Section-A: Research Paper

SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME NOVEL 2, 5-DISUBSTITUTED-1, 3, 4-OXADIAZOLES

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Abstract

Oxadiazole and its derivatives exhibit potent bactericidal activity against a wide range of microorganisms. A number of fresh 1,3,4-oxadiazole derivatives and analogues were created and their effectiveness as antimicrobials against a variety of pathogenic gram-positive and gram-negative organisms was examined. By using QSAR analysis, it is possible to correlate the physicochemical and structural characteristics of produced compounds with their antibacterial activity. The peptide deformylase inhibitory potential of the derivatives with strong antibacterial activity was examined using molecular docking experiments, which describe the interaction between the active derivatives and amino acid residues found in the active site of the enzyme. Oxadiazoles are common motifs in drug-like compounds and are regularly employed to replace the ester and amide functions with bioisosteric alternatives. 1,3,4-Oxadiazole and its derivatives have become significant pharmacological scaffolds, particularly in the treatment of cancer disease, because to their extensive and powerful activity. Several 1,3,4-oxadiazole compounds with di-, tri-, aromatic- and heterocyclic substitutions have been found to have strong anticancer properties. These substituted 1,3,4-oxadiazoles contributed to the discovery and development of anticancer drugs and have diverse mechanisms of action.

Keywords: Oxadiazole, Scaffold, QSAR, Docking, Bioisosteric

Introduction

The primary objective of medicinal chemistry is the design and discovery of new compounds that are suitable for use as drugs. Medicinal chemistry, according to Burger, “Tries to be based

on the ever-increasing hope that biochemical rationales for drug discovery may be found. In contrast he described pharmaceutical chemistry as being concerned primarily with modification of structures having known Physiological or Pharmacological effects and with analysis of drugs."[1-2]

After advancement in the field of chemistry the man started knowing the active constituents of the plants. In this process he started.

(a) Identifying the active constituents from plant origin.

(b) Evaluating them to define their pharmacological properties and

(c) Chemical modification of natural substances to improve their pharmacological profile eg. Chemical modification of morphine leads to morphinan, diethyl morphine and heroin.

Heterocyclic compounds are those cyclic compounds whose ring contains besides carbon, one or more atom of other elements (heteroatoms). The most common heteroatoms are nitrogen, sulphur and oxygen.[3-5] Heterocyclic compound can be aliphatic or aromatic in character depending upon the electronic constitution.[6-8]

1,3,4-Oxadiazoles have a wide variety of uses, in particular as biologically active compounds in medicine and in agriculture, as dyestuffs, UV absorbing and fluorescent materials, heat resistant polymers and scintillators.[9]

1,3,4-Oxadiazoles were associated with broad spectrum of biological activities, including antituberculosis[10], anticonvulsant[11], anti-inflammatory[12], antifungal[13], analgesic[14], antitumor[15], antibacterial[16], antihypertensive activities[17], CNS-stimulant[18], antimalarial[19], anticancer[20] properties.

ANTIMICROBIAL ACTIVITY OF HETEROCYCLIC COMPOUNDS:

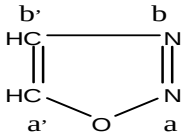
Some heterocyclic chemicals and compounds show little antimicrobial activity. Among them some antimicrobial agents are safe, potent and they must be encouraged. Further all future efforts to produce an effective antimicrobial agent must strictly take the following things must be strictly taken as major concerns.

- To have the best suitable compounds which can destroy or prevent the activity of a practice without injuring the cell of the host or with only minor injury to host cell.
- The design of compounds that must be able to come in contact with the parasite by penetrating the cell and tissue of the host in effective concentration.
- These compounds should leave unaltered the host's natural defence mechanism such as phagocytosis and production of antibodies.
- The compound should have the ability to destroy or inhibit many different species of pathogenic microorganisms.
- The compound should prevent the ready development of resistance forms of parasite.

- The agents should not produce undesirable effects in the host, such as sensitivity or allergic reactions, nerve damage or irritation of the kidney and GIT.
- These agents should not eliminate the normal flora of the host.

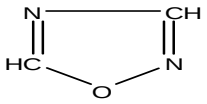
Chemistry of Compound (OXADIAZOLE RING)

Compounds having a five membered ring containing one oxygen and two nitrogen atoms are called oxadiazoles or in the older literature furadiazoles.[21] Four types of oxadiazoles are known as following.[22]



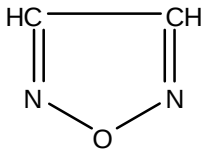
1,2,3-Oxadiazole diazo-oxides

Furo (ab) diazole



1,2,4-Oxadiazole azoximes

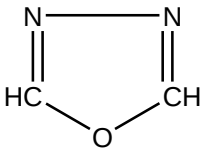
Furo (ab') diazole



1,2,5-Oxadiazole

Furo (aa') diazole

Furazans



1,3,4-Oxadiazole

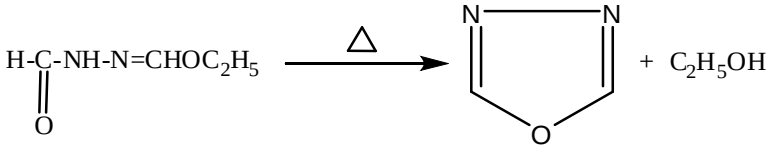
Furo (bb') diazole

biazole , oxybiazole, Oxadiazole

Name for oxadiazole ring such as "azoxime" (for 1,2,4-oxadiazole), "Furazan" (for 1,2,5-oxadiazole) has gained acceptance, therefore, the literature is full of multiplicity of name for this nucleus. Amongst these are "oxybiazole", "diazoxole" "furo (bb') diazole and "biozole". The systematic name of 1,3,4-oxadiazole has gradually become prevalent and is used exclusively.[23]

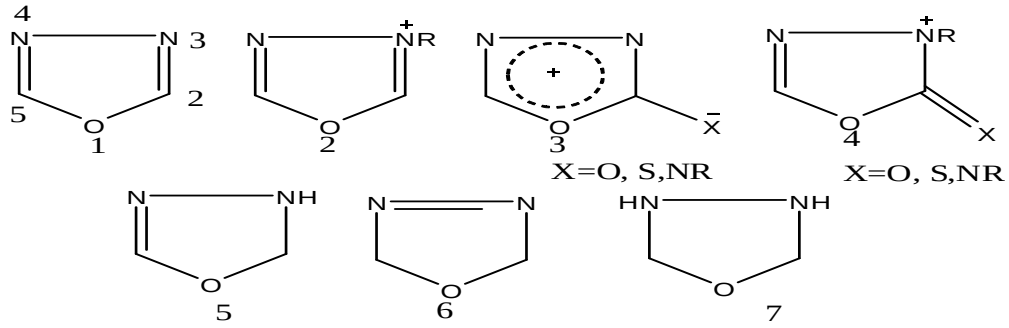
1,3,4-Oxadiazole

1,3,4-Oxadiazole is a liquid, which boils at 150^0C. Ainsworth first prepared it in 1965 by the thermolysis of ethylformate formyl hydrazone at atmospheric pressure.[24]



1,3,4-Oxadiazole (1) is a thermally stable neutral aromatic molecule. Other aromatic systems are 1,3,4-oxadiazolium cations (2) and the exocyclic-conjugated mesoionic-1,3,4-oxadiazoles (3) and 1,3,4-oxadiazolines (4). Also known are derivatives of the non-aromatic reduced

systems, 2,3-dihydro-1,3,4-oxadiazole(5), 2,5- dihydro-1,3,4-oxadiazole (6) and 2,3,4,5-tetrahydro-1,3,4-oxadia-zole (7).[25]



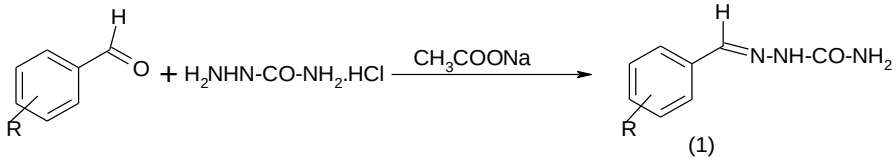
Objectives

1. To observe the effect of various substituted aryl group (R_2) on antimicrobial activity.
2. To observe the biological effect of chain lengthening at 2nd position of oxadiazole ring.
3. To observe the biological effect of semicarbazone group placed at 2nd position of the 1,3,4-oxadiazole.

Experimental

Synthesis of compound

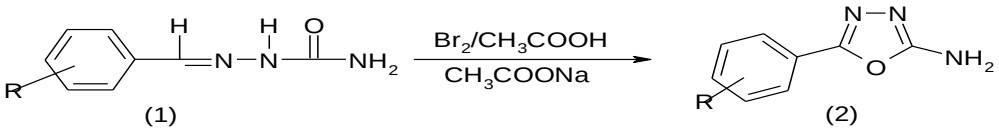
Step 1- Synthesis of Semicarbazone



R=

2,5-Dimethoxy
2-Chloro-3,4-Dimethoxy

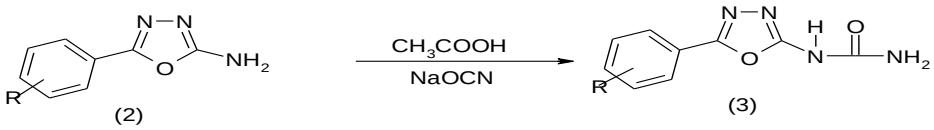
Step 2- Synthesis of 5-Phenyl-1,3,4,-oxadiazole-2-amine



R=

2,5-Dimethoxy
2-Chloro-3,4-Dimethoxy

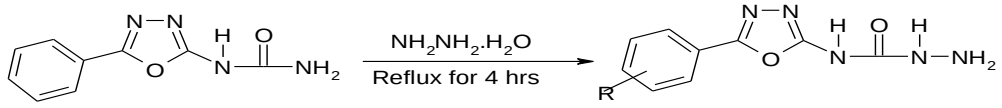
Step 3- Synthesis of 5-Phenyl-1,3,4,-oxadiazole-2-yl-urea



(2) (3)

R=	2,5- dimethoxy
	2-Chloro-3,4-dimethoxy

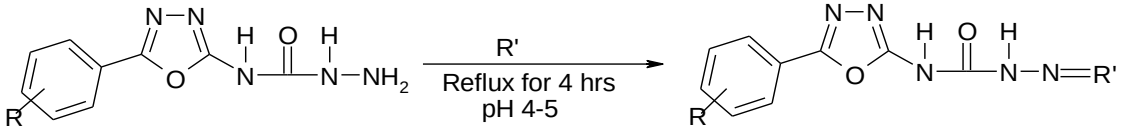
Step 4- Synthesis of 5-Phenyl-1,3,4,-oxadiazole-2-yl-semi-carbazide



(3) (4)

R=	2,5 -dimethoxy
	2-Chloro-3,4-dimethoxy

Step 5- Synthesis of final compounds (N_1-N_4)

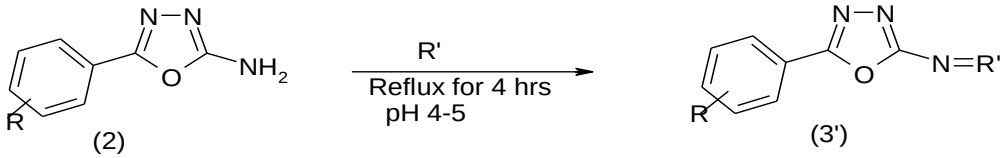


(4) (5)

R=	2,5-dimethoxy
	2-chloro-3,4-dimethoxy

R'=	Camphor
	Coumarine

Step 6:- Synthesis of final compound (J_1-J_4)

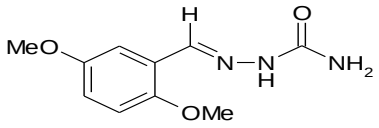
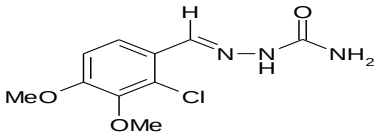
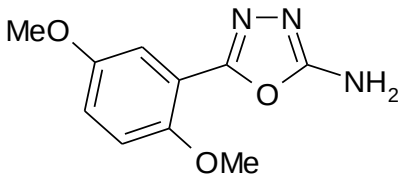
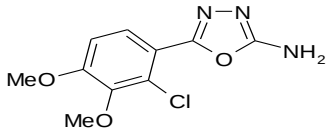
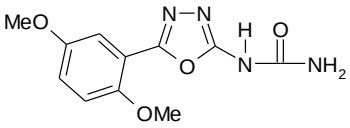
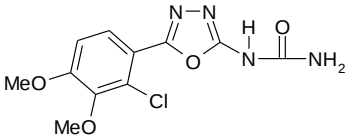
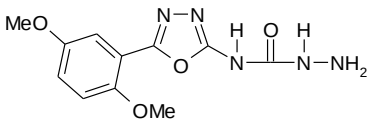
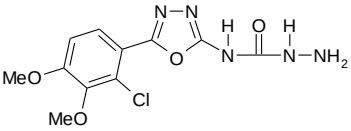


(2) (3')

R=	2,5-dimethoxy
	2-chloro-3,4-dimethoxy

R'=	Camphor
	Coumarine

Table 1: List of Synthesized compounds

Compound	Structure	Yield (%)	Solvent for recrystallization	M.P (°C)
Semicarbazole		71.81	75% ethanol	224-226
		76.56	75% ethanol	232-234
5-Phenyl-1,3,4-oxadiazole-2-yl-amine		88.8	75% ethanol	192-194
		89.6	75% ethanol	222-224
5-Phenyl-1,3,4-oxadiazole-2-yl-urea		74.80	75% ethanol	222-224
		70.13	75% ethanol	240-242
5-Phenyl-1,3,4-oxadiazole-2-yl-semicarbazide		85.17	75% ethanol	140-142
		84.80	75% ethanol	161-163

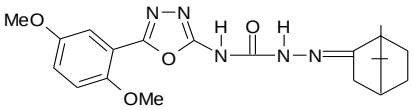
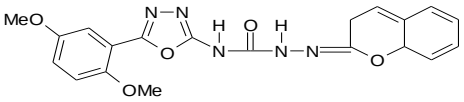
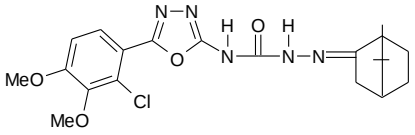
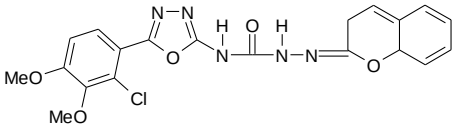
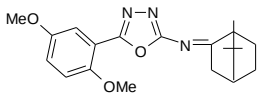
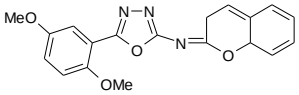
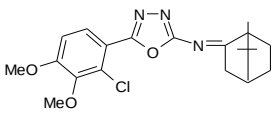
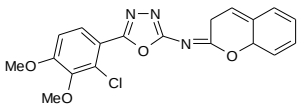
Final compounds (N_1- N_4) (N_1)		59.09	75% ethanol	237-240
N_2		52.57	75% ethanol	238-240
N_3		34.17	75% ethanol	259-260
N_4		59.17	75% ethanol	245-247
Final compounds(J_1-J_4) J_1		38.57	75% ethanol	256-258
J_2		31.72	75% ethanol	259-261
J_3		31.02	75% ethanol	288-290
J_4		24.69	75% ethanol	260-262

Table 2: Evaluation Parameters

1. **Physio-chemical properties**
   a) Solubility

Comp	Cool Water	Hot Water	Methanol	Ethanol	Hot Ethanol	DMF	Chloroform	DMSO
N_1	—	—	+	+ +	+++	+++	—	+++
N_2	———	—	+	+ +	+++	+++	—	+++
N_3	—	—	+	+ +	+++	+++	—	+++
N_4	—	—	+	+ +	+++	+++	—	+++
J_1	+	++	+	+ +	+++	+++	—	+++
J_2	+	++	+	+ +	+++	+++	—	+++
J_3	+	++	+	+ +	+++	+++	—	+++
J_4	+	++	+	++	+++	+++	—	+++

- **= Practically insoluble, + = Slightly soluble, ++ = Soluble, +++ = Freely soluble**

   b) Melting Point

Code	% Yield	Rf value	MeltingPoint(°C)	Molecular Formula	Molecular Weight
N_1	59.09%	0.62	237-240	$C_{20}H_{27}O_4N_5$	401
N_2	52.57%	0.55	238-240	$C_{20}H_{17}O_5N_5$	407
N_3	34.25%	0.59	259-260	$C_{20}H_{26}O_4N_5Cl$	435.5
N_4	59.17%	0.65	245-247	$C_{20}H_{16}O_5N_5Cl$	441.5
J_1	38.57%	0.58	256-258	$C_{20}H_{25}O_3N_3$	355
J_2	31.72%	0.50	259-261	$C_{19}H_{15}O_4N_3$	349
J_3	31.02%	0.50	288-290	$C_{20}H_{24}O_3N_3Cl$	389.5
J_4	24.69%	0.38	260-262	$C_{19}H_{14}O_4N_3Cl$	383.5

c) TLC Studies

2. **Structural elucidation of synthesized compound**
   a) IR spectra study
   b) ^{1}H-NMR spectra study
   c) Mass spectra study
3. **Antimicrobial study of synthesized compound**
   a) Antibacterial activity
   b) Antifungal activity

Activity/ Screening

ANTIMICROBIAL ACTIVITY

Antibacterial Activity of Synthesized Compounds

Bacteria are very small (0.5-1.0 µm in diameter) unicellular, prokaryotic organism with rigid cell wall. Bacteria are of different nutritional types like saprophytic, parasitic, phototrophic and autotrophic. They can be non-motile or motile with simple flagella, axial filament or gliding motility[26-28].

For the bacterial screening of the synthesized compounds the following bacterial species were taken

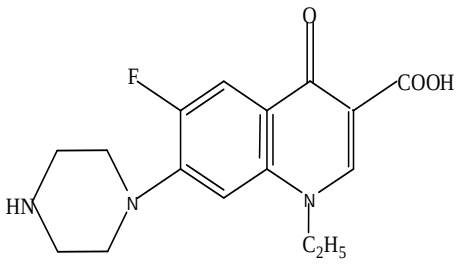
Table 3: List of Bacterial Strains[29]

S. No.	Microbial strains	ATCC No.*	Gram	Incubation period
1.	*Staphylococcus aureus*	33592	+ve	48h
2.	*Pseudomonas aeruginosa*	15442	-ve	24h
3.	*Escherichia coli*	DT-01	-ve	24h

The Standard drug selected for antibacterial activity[30]

Norfloxacin, a broad-spectrum chemotherapeutic agent was being used as a standard drug for the present study of antibacterial activity. It is active against Gram +ve and Gram –ve bacteria both. Its chemical name is 1-Ethyl-6-fluro-1,4-dihydro-4-oxo-7-(1-piperazinyl) -3 quinoline carboxylic acid.

Norfloxacin



Molecular formula	$C_6H_{18}FN_3O_3$
Molecular weight	319.34
Melting point	227-228°C
Solubility	In water, ethanol and chloroform
Category	Fluoroquinolone Antibacterial
Mechanism of action	By inhibiting the subunit of DNA Gyrase which is essential for the reproduction of bacterial strain
Zone of inhibition (ZOI)	28mm

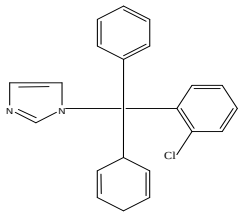
Antifungal Activity Of The Synthesized Compounds

Table 4: List of Fungal Strains

S. No.	Fungal strain	MTCC* No.	Incubation Temperature (°C)	Incubation period
1.	*Aspergillus niger*	1344	25	5 days

Standard Drug for Antifungal Activity [31]

Clotrimazole (1-(*o*-chloro-α, α-diphenyl) benzyl imidazole) was selected as standard drug for antifungal study. It is a broad-spectrum antifungal agent.



Molecular formula	$C_{22}H_{17}ClN_2$

Molecular weight	344.8
Melting Point	142^0 C
Solubility	In ethanol, methanol, acetone and chloroform.
Category	Imidazole antifungal agent
Mechanism of action	They inhibit fungal cytochrome P-450 enzyme lanosterol 14- demethylase and thus impair ergosterol synthesis leading to a cascade of membrane abnormalities in the fungus.
ZOI	21mm

Result & Discussion:

The success of the syntheses was confirmed through physical and spectral characterization. In synthesized compound N_1, the peaks observed at 3267cm^{-1} for C-H group and 2332 cm^{-1} for N-N group in IR spectra matched well with its structure. Chemical shift observed in range of 7.51-7.98 ppm in NMR spectra of synthesized compound has delta value assignable to corresponding protons in its structure. This is supported by mass spectra, where characteristic $(M+H)^+$peak was observed clearly at 402.16 m/z. Further, in synthesized compound N_2, the peaks observed at 2947.5 cm^{-1} for C-H group and 1650.6 cm^{-1} for C=O group in IR spectra determined its structure. Chemical shift observed in range of 7.00-7.96 ppm in NMR spectra. In mass spectra, characteristic $(M+H)^+$peak was observed at 407.41 m/z. Similarly, in synthesized compound N_3, the peaks observed at 2886.5 cm^{-1} for C=O group and 1509.8 cm^{-1} for N-H group in IR spectra. Chemical shift observed in range of 7.50-7.98 ppm in NMR spectra. In mass spectra, characteristic $(M+H)^+$peak was observed at 438.53 m/z. In synthesized compound N_4, the peaks observed at 3179.83 cm^{-1} for C-H group and 1717.47 cm^{-1} for C=O group in IR spectra. Chemical shift observed in range of 7.21-7.73 ppm in NMR spectra. In mass spectra, characteristic $(M+H)^+$peak was observed at 444.31 m/z. In synthesized compound J_1, the peaks observed at 3001.80 cm^{-1} for C-H group and 2469.58 cm^{-1} for N-N group in IR spectra. Chemical shift observed in range of 7.13-7.44 ppm in NMR spectra. In mass spectra, characteristic $(M+H)^+$peak was observed at 356.28 m/z. In synthesized compound J_2, the peaks observed at 2938.1 cm^{-1} for C-H group and 1509.8,1448.7cm^{-1} for C=C group in IR spectra. Chemical shift observed in range of 7.00-7.43 ppm in NMR spectra. In mass spectra, characteristic $(M+H)^+$peak was observed at 349.27 m/z. In synthesized compound J_3, the peaks observed at 2881.8 cm^{-1} for C-H group and 1486.3, 1406.5 cm^{-1} for C=C group in IR spectra. Chemical shift observed in range of 7.02-7.87 ppm in NMR spectra. In mass spectra, characteristic $(M+H)^+$peak was observed at 389.14 m/z. In synthesized compound J_4, the peaks observed at 2919.4 cm^{-1} for C-H group and 1594.4 cm^{-1} for C-CN group in IR spectra. Chemical shift observed in range of 7.01-7.88 ppm in NMR spectra. In mass spectra, characteristic $(M+H)^+$peak was observed at 383.22 m/z. All these spectral analysis expressed the structures of potent synthesized compounds efficiently.

Conclusion

From exhaustive research on antibacterial and antifungal activity of 1,3,4-Oxadiazole, it may be concluded that-.

In case of Gram positive bacteria compound N_2 , J_1 showed significant activity against *S. aureus* with ZOI= 17,15 mm 100µg/ml and showed maximum activity. Similarly,in case of Gram negative*P. aerugenosa* (ATCC-15442) and *E. coli* (DT-01) bacteria compounds N_2, N_3, N_4, J_1, J_2, J_4 (24, 25,17, 20,14, 24 mm) and N_1, N_2, N_3, N_4, J_1, J_2, J_3, J_4 (21,15, 20,14, 20, 19, 25, 26 mm) was showed zone of inhibition. In case of fungus *A. Niger,* Clotrimazole was selected as the standard drug for antifungal studies. The highest significant activity was showed by the synthesized compounds code no. N_1,N_2,N_3,N_4 (18, 21, 15, 14 mm) and J_1, J_2, J_3, J_4 (10,11,10,12 mm) showed moderate singnificant activity compare to N_1,N_2,N_3,N_4.

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References

1. Hetzhein A. and Mockel K., **"Adv. Heterocyclic Chem."**, 1966, **7**, 83.
2. Kenneth R., Scott In Abraham, Donald J. Eds.*, "Burger's Medicinal Chemistry and Drug Discovery*", 6th ed., John **Willey and Sons Publication**, 2005, 384.
3. Tripathi K.D.*, "Essential of Medical Pharmacology",* 5th ed., J. P. Brothers's Prakashan, 2003.
4. Chatwal G.R., *"Synthetic Drugs",* 2nd ed., **Himalaya Publishing House**, Bombay, 1988, 01.
5. Ariens E.J., "*Drug Design*", 1st ed**., Academic Press**, New York, 1971, 42.
6. Katritzky A.R., *"Handbook of Heterocyclic Chemistry"*, 1st ed., **Pergamon press**, Oxford, 1985, 02.
7. Morisson R.T. and Boyd R.N.*, "Organic Chemistry",* 4th ed., Allyn and Bacon INC Universal Book stall, New Delhi, 1987, 1268.
8. Foye W.O., "*Principles of Medicinal Chemistry",* 3rd ed., **Varghese Publishing House**, Bombay, 1989, 06.
9. Katritzky A.R. and Rees C.W.*, "Comprehensive Heterocyclic chemistry"*, 1st ed., **Pergamon Press,** Oxford, 1984, **6**, 445.
10. Zhang Z.Y. and Chen L.M.*,* **"Chem. J. Chin. Univ."**, 1981, 2, 50.
11. Chopleo C.B., Myers P.L., Smith A.C.B., Stillings M.R., Tullochi F. and walter D.S., **"J. Med. Chem."**, 1988, **7**, 31.
12. Ramalingam R.T., Deshmukh A.A., Sattur P.B., Sheth U. K. and Naik S.R., **"J. Indian Chem. Soc."**, 1981, **58**, 269.
13. Adams S.S., Cliffe E.E., Lessel B. and Nicholson J.S., **"J. Pharm. Sci."***,* 1967, **56**, 1986.

14. Clemence F., Joliveau-Maushart C., Meier J., Cerede J., Deraldt R., **"Eur. J. Med. Chem.",** 1985, **20**, 257.
15. Miyahara M., Nakadate M., Tanno M., and Kamiya S., **"Chem. Pharm. Bull."**, 1982, **30**, 4402.
16. Hill J., "*Comprehensive Heterocyclic Chemistry",* edited by A.R. Katritzky and C.W. Rees (Pergmon Press) 1986, **6**, 267.
17. Ponticello G.S., Englehardt E.L. and Baldwin J.J., **"J. Heterocyclic Chem."**, 1980, **17**, 425.
18. Dubey A.K. and Sangwan N.K., **"Indian J. Chem."**,1994, **33B**, 1043.
19. Chem. B., Qin W., Shen Z. and Lei X., 1985, **16**, 305, *"Chem. Abstr."*, 1986, **104**, 186357.
20. Bhat K.S., Karthikeyan M.S., Holla B.S. and Shetty N.S., "**Indian J. Chem."***,* 2004, **43B**, 1765.
21. Rodd E.H., *"Chemistry of Carbon Compounds,"* Part A, **Elsevier Publishing Co.,** Adsterdam, 1957, **4**, 464.
22. Finar I.L., *"Organic Chemistry"*, 2nd ed., 1959, **2**, 525.
23. Katritzky A.R. & Lagowski J.M.*, "Heterocyclic Chemistry",* **Oxford University of Cambridge**, 1960, 206.
24. Ainworth C., **"J. Am. Chem. Soc."**, 1965, **67**, 5800-5801.
25. Katritzky A.R. and Rees C.W., *"Comprehensive Heterocyclic Chemistry",* 1st ed.,**Pergamon Press**, Oxford, 1984, **6**, 427-428.
26. Pelczar, M.J., Chan, E.C.S., Krieg, N.R., "*Microbiology"*, **Tata Mc. Graw Hill Publishing Company Ltd.**, New Delhi, 5th ed., 1993, 66-796
27. Reynolds, J.E.F., In "*Martindale the Extra Pharmacopoeia*". **The Pharmaceutical Press London,** 29th ed., 1989, 274-714
28. Reynolds, J.E.F., In "*Martindale the Extra Pharmacopoeia*". **The Pharmaceutical Press London,** 29th ed., 1989, 274-714
29. Rawlins, E.A., *"Bentley's Textbook of Pharmaceuticals",***The English Book Society and Baillier's Tindall, London,** 8th ed., 1980, 540-555
30. Kalsi, P.S., *"Spectroscopy of Organic Compounds",***New Age International Publishers Ltd.,** New Delhi, 2nd ed., 1995, 35.
31. Rawlins, E.A., "*Bentley's Textbook of Pharmaceuticals*", **The English Book Society and Baillier's Tindall,** London, 8th ed., 1980, 432-41