recorded on various spectrometers at 300 & 400MHz using TMS as an internal standard.

General procedure for the synthesis of 9-substituted derivatives of pyrimido [4,5-d] [1,3,4]thiadiazolo[3,2-a]pyrimidinedione(4a-j) :

A mixture of barbituric acid **(1)**, different substituted aromatic aldehydes **(2a-j)** and 2-amino-5-(4-chlorophenyl) 1,3,4-thiadiazole **(3)** was refluxed in presence of 1-Butyl-3-methyl Imidazolium Chloride [BMIM]Cl (10 mol %) in ethanol-water (10 ml ethanol and 10 ml) for 4hrs. The progress of reaction was monitored by TLC. The reaction mixture was cooled to room temperature and separated solid product was filtered, washed with water and recrystallized from ethanol to afford **(4a-j).** These synthesized products **(4a-j)** were completely characterized from spectroscopic technique and also elemental analysis.

III. RESULT AND DISCUSSION:



(Scheme-1)

Initially, we efforts were focused on optimization reaction condition. The reaction mixture of barbituric acid (**1**), 2-amino 5-(4-chlorophenyl)-1,3,4-thiadiazole (**3**) and substituted aromatic aldehydes **(2a-j)** were refluxed in aqueous ethanol using 1-Butyl-3-methyl Imidazolium Chloride [BMIM]Cl (10 mol%) was considered as a model reaction (**Scheme1**). Then further investigating the effectiveness of different polar and non polar solvent using 1-Butyl-3-methyl Imidazolium Chloride [BMIM]Cl (10 mol%). In solvent optimization observed that that ethanol-water is the best solvent for the desired transformation and give

high yield (Table1, entry 6) but other polar protic solvents gives moderate yield (Table1, entry 5). On other side aprotic solvent such as toluene, DCM and DMF exhibit slow reaction rates leading lower yield (Table1, entry 1-3).

We have performed the model reaction using different stoichiometric amount of catalyst. The catalyst screening result are summarized in (Table 2). It was observed that the excellent yield was gained by using 1-Butyl-3-methyl Imidazolium Chloride [BMIM]Cl (Table 2, entry 5). The use of aqueous ethanol as solvent in reaction protocol exhibit remarkable benefits like environmentally safe, comparatively cheaper to operate and easy work up.

Analyzed the influence of different parameters on the model reaction, we revolved our focus towards the 9-substituted derivatives of pyrimido [4,5-*d*] [1,3,4] thiadiazolo [3,2-*a*] pyrimidinedione **(4a-j)** using one pot three component reaction of barbituric acid **(1),** different substituted aldehydes **(2a-j)**, and 5-(4-chlorophenyl)-1,3,4-thiadiazole **(3)** were refluxed in the presence of 1-Butyl-3-methyl Imidazolium Chloride [BMIM]Cl (10 mol%) in aqueous ethanol **(Scheme 1)**, the result are summarized in Table **3**. The desired products (**4a-j**) were obtained to excellent yields. These synthesized products **(4a-j)** were completely characterized from IR, ^{1}H-NMR, Mass and ^{13}C-NMR spectroscopic technique and also elemental analysis.

Table 1. Optimization of the reaction conditions using different solvents.[a]

Entry	Solvent	Reaction Time (h)	Yield (%)[b]
1	Toluene	7.0	35
2	DCM	6.5	42
3	DMF	6.0	48
4	Water	6.0	56
5	Ethanol	5.5	66
6	Ethanol-Water	4.0	85

[a] ***Reaction conditions:*** Barbituric acid (1 mmol), 2-amino-5-(4-chlorophenyl)1,3,4-thiadiazole (1 mmol), with substituted benzaldehydes (1 mmol) in EtOH-H_2O and 1-Butyl-3-methyl Imidazolium Chloride [BMIM]Cl were refluxed at 70 ^{0}C. [b] Isolated yields.

Table 2: Optimization Study for the amount of 1-Butyl-3-methyl Imidazolium Chloride.[a]

Entry	Catalyst (mole %)	Temperature (^{0}C)	Reaction Time (h)	Yield %[b]
1	01	70	3.0	36
2	02	70	3.0	46
3	05	70	3.0	66
4	08	70	3.0	78
5	10	70	3.0	85
6	15	70	3.0	85
7	20	70	3.0	85

[a] ***Reaction conditions:*** Barbituric acid (1 mmol), 2-amino-5-(4-chlorophenyl)1,3,4-thiadiazole (1 mmol), with substituted benzaldehydes (1 mmol) in EtOH-H_2O and 1-Butyl-3-methyl Imidazolium Chloride [BMIM]Cl were refluxed at 70 ^{0}C. [b] Isolated yields.

Table 3: Three component reaction of barbituric acid **(1),** aromatic aldehydes **(2a-j)** and 2-amino-5-(4-chlorophenyl)1,3,4-thiadiazole **(3),** for the synthesis of **(4a-4j).**[a]

Entry	Aldehydes	Time (Hrs)	Yield (%)[b]	Mp (^{0}C)
4a	$-C_6H_5$	3.5	61	205-207
4b	4’ $-OCH_3$ $-C_6H_4$	3.0	85	209-211
4c	4’ $-CH_3$ $-C_6H_4$	3.5	75	239-241
4d	4’ -Br $-C_6H_4$	3.5	70	269-271
4e	3’ - Br $-C_6H_4$	3.0	78	221-223
4f	4’ -Cl $-C_6H_4$	3.5	72	277-279
4g	4’ , 3’ -di Cl $-C_6H_3$	2.5	76	231-233
4h	4’ -OH $-C_6H_4$	3.0	78	239-241
4i	4’ -F $-C_6H_4$	3.5	64	244-246
4j	4’ $-NO_2$ $-C_6H_4$	4.0	62	285-287

[a] ***Reaction conditions:*** Barbituric acid (1 mmol), 2-amino-5-(4-chlorophenyl)1,3,4-thiadiazole (1 mmol), with substituted benzaldehydes (1 mmol) in EtOH-H_2O and 1-Butyl-3-methyl Imidazolium Chloride [BMIM]Cl were refluxed at 70 ^{0}C. [b] Isolated yields.

SPECTRAL ANALYSIS:

2-(4-chlorophenyl)-9-phenyl-5*H*-pyrimido[4,5-*d*][1,3,4]thiadiazolo[3,2-*a*]pyrimidine-6,8 (7*H*,9*H*)-dione(4a) :

M.P. 205-207 ^{0}C, Yield 61%. IR (KBr/ cm^{-1}) 3240 (-NH), 1720,1635 (2 C=O); ^{1}H NMR (400MHz, DMSO-d_6 / ppm) δ 6.6 (s, 1H, -CH), 6.5-8.2 (m, 09 H, Ar-H), 10.4 and 11.5 (2 bs,2H,-NH); EI-MS (m/z: RA %): 409 ($M^{+\cdot}$, 100%), 409 ($M^{+\cdot}$, 37%). Elemental analysis calculated data for $C_{19}H_{12}ClN_5O_2S$; C, 55.68; N, 17.09. Found: C, 5570; N, 17.10.

2-(4-chlorophenyl)-9-(4-methoxyphenyl)-5*H*-pyrimido[4,5-*d*][1,3,4]thiadiazolo[3,2-*a*] pyrimidine-6,8(7*H*,9*H*)-dione(4b) : M.P. 209-211 ^{0}C , Yield 85 %. IR (KBr/ cm^{-1}) 3220 (-NH), 1722, 1680 (2 C=O),1268 (-O-R); ^{1}H NMR (400MHz, DMSO-d$_6$ / ppm) δ 3.1 (s, 3H, -Ar-OCH$_3$), 6.4 (s, 1H, -CH), δ 7.0-8.5 (m, 8H, Ar-H), 11.0 and 11.2 (2 bs, 2H,-NH); EI-MS (m/z: RA %): 439 (M$^{+\cdot}$, 100%). ^{13}C NMR (400 MHz, DMSO–d$_6$/ ppm) δ: 162, 160, 150, 148, 142, 129, 125, 90 ,52, 50, 40, 30. Elemental analysis calculated data for $C_{20}H_{14}ClN_5O_3S$; C, 54.61; N, 15.52. Found: C, 54.62; N, 15.94.

2-(4-chlorophenyl)-9-(4-methylphenyl)-5*H*-pyrimido[4,5-*d*][1,3,4]thiadiazolo[3,2-*a*] pyrimidine-6,8 (7*H*,9*H*)-dione (4c):M.P. 239-241 ^{0}C, Yield 75 %. IR (KBr/ cm^{-1}) 3210 (-NH), 1715, 1665 (2 C=O) ; ^{1}H NMR (400MHz, DMSO-d$_6$ / ppm) δ 2.30 (s, 3H, -Ar-CH$_3$), 6.40 (s, 1H, -CH), 7.5-8.9 (m, 8H, Ar-H), 11.4 and 11.8 (2 bs, 2H,-NH); EI-MS (m/z: RA %): 423 (M$^{+\cdot}$, 100%). ^{13}C NMR (400 MHz, DMSO–d$_6$/ ppm) δ: 165, 164, 150, 145, 140, 130, 126, 92 ,50, 48, 40, 30. Elemental analysis calculated data for $C_{20}H_{14}ClN_5O_2S$; C, 56.67; N, 16.52. Found: C, 56.69; N, 16.94.

9-(4-bromophenyl)-2-(4-chlorophenyl)-5*H*-pyrimido[4,5-*d*][1,3,4]thiadiazolo[3,2-*a*] pyrimidine-6,8(7*H*,9*H*)-dione (4d) : M.P. 269-271 ^{0}C, Yield 70%. IR (KBr/ cm^{-1}) 3135 (-NH), 1690,1640 (2C=O); ^{1}H NMR (400MHz, DMSO-d$_6$ / ppm) δ 5.6 (s, 1H, -CH), 7.1-8.9 (m, 8H, Ar-H), 10.3 and 11.5 (2 bs, 2H,-NH); EI-MS (m/z: RA %): 486, (M$^{+\cdot}$+3, 100) ; ^{13}C NMR (400 MHz, DMSO–d$_6$/ppm) δ: 164, 162, 160, 150, 145, 130, 126, 125, 90 ,60, 55, 40,35. Elemental analysis calculated data for $C_{19}H_{11}BrClN_5O_2S$; C, 46.69; N, 14.33. Found: C, 46.72; N, 14.35.

9-(3-bromophenyl)-2-(4-chlorophenyl)-5*H*-pyrimido[4,5-*d*][1,3,4]thiadiazolo[3,2-*a*] pyrimidine-6,8(7*H*,9*H*)-dione (4e) :M.P. 221-223 ^{0}C, Yield 78%. IR (KBr/ cm^{-1}) 3140 (-NH), 1680,1625 (2C=O); ^{1}H NMR (400MHz, DMSO-d$_6$ / ppm) δ 5.6 (s, 1H, -CH), 7.3-8.6(m, 8H, Ar-H), 10.8 and 11.6 (2 bs, 2H,-NH); EI-MS (m/z: RA %): 486 (M$^{+\cdot}$+3, 100); ^{13}C NMR (400 MHz, DMSO–d$_6$/ppm) δ: 168, 166, 164, 162,154, 152, 150, 145, 130, 129,125, 121, 120, 94 ,40, 30. Elemental analysis calculated data for $C_{19}H_{11}BrClN_5O_2S$; C, 46.69; N, 14.33. Found: C, 46.71; N, 14.34.

2,9-bis(4-chlorophenyl)-5*H*-pyrimido[4,5-*d*][1,3,4]thiadiazolo[3,2-*a*]pyrimidine-6,8(7*H*,9*H*)-dione (4f) : M.P. 277-279 ^{0}C, Yield 72% .IR (KBr/ cm^{-1}) 3265(-NH) 1725, 1670 (2C=O); ^{1}H NMR (400MHz, DMSO-d$_6$ / ppm) δ 5.7 (s, 1H, -CH), 7.2-8.6 (m, 8H, Ar-H), 10.7 and 11.4 (2 bs, 2H,-NH); EI-MS (m/z: RA %): 443 (M$^{+\cdot}$, 100%); ^{13}C NMR (400 MHz, DMSO–d$_6$/ ppm) δ: 170,165,164, 150,140, 135, 130, 125,117,114, 90 ,56, 45, 30.Elemental analysis calculated data for $C_{19}H_{11}Cl_2N_5O_2S$; C, 51.36; N, 15.76. Found: C, 51.38; N, 15.78.

2-(4-chlorophenyl)-9-(2,4-dichlorophenyl)-5*H*-pyrimido[4,5-*d*][1,3,4]thiadiazolo[3,2-*a*] pyrimidine-6,8(7*H*,9*H*)-dione (4g) : M.P. 231-233 ^{0}C, Yield 76%.IR (KBr/cm^{-1}) 3150 (-NH), 1680,1630 (2C=O); ^{1}H NMR (400MHz, DMSO-d$_6$ / ppm) δ 5.9 (s, 1H, -CH), 7.2-8.7 (m, 7H, Ar-H),10.4 and 11.4 (2 bs, 2H,-NH); EI-MS (m/z: RA %): 476 (M$^{+\cdot}$ +1, 100%); ^{13}C NMR (400 MHz, DMSO–d$_6$/ppm) δ: 167, 164 162,155, 140, 130, 125,122, 125, 85,45,30. Elemental analysis calculated data for $C_{19}H_{10}Cl_3N_5O_2S$; C, 47.67; N, 14.63. Found: C, 47.69; N, 14.65.

2-(4-chlorophenyl)-9-(4-hydroxyphenyl)-5*H*-pyrimido[4,5-*d*][1,3,4]thiadiazolo[3,2-*a*] pyrimidine-6,8(7*H*,9*H*)-dione(4h) M.P. 238-240 ^{0}C , Yield 78 %. IR (KBr/ cm^{-1}) 3513(-OH), 3235 (-NH), 1740, 1670 (2 C=O) ; ^{1}H NMR (400MHz, DMSO-d$_6$ / ppm) δ 4.3 (s, 1H, -Ar-OH), 6.1 (s, 1H, -CH), 7.4-8.9 (m, 8H, Ar-H), 11.4 and 11.6 (2 bs, 2H,-NH); EI-MS (m/z: RA %): 425 (M$^{+\cdot}$, 100%). ^{13}C NMR (400 MHz, DMSO–d$_6$/ ppm) δ: 170, 166,152, 140, 136,125,55, 45, 35, 30. Elemental analysis calculated data for $C_{19}H_{12}ClN_5O_3S$; C, 53.59; N, 16.45. Found: C, 53.61; N, 16.46.

2-(4-chlorophenyl)-9-(4-fluorophenyl)-5*H*-pyrimido[4,5-*d*][1,3,4]thiadiazolo[3,2-*a*] pyrimidine-6,8(7*H*,9*H*)-dione(4i): M.P. 244-246 ^{0}C, Yield 64%. IR (KBr/ cm^{-1}) 3145 (-NH), 1660,1640 (2C=O); ^{1}H NMR (400MHz, DMSO-d$_6$ / ppm) δ 5.5 (s, 1H, -CH), 7.2-8.8 (m, 8H, Ar-H), 10.4 and 11.5 (2 bs, 2H,-NH); EI-MS (m/z: RA %): 427, (M$^{+\cdot}$+3, 100); ^{13}C NMR (400 MHz, DMSO–d$_6$/ppm) δ: 168, 165, 162, 155, 142, 130, 120, 90 ,50, 45,32. Elemental analysis calculated data for $C_{19}H_{11}ClFN_5O_2S$; C, 53.34; N, 16.37. Found: C, 53.36; N, 16.39.

2-(4-chlorophenyl)-9-(4-nitrophenyl)-5*H*-pyrimido[4,5-*d*][1,3,4]thiadiazolo[3,2-*a*] pyrimidine-6,8(7*H*,9*H*)-dione(4j): M.P. 285-287 ^{0}C, Yield 62%. IR (KBr/ cm^{-1}) 3250 (-NH), 1670,1620 (2C=O); ^{1}H NMR (400MHz, DMSO-d$_6$ / ppm) δ 5.8 (s, 1H, -CH), 7.4-8.9 (m, 8H, Ar-H), 10.4 and 11.6 (2 bs, 2H,-NH); EI-MS (m/z: RA %): 454, (M$^{+\cdot}$+3, 100); ^{13}C NMR (400 MHz, DMSO–d$_6$/ppm) δ: 170, 166, 164, 162, 158, 150, 140, 125, 92 ,52, 45,25. Elemental analysis calculated data for $C_{19}H_{11}ClN_6O_4S$; C, 50.17; N, 18.48. Found: C, 50.19; N, 18.50.

ANTIMICROBIAL ACTIVITY:

We have used the Agar well diffusion method for assessment of the antimicrobial activity of newly synthesized compounds. On Muller-Hinton agar medium zone of inhibition were observed and zone diameter was recorded in mm against specific test microorganisms.

The synthesized compounds were accessed antimicrobial activity particularly antibacterial. The antibacterial activity against gram positive species *S. aureus* and *B.subtilis* and gram negative species *E.coli* and S.*typhi* using standard drugs are Penicillin and Streptomycin.

The synthesized compounds **4d, 4e, 4f and 4j** show good antibacterial activity against *S. aureus* as compaired to standard drugs Penicillin and Streptomycin. The compounds **4d, 4e and 4j** show good antibacterial activity against *B.subtilis* as compaired to standard drugs Penicillin and Streptomycin. The compounds **4d and 4j** show good antibacterial activity against *E.coli* as compaired to standard drugs Penicillin and Streptomycin. The synthesized compounds **4d, 4f and 4j** show good zone of inhibition against *B.subtilis* as compaired to Penicillin and Streptomycin.

Table 4 : Antimicrobial activity of tested compounds (4a-j).

Entry	Compounds	Zone of Inhibition in mm			
		S.aureus	***B.subtilis***	***E. coli***	***S.typhi***
1	**4a**	12	10	12	12
2	**4b**	12	ND	10	ND
3	**4c**	ND	12	10	10
4	**4d**	18	14	16	16
5	**4e**	20	16	ND	14
6	**4f**	16	ND	14	16
7	**4g**	ND	10	12	10
8	**4h**	12	ND	12	ND
9	**4i**	12	14	10	12
10	**4j**	18	18	14	18
11	**Penicillin**	26	20	18	18
12	**Streptomycin**	30	26	34	26

ND = Not Detected zone of inhibition under experimental condition.

IV.CONCLUSION:

In conclusion, We have developed a green, efficient and eco-friendly synthesis for the preparation of pyrimido [4,5-*d*] [1,3,4] thiadiazolo [3,2-*a*] pyrimidinedione **(4a-j)** derivatives by one-pot three component condensation reactions of barbituric acid, substituted aromatic aldehyde, and 2-amino-5-(4-chlorophenyl) 1,3,4-thiadiazole in presence of 1-Butyl-3-methyl Imidazolium Chloride [BMIM]Cl in aqueous ethanol medium. The product can be easily isolated by simple workup technique, short time, less expensive, ambient reaction condition, and give excellent isolated yields. These synthesized compounds screened Antimicrobial activity.

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REFERENCES:

1. M. Jha, S. Guy, U.C. Ting, Microwave assisted synthesis of indole-annulated dihydropyrano[3,4-c]chromene derivatives via hetero-Diels– Alder reactionTetrahedron Letters., **2011**,52,4337-4371.
2. K .Zamani, K. Faghli, M.S. Mehranjani, Synthesis of some new 2,5-disubstituted 1,3,4-thiadiazoles containing isomeric pyridyl as potent antimicrobial agents, Polish J. Pharm. **2003,** 55, 1111.
3. A. Faroumadi, M. Mirzaei, A. Shafiee, Reaction of thiocarboxanilide derivatives of 2-phenylimino-3-phenyl-4-thiazolidinone and 1,3-diphenyl-2-thioxo-4-imidazolone with hydrazonoyl halides and active chloromethylene compounds, Pharmazie. **2001,** 56, 610.
4. G. Sorba, A. Stilo, A.M. Gasco, M. Gill, 2-substituted 1H-pyrrolo [3,2-h] quinoline derivatives: synthesis and aspects of their biological activity, Farmaco., **1992** , 47(12):1513-28.
5. Q. Chen, X.L. Zhu, Z.M. Liu, G.F. Yang, Synthesis, antifungal activity and CoMFA analysis of novel 1, 2, 4-triazolo [1, 5-a] pyrimidine derivatives. Eur. J. Med. Chem, 2008, 43(3):595-603.
6. X.L. Zhao, Y.F. Zhao, S.C. Guo, H.S. Song, D. Wang, P. Gong, Synthesis and Anti-tumor Activities of Novel [1,2,4]triazolo[1,5-a]pyrimidines, Molecules, **2007**,12,1136-1146.
7. V.N. Bhosale, G.S. Khansole, J.A. Angulwar, In Vitro Antioxidant activity of 9-substituted derivatives of 9-phenyl-5,9-dihydropyrimido[4,5-d][1,2,4] triazolo[1,5-a] pyrimidine-6,8(1H,7H)-dione, World J. Pharmacy & Pharmaceutical Sciences., **2016**,5(5),1434-1441.
8. X.Yu. Dun, Xi-Wei Cheng, X.Q.Deng, Evaluation of the anticonvulsant activity of 6-(4-chlorophenyoxy)-tetrazolo[5,1-a]phthalazine in various experimental seizure models in mice Pharmacological reports, **2010**, 62, 273-277.
9. S. Sharma, M.C. Sharma, D.V. Kolhi, Wound healing activity and formulation of ether-benzene-95% ethanol extract of herbal drug Madhuca longifolia leaves in albino rats, Journal of Optoelectronics and Biomedical Material, **2010**; 1(3),151-160.
10. [10] M.J. Hour, L.J. Huang, S.C. Kuo, Y. Xia, K. Bastow, Y. Nakanishi, E. Hamel, K.H.Lee, 6-Alkylamino- and 2,3-dihydro-3'-methoxy-2-phenyl-4-quinazolinones and related compounds: their synthesis, cytotoxicity, and inhibition of tubulin polymerization.
11. J. Med. Chem., **2000**, 43 (23), 4479– 4487.
12. V. Alagarsamy, R. Revathi, S. Meena, K.V. Ramasheshu, S. Rajeshekarn, De E. Clercq, Anti HIV, antibacterial and antifungal activities of some 2, 3-disubstituted quinazolin-4 (3H)-ones, Indian Journal of Pharmaceutical Sciences, **2004**, 459-462.
13. M. Cei , R. Pardelli , S. Sani , N., Local resistance patterns to antimicrobials in internal medicine: a focused report from the REGIMEN (REGistro Infezioni in MEdicina INterna) study, Clin.Exp.Med., **2014**, 77-82.
14. S.V. Tiwari, J.A. Seijas, M.P. Vazquez, A.P. Sarkate, D.K. Lokwani, A.G Nikalje, Ultrasound Mediated One-Pot, Three Component Synthesis, Docking and ADME Prediction of Novel 5-Amino-2-(4-chlorophenyl)-7-Substituted Phenyl-8,8a-dihydro-7H-(1,3,4)thiadiazolo(3,2-α)pyrimidine-6-carbonitrile Derivatives as Anticancer Agents, Molecules, **2016**, 21,894..

15. V.N. Bhosale, S.S. Choudhare, G.S.Khansole, N.B.Wadwale, Molecular Iodine Catalyzed Synthesis of 9-Substituted Derivatives of 9-Phenyl-2,9-Diphenyl-5,9-Dihydro-6 H-Pyrimido[4,5-d] [1,3,4]Thiadiazolo[3,2-a] Pyrimidine-6,8(7H)-dione, Der Pharma Chemica, **2017**, 9(5): 66-70
16. S. SAHI, S. PAUL, SYNTHESIS AND BIOLOGICAL EVALUATION OF QUINOLINES, THIAZOLO[3,2-***A***] PYRIMIDINES, THIADIAZOLO[3,2-***A***]PYRIMIDINES AND TRIAZOLO[3,4-***B***][1,3,4]THIADIAZEPINES AS ANTIMICROBIAL AGENTS,MEDICINAL CHEMISTRY RESEARCH, 2016, 25 (5), 951-969.
17. R.G. PULIGOUNDLA, S. KARNAKANTI, R. BANTU, N. KOMMU, S.B. KONDA, L. NAGARAPU, A SIMPLE, CONVENIENT ONE-POT SYNTHESIS OF 1, 2, 4-TRIAZOLO [5,1-B] QUINAZOLIN-8(4H)-ONEAND DERIVATIVES BY USING MOLECULAR IODINE, TETRAHEDRON, **2013**,54,2480-2483.