EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 7
714-720
Year: 2017

SYNTHESIS, CHARACTERIZATION AND CYTOTOXIC EVALUATION OF 4-[4-(1*H*-IMIDAZOL-1-YL) PHENYL]-6-PHENYL-1,6-DIHYDROPYRIMIDIN-2-OL DERIVATIVES BY MTT ASSAY METHOD

Gondu Eswara Rao[1,2]*, S. A. Rahaman[3] and A. Prameela Rani[4]

[1]Research scholar, School of Pharmaceutical Sciences, JNTUK, Kakinada- 533003.
[2]Assistant Professor, Vignan Pharmacy College, Vadlamudi post, Chebrolu Mandal, Guntur District. AP. 522213. India.
[3]Principal and Professor, Nirmala College of Pharmacy, Atmakur Village, Mangalagiri Mandal, Guntur Dt, A.P-522503.
[4]Principal and Professor, ANU College of Pharmaceutical Science, Nagarjuna Nagar, ANU, Guntur-530003, (A.P), India.

***Corresponding Author: Gondu Eswara Rao**
Research scholar, School of Pharmaceutical Sciences, JNTUK, Kakinada- 533003.

Article Received on 18/05/2017 **Article Revised on 07/06/2017** **Article Accepted on 28/06/2017**

ABSTRACT

The pyrimidine derivatives were synthesized from chalcone as intermediate compound .the chalcones were reacted with urea in presence of glacial acetic acid and sodium acetate to form cyclic compound pyrimidine-2-ol derivatives and the structures were confirmed by spectral evidence. The compounds were tested for cytotoxic evolution by MTT assay method. In these test compounds, The compound PY-02, (4-[4-(1H-imidazol-1-yl)phenyl]-6-[4-(methylsulfanyl)phenyl]-1,6-dihydropyrimidin-2-ol) 15±2 µg/ml,18±1 µg/ml,12±1 µg/ml against the Breast cancer (MDA MB, Colon cancer (HT-29), Prostate cancer(DU-145).

KEYWORDS: Pyrimidine-2- ol derivatives, urea, cytotoxicity, MTT assay method.

INTRODUCTION

Heterocyclic compounds are abundant in nature and are of great significance to life because their structural subunits exist in many natural products such as vitamins, hormones, and antibiotics.[1,2] Hence, they have attracted considerable attention in the design of biologically active molecules[3,4] and advanced organic chemistry.[5,6] Also in the family of heterocyclic compounds nitrogen containing Heterocyclic compounds are an important in the medicinal chemistry and also contributed to the society from biological and industrial point which helps to understand life processes.[7] However, the current review intends to focus on the significance of Pyrimidines class of antimicrobial agents along with clinical andin vitro applications of pyrimidine derivatives to facilitate the development of more potent as well as effective antimicrobial agents.

Pyrimidines[10] are the heterocyclic aromatic compounds similar to benzene and pyridine containing two nitrogen atoms at positions 1 and 3 of the six membered rings. Heterocycles containing pyrimidine moiety are of great interest because they constitute an important class of natural and non natural products, many of which exhibit useful biological activities and clinical applications.[11,12] Substituted purines and pyrimidines occur very widely in living organisms and were some of the first compounds studied by the organic chemists.[13] Pyrimidines are biologically very important Heterocycles and represent by far the most important of the di azine family with uracil[14] and thymine[15] being constituents of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) and with cytosine.[16] In addition to this, pyrimidines skeleton is also present in many natural products such as vitamin B1 (thiamine) and many synthetic compounds, such as barbituric acid[17] and Veranal[18] which are used as hypnotics.[19] The Pyrimidines represent one of the most active classes of compounds possessing wide spectrum of biological activities like significantin vitroactivity against unrelated DNA and RNA, viruses including polioherpes viruses, diuretic, antitumour, anti-HIV, and cardiovascular.[20] The literature survey indicated that a wide range of pharmacological activities are exhibited by the compounds encompassing pyrimidines nucleus. In addition to this, various analogs of pyrimidines have been found to posses antibacterial,[21] antifungal,[22] antileishmanial,[23] anti-inflammatory,[24] analgesic,[25] antihypertensive,[26] antipyretic,[27] antiviral,[28] antidiabetic,[29] antiallerggic,[30] anticonvulsant,[31] antioxidant,[32] antihistaminic,[33] herbicidal,[34] and

anticancer activities[35] and many of Pyrimidines derivatives are reported to possess potential central nervous system (CNS) depressant properties[36] and also act as calcium channel blockers.[36]

MATERIALS AND METHODS

Experimental work

4-(1*H*-imidazol-1-yl Acetophenone, Urea, sodium acetate, glacial acetic acid conc. HCl , DMSO, DPPH reagent .all the reagents were purchased analytical grade. Melting points were determined on a capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded in the indicated solvent on Bruker WM 400 MHz spectrometer with TMS as internal standard. Infrared spectra were recorded in KBr on Perkin-Elmer AC-1 spectrophotometer. Column chromatography was performed on silica gel (Merck, 60-120 mesh).

General method of preparation:[4]

A mixture of (2*E*)-1-[4-(1*H*-imidazol-1-yl)phenyl]-3-phenylprop-2-en-1-one (0.001moles) and urea (0.001moles) were dissolved in sodium acetate in glacial acetic acid (20ml) reflux it for 6hr.afetr that add the solution to the cooling water. The mixture was kept for 24hours and it was acidified with 1:1 HCl and water, then it was filtered through vacuum by washing with water.

Chemical reaction

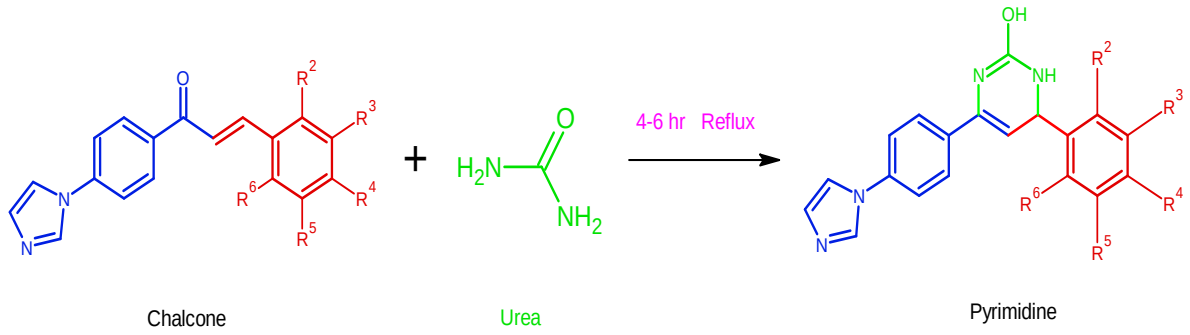
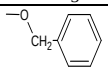


Table 1: List of aldehydes.

Chalcone	Radicals				
	R_2	R_3	R_4	R_5	R_6
PR-01	-O-CH_3	-H	-O-CH_3	-H	-O-CH_3
PR-02	-H	-O-CH_3	-O-CH_3	-O-CH_3	-H
PR-03	-H	-H	-S-CH_3	-H	-H
PR-04	-H	-H	-CF_3	-H	-H
PR-05	-H	-H		-H	-H
PR-06	-CF_3	-H	-H	-H	-H
PR-07	-Cl	-H	-H	-H	-F
PR-08	-H	-H	-C_2H_5	-H	-H
PR-09	-H	-H	-Cl	-H	-H
PR-10	-H	-H	-O-CH_3	-H	-H

Biological evolution of compounds

Based on the literature, chalcones were reported to possess antimicrobial activity, anti oxidant, anti inflammatory, analgesic, anti cancerous, etc. Therefore the present work performs the anti cancerous activities.

Cytotoxicity studies[37]

The *in vitro* cyto toxicity of the test compounds (PY_1-PY_{10}) were performed based on MTT assay method on Breast cancer (MDA MB, Colon cancer (HT-29), Prostate cancer(DU-145). The cell lines were obtained from National Centre for Cell Science (NCCS), Pune, India. Methotrexate was used as reference drug for comparison. Assay was performed in triplicate for three independent determinations. The cytotoxicity was expressed as IC_{50} (µg/mL) which is the concentration of the compound that inhibited proliferation rate of the tumour cells by 50% as compared to the control untreated cells. IC_{50} values were determined from the plot: % inhibition versus concentration.

$$\% \text{ inhibition at the given concentration} = \frac{1 - (\text{Absorbance average})}{(\text{Control absorbance average})} \times 100$$

IC_{50}=Inv.log(50-c) / m; c and m derived from y=mx+c of plot of % inhibition Vs log C.
The results were tabulated.

Table 2: Physical Data of Synthesised Compounds.

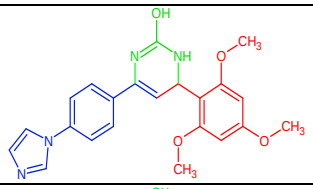
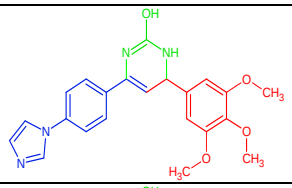
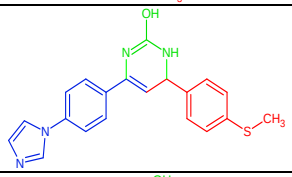
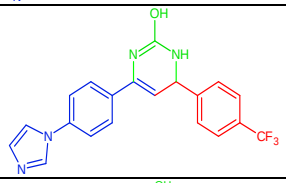
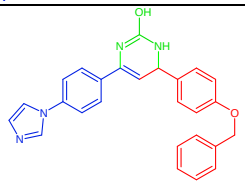
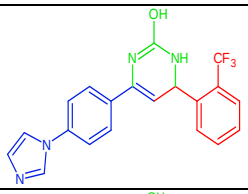
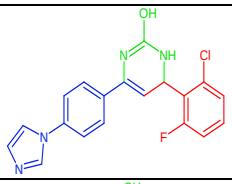
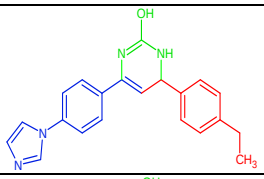
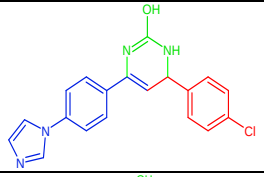
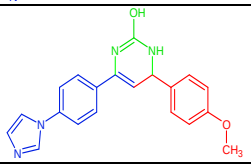
Compound Code	Compound	Molecular Formula	Mol. Wt	M.P (^{O}C)	% Yield
PR 01		$C_{22}H_{22}N_4O_4$	406.4	186-188	85
PR 02		$C_{22}H_{22}N_4O_4$	406.4	184-185	84
PR 03		$C_{20}H_{18}N_4OS$	362.4	178-179	81
PR 04		$C_{20}H_{15}F_3N_4O$	384.3	175-176	79
PR 05		$C_{26}H_{22}N_4O_2$	422.4	172-173	78
PR 06		$C_{20}H_{15}F_3N_4O$	384.3	165-167	75
PR 07		$C_{19}H_{14}ClFN_4O$	368.7	155-156	69
PR 08		$C_{21}H_{20}N_4O$	344.4	164-165	84
PR 09		$C_{19}H_{15}ClN_4O$	350.8	166-168	84
PR 10		$C_{20}H_{18}N_4O_2$	346.3	184-185	88

Table 3: Elemental Compositions.

Compound		C	H	N	O	S	Cl	F
PR 01	%Calculated	65.01	5.46	13.78	15.75	-	-	-
	%Found	65.33	5.50	13.70	15.72	-	-	-
PR 02	%Calculated	65.01	5.46	13.78	15.75	-	-	-
	%Found	65.20	5.48	13.70	15.73	-	-	
PR 03	%Calculated	66.28	5.01	15.46	4.41	8.85	-	-
	%Found	66.26	5.30	15.48	4.50	8.87	-	-
PR 04	%Calculated	62.50	3.93	14.58	4.16	-	-	14.83
	%Found	62.54	3.90	14.60	4.14	-	-	14.84
PR 05	%Calculated	73.92	5.25	13.26	7.57	-	-	-
	%Found	73.90	5.27	13.28	7.56	-	-	-
PR 06	%Calculated	62.50	3.93	14.58	4.16	-	-	14.83
	%Found	62.51	3.91	14.60	4.19	-	-	14.85
PR 07	%Calculated	61.88	3.83	15.19	4.34	-	9.61	5.15
	%Found	61.83	3.79	15.21	4.36	-	6.69	5.16
PR O8	%Calculated	73.23	5.85	16.27	4.65	-	-	-
	%Found	73.21	5.88	16.26	4.66	-	-	-
PR O9	%Calculated	65.05	4.31	15.97	4.56	-	10.11	-
	%Found	65.08	4.29	15.99	4.53	-	10.14	-
PR 1O	%Calculated	69.35	5.24	16.17	9.24	-	-	-
	%Found	69.36	5.20	16.20	9.27	-	-	-

Table 4: Spectral data of compounds.

Compound	IR, NMR data
PR-01	C=O,str. – 1660.76cm^{-1} ;C=C, str. – 1602.33cm^{-1}, N-H stretching :3365.01 cm^{-1} , C-H stretching: 3105 cm^{-1} ,C-H stretching:3048.5 cm^{-1} , C-H stretching: 2936.16 cm^{-1}, C-C stretching:1583.83 cm^{-1},C-N stretching: 1461.77cm^{-1} C-N stretching: 1371.05 cm^{-1}, C-N stretching:1318.09 cm^{-1} :(H^1 NMR(CHCl3):7.05 (1H, s, C-2 of imidazole), 7.58 (1H, d, C-4 of imidazole), 7.44-7.89 (6H, m, Ar-H), 7.59 (1H, d, α-H), 8.06 (1H, d, β-H), 3.83 (9H, s, 3-OCH_3)
PR-02	C=O,str. – 1661.12cm-1 ,C=C str. – 1588.75cm-1 C-O str. – 828.23cm-1; N-H stretching : 3364.71 cm^{-1} , C-H stretching:3364 cm^{-1} C-H stretching: 3119.04 cm^{-1} , C-H stretching: 2937.74 cm^{-1} C-C stretching: 2834.04 cm^{-1} ,C-N stretching: 1587.08 cm^{-1} ,C-N stretching: 1486.59 cm^{-1} ,C-N stretching: 1420.91 cm^{-1}:H^1 NMR(CHCl3): 7.15 (1H, s, C-2 of imidazole), 7.16 (1H, d, C-4 of imidazole), 7.44-7.89 (6H, m, Ar-H), 7.59 (1H, d, α-H), 8.06 (1H, d, β-H), 3.83 (9H, s, 3-OCH_3)
PR-03	C=O: str. – 1657.87cm-1 C=C str. – 1600.46 cm-1 C-S str. – 1333.18 cm-1; N-H stretching :3404 cm^{-1} , C-H stretching: 3144.43 cm^{-1} C-H stretching :3051.37 cm^{-1} , C-H stretching:2926.41 cm^{-1}, C-C stretching:1588.82 cm^{-1} ,C-N stretching: 1491.32 cm^{-1} ,C-N stretching: 1491.32 cm^{-1},C-N stretching:1426.08 cm^{-1} H^1 NMR(CHCl3): 7.15 (1H, s, C-2 of imidazole), 7.16 (1H, d, C-4 of imidazole), 7.44-7.89 (8H, m, Ar-H), 7.59 (1H, d, α-H), 8.06 (1H, d, β-H), -CH_3), 2.53 (3H, s, -CH_3)
PR-04	C=O,str. – 1661.12cm-1 ,C=C str. – 1588.75cm-1 C-O str. – 828.23cm-1; N-H stretching : 3379.04 cm^{-1} , C-H stretching:2971.04 cm^{-1} C-H stretching : 2922.0 cm^{-1} , C-H stretching: 2866.04 cm^{-1} C-C stretching: 1603.2 cm^{-1} ,C-N stretching: 1455.99 cm^{-1} ,C-N stretching: 1325.03 cm^{-1} : H^1 NMR(CHCl3): 7.15 (1H, s, C-2 of imidazole), 7.16 (1H, d, C-4 of imidazole), 7.44-7.89 (8H, m, Ar-H), 7.59 (1H, d, α-H), 8.06 (1H, d, β-H)
PR-05	C=O: str. – 1657.87cm-1 C=C str. – 1600.46 cm-1 C-O str. – 1333.18 cm-1; N-H stretching : 3330.60 cm^{-1} , C-H stretching: 3115.98 cm^{-1} C-H stretching: 3034 cm^{-1} , C-H stretching: 2931.65 cm^{-1} C-C stretching: 1595.88 cm^{-1} ,C-N stretching: 1451.59 cm^{-1} ,C-N stretching: 1422.70 cm^{-1} ,C-N stretching: 1347.88 cm^{-1}, H^1 NMR(CHCl3): 7.15 (1H, s, C-2 of imidazole), 7.16 (1H, d, C-4 of imidazole), 7.38-7.89 (13H, m, Ar-H), 7.59 (1H, d, α-H), 8.06 (1H, d, β-H), 3.83 (2H, s, -OCH_2-)
PR-06	C=O: str.- 1649.38cm-1 C=C str. – 1598.05cm-1 C-O str.- 1376.52cm-1; N-H stretching :3368.84 cm^{-1} , C-H stretching: 2971.98 cm^{-1} C-H stretching: 2834.04 cm^{-1} , C-H stretching: 11587.08 cm^{-1},C-C stretching: 1486.59 cm^{-1},C-N stretching: 1420.91 cm^{-1} ,C-N stretching: 1370.02 cm^{-1} ,C-N stretching:1326.02 cm^{-1}, H^1 NMR(CHCl3): 7.15 (1H, s, C-2 of imidazole), 7.16 (1H, d, C-4 of imidazole), 7.31-7.89 (8H, m, Ar-H), 7.42 (1H, d, α-H), 8.33 (1H, d, β-H)
PR-07	C=O: str.- 1649.38cm-1 C=C str. – 1598.05cm-1 C-O str.- 1376.52cm-1; N-H stretching : 3077.88 cm^{-1} , C-H stretching: 3116.5 cm^{-1} C-H stretching: 3025.22 cm^{-1} , C-H stretching: 2969.61 cm^{-1} C-C stretching: 1599.52 cm^{-1} ,C-N stretching: 1479.27 cm^{-1} ,C-N stretching: 1370.88 cm^{-1} ,C-N

	stretching: 1308.03 cm^{-1} H^1 NMR(CHCl3): 7.15 (1H, s, C-2 of imidazole), 7.16 (1H, d, C-4 of imidazole), 7.07-7.89 (7H, m, Ar-H), 7.42 (1H, d, α-H), 8.33 (1H, d, β-H)
PR-08	C=O,str. – 1661.12cm-1 ,C=C str. – 1588.75cm-1 C-O str. – 828.23cm-1; N-H stretching : 3364.71 cm^{-1} , C-H stretching:3364 cm^{-1} C-H stretching: 3119.04 cm^{-1} , C-H stretching: 2937.74 cm^{-1} C-C stretching: 2834.04 cm^{-1} ,C-N stretching: 1587.08 cm^{-1} ,C-N stretching: 1486.59 cm^{-1} ,C-N stretching: 1420.91 cm^{-1}:H^1 NMR(CHCl3): 7.15 (1H, s, C-2 of imidazole), 7.16 (1H, d, C-4 of imidazole), 7.44-7.89 (8H, m, Ar-H), 7.59 (1H, d, α-H), 8.06 (1H, d, β-H), 1.25 (3H, t, $-CH_3$), 2.60 (2H, q, $-CH_2-$)
PR-09	C=O: str. – 1657.87cm-1 C=C str. – 1600.46 cm-1 C-O str. – 1333.18 cm-1; N-H stretching :3404 cm^{-1} , C-H stretching: 3144.43 cm^{-1} C-H stretching :3051.37 cm^{-1} , C-H stretching:2926.41 cm^{-1}, C-C stretching:1588.82 cm^{-1} ,C-N stretching: 1491.32 cm^{-1} ,C-N stretching: 1491.32 cm^{-1},C-N stretching:1426.08 cm^{-1} H^1 NMR(CHCl3): 7.15 (1H, s, C-2 of imidazole), 7.16 (1H, d, C-4 of imidazole), 7.44-7.89 (8H, m, Ar-H), 7.59 (1H, d, α-H), 8.06 (1H, d, β-H)
PR-10	C=O: str.- 1658.95cm-1C=Cstr.- 1594.05cm-1 C-O str.- 756.23cm-1; N-H stretching : 3392.75 cm^{-1} , C-H stretching: 3123.41 cm^{-1} C-H stretching: 2942.19 cm^{-1} , C-H stretching: 2829.8 cm^{-1} C-C stretching: 1602.2 cm^{-1} ,C-N stretching: 1486.58 cm^{-1},C-N stretching:1587.10 cm^{-1} :H^1 NMR(CHCl3): 7.15 (1H, s, C-2 of imidazole), 7.16 (1H, d, C-4 of imidazole), 7.44-7.89 (8H, m, Ar-H), 7.59 (1H, d, α-H), 8.06 (1H, d, β-H), 3.83 (3H, s, $-CH_3$)

Cytotoxicity Evolution
MTT assay method
Table 5: anti cancerous activity.

S. No	Compound code	Cell line		
		Breast cnacer (MDA MB)	Colon cancer (HT-29)	Prostate cancer (DU-145)
1	PR-01	20±1	22±2	18±2
2	PR-02	15±2	18±1	12±1
3	PR-03	56±1	42±2	30±2
4	PR-04	64±1	52±1	46±1
5	PR-05	24±1	26±1	23±2
6	PR-06	84±2	64±1	54±1
7	PR-07	188±1	174±2	134±1
8	PR-08	96±1	72±1	62±2
9	PR-09	170±2	98±2	120±1
10	PR-10	32±1	30±1	26±2
11	Methothrexate	12 ± 1	9 ± 1	5 ± 1

Data presented as mean ± SD (n=3). All the compounds and the standard dissolved in DMSO, diluted with culture medium containing 0.1% DMSO. The control cells were treated with culture medium containing 0.1% DMSO. NA- No Activity (i.e IC_{50} > 200 µg/mL)

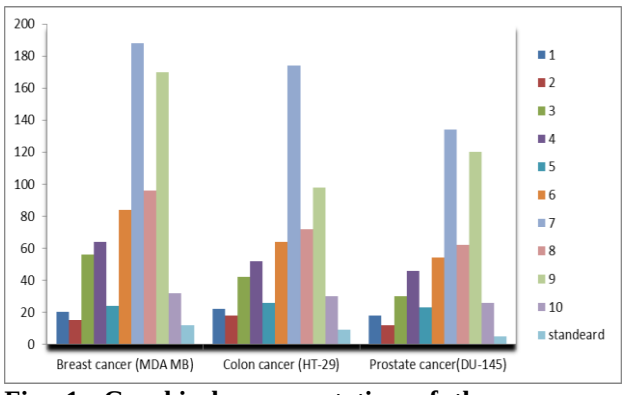


Fig: 1: Graphical representation of the cancerous activity

DISCUSSION

Based on the results conclude that the compounds shows better anti cancerous activity different cell lines like Breast cancer (MDA MB, Colon cancer (HT-29), Prostate cancer(DU-145) . In all of these compounds, compound-02 (4-[4-(1H-imidazol-1-yl)phenyl]-6-[4-(methylsulfanyl)phenyl] -1,6-dihydropyrimidin-2-ol) 15±2 µg/ml, ,18±1 µg/ml,12±1 µg/ml respectively and compound - 01 4-[4-(1-imidazolyl)phenyl]-6-(2,4,6-trimethoxyphenyl)-1,6-dihydropyrimidin-2-ol 20±1 µg/ml, 22±2 µg/ml,18±2 µg/ml respectively.

CONCLUSION

The compound PY-02, (4-[4-(1H-imidazol-1-yl) phenyl]-6-[4-(methylsulfanyl)phenyl]-1,6-dihydropyrimidin-2-ol) 15±2 µg/ml, ,18±1 µg/ml,12±1 µg/ml against the Breast cancer (MDA MB, Colon cancer (HT-29), Prostate cancer(DU-145).

ACKNOWLEDGMENT

The author thankful to Principal, Management of Vignan College of Pharmacy, for provide all the facilities and supports for accomplishment and completion of this research work.

REFERENCES

1. Y. Ju and R. S. Varma, “Aqueous N-heterocyclization of primary amines and hydrazines with dihalides: microwave-assisted syntheses of N-azacycloalkanes, isoindole, pyrazole, pyrazolidine, and phthalazine derivatives,” Journal of Organic Chemistry, 2006; 71(1): 135–141.
2. Y. Ju, D. Kumar, and R. S. Varma, “Revisiting nucleophilic substitution reactions: microwave-assisted synthesis of azides, thiocyanates, and sulfones in an aqueous medium,” Journal of Organic Chemistry, 2006; 71(17): 6697–6700.
3. P. D. Lokhande, B. Y. Waghamare, and S. S. Sakate, “Regioselective one-pot synthesis of 3,5-

diarylpyrazoles," Indian Journal of Chemistry B, 2005; 44(11): 2338–2342.
4. G. J. Reddy, D. Manjula, K. S. Rao, M. Khalilullah, and D. Latha, "A Direct single step synthesis of 1,3-diaryl-4-cyanopyrazoles and their conversion to 1,3-diaryl-4-(4,6-diamino 1,3,5-triazin-2-yl)pyrazoles," Indian Journal of Chemistry B, 2005; 44: 2412–2415.
5. C. A. Zificsak and D. J. Hlasta, "Current methods for the synthesis of 2-substituted azoles," Tetrahedron, 2004; 60(41): 8991–9016.
6. T. Haino, M. Tanaka, K. Ideta, K. Kubo, A. Mori, and Y. Fukazawa, "Solid-phase synthesis of liquid crystalline isoxazole library," Tetrahedron Letters, 2004; 45(11): 2277–2279.
7. M. García-Valverde and T. Torroba, "Special issue: sulfur-nitrogen heterocycles," Molecules, 2005; 10(2): 318–320.
8. D. W. Hopper, A. L. Crombie, J. J. Clemens, and S. Kwon, "Six-membered ring systems: pyridine and benzo derivatives," Progress in Heterocyclic Chemistry, 2009; 21: 330–374.
9. Manlove and M. P. Groziak, "Six-membered ring systems: diazines and benzo derivatives," Progress in Heterocyclic Chemistry, 2009; 21: 375–414.
10. D. Russell, "Mechanisms of bacterial insusceptibility to biocides," The American Journal of Infection Control, 2001; 29(4): 259–261.
11. D. J. Brown, Comprehensive Heterocyclic Chemistry, Pergamon Press, Oxford, UK, 1984, 14.
12. R. C. Elderfield, Heterocyclic Compounds, John Wiley and Sons, New York, NY, USA, 1957, 6.
13. P. Y. Bruice, Organic Chemistry, Pearson Education, Singapore, 3rd edition, 2007.
14. H. P. Schweizer, "Triclosan: a widely used biocide and its link to antibiotics," FEMS Microbiology Letters, 2001; 202(1): 1–7.
15. S. B. Levy, "Antibiotic and antiseptic resistance: impact on public health," Pediatric Infectious Disease Journal, 2000; 19(10): S120–S122.
16. S. B. Levy, "Active efflux, a common mechanism for biocide and antibiotic resistance," Journal of Applied Microbiology, 2002; 92(1): 65S–71S.
17. K. Poole, "Mechanisms of bacterial biocide and antibiotic resistance," Journal of Applied Microbiology, 2002; 92(1): 55S–64S.
18. M. Hassan, D. van der Lelie, D. Springael, U. Römling, N. Ahmed, and M. Mergeay, "Identification of a gene cluster, CZR, involved in cadmium and zinc resistance in Pseudomonas aeruginosa," Gene, 1999; 238(2): 417–425.
19. E. A. Porter, Diazines and Benzodiazines, Pregamon Press, Elsevier Science BV, Amsterdam, The Netherlands, 1979, 14.
20. O. Kappe, "100 years of the biginellidihydropyrimidine synthesis," Tetrahedron, 1993; 49(32): 6937–6963.
21. P. Sharma, N. Rane, and V. K. Gurram, "Synthesis and QSAR studies of pyrimido[4,5-d]pyrimidine-2,5-dione derivatives as potential antimicrobial agents," Bioorganic and Medicinal Chemistry Letters, 2004; 14(16): 4185–4190.
22. S. Ito, K. Masuda, S. Kusano et al., "Pyrimidine derivative, process for preparing same and agricultural or horticultural fungicidal composition containing same," U.S. Patent, 1991; 4: 988-704.
23. V. J. Ram, N. Haque, and P. Y. Guru, "Chemotherapeutic agents XXV: synthesis and leishmanicidal activity of carbazolylpyrimidines," European Journal of Medicinal Chemistry, 1992; 27(8): 851–855.
24. M. Amir, S. A. Javed, and H. Kumar, "Pyrimidine as anti-inflammatory agent: a review," Indian Journal of Pharmaceutical Sciences, 2007; 68: 337.
25. S. Vega, J. Alonso, J. A. Diaz, and F. Junquera, "Synthesis of 3-substituted-4-phenyl-2-thioxo-1,2,3,4,5,6,7,8-octahydrobenzo[4,5]thieno[2,3-d]pyrimidines," Journal of Heterocyclic Chemistry, 1990; 27(2): 269–273.
26. D. R. Hannah and M. F. G. Stevens, "Structural studies on bioactive compounds-part 38.1: reactions of 5-aminoimidazole-4-carboxamide: synthesis of imidazo[1,5-a]quinazoline-3-carboxamides," Journal of Chemical Research S, 2003; 7: 398–401.
27. P. A. S. Smith and R. O. Kan, "Cyclization of isothiocyanates as a route to phthalic and homophthalic acid derivatives," Journal of Organic Chemistry, 1964; 29(8): 2261–2265.
28. J. Balzarini and C. McGuigan, "Bicyclic pyrimidine nucleoside analogues (BCNAs) as highly selective and potent inhibitors of varicella-zoster virus replication," Journal of Antimicrobial Chemotherapy, 2002; 50(1): 5–9.
29. H. W. Lee, Y. K. Bok, B. A. Joong et al., "Molecular design, synthesis, and hypoglycemic and hypolipidemic activities of novel pyrimidine derivatives having thiazolidinedione," European Journal of Medicinal Chemistry, 2005; 40(9): 862–874.
30. P. F. Juby, T. W. Hudyma, M. Brown, J. M. Essery, and R. A. Partyka, "Antiallergy agents. 1. 1, 6-dihydro-6-oxo-2-phenylpyrimidine-5-carboxylic acids and esters," Journal of Medicinal Chemistry, 1979; 22(3): 263–269.
31. K. Gupta, Sanjay, H. P. Kayath, A. Singh, G. Sharma, and K. C. Mishra, "Anticonvulsant activity of pyrimidine thiols," Indian Journal of Pharmacology, 1994; 26(3): 227–228.
32. Abu-Hashem, M. M. Youssef, and H. A. R. Hussein, "Synthesis, antioxidant, antituomer activities of some new thiazolopyrimidines, pyrrolothiazolopyrimidines and triazolopyrrolothiazolopyrimidines derivatives," Journal of the Chinese Chemical Society, 2011; 58(1): 41–48.
33. S. A. Rahaman, Y. R. Pasad, P. Kumar, and B. Kumar, "Synthesis and anti-histaminic activity of some novel pyrimidines," Saudi Pharmaceutical Journal, 2009; 17(3): 255–258.

34. Y. Nezu, M. Miyazaki, K. Sugiyama, and I. Kajiwara, "Dimethoxypyrimidine as novel herbicides—part1:synthesis and herbicidal activity of dimethoxy phenoxy phenoxy pyrimidines and analogues," Pesticide Science, 1996; 47: 103–113.
35. J. W. Coe, A. F. J. Fliri, T. Kaneko, and E. R. Larson, "Pyrimidine derivatives enhancing antitumour activity," U.S. Patent, 1996; 5: 491-234.
36. L. S. Rodrigues, J. M. Rosa, V. M. Gadotti et al., "Antidepressant-like and antinociceptive-like actions of 4-(4′-chlorophenyl)-6-(4″-methylphenyl)-2-hydrazinepyrimidine Mannich base in mice," Pharmacology Biochemistry and Behavior, 2005; 82(1): 156–162.
37. CH. M. M. Prasada Rao. et al. Synthesis, Characterization And Anti Bacterial And Cytotoxic Studies Of Novel 1, 5 Benzothiazepines From Chalcones Of 2,4 Di Fluoro Acetophenone. Asian Journal of Research in Chemistry and Pharmaceutical Sciences, 2016; 4(4): 128-139.