

## DESIGN, SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF SCHIFF BASE METAL COMPLEXES

**B. Jagadeeswara Reddy\*<sup>1</sup> and S. Rashida begum<sup>2</sup>**

\*<sup>1</sup>Faculty of Engineering Chemistry, Ravindra College of Engineering for Women, Kurnool(dt), Kurnool. Affiliated to JNTUA, Anthapuram, Kurnool, PIN 518501, Kurnool (Dist), A.P., INDIA.

<sup>2</sup>Faculty of Chemistry, Ravindra College of Engineering for Women, Kurnool(dt), Kurnool. Affiliated to JNTUA, Anthapuram, Kurnool, PIN 518501.

**\*Corresponding Author: B.Jagadeeswara Reddy**

Faculty of Engineering Chemistry, Ravindra College of Engineering for Women, Kurnool(dt), Kurnool. Affiliated to JNTUA, Anthapuram, Kurnool, PIN 518501, Kurnool (Dist), A.P., INDIA.

Article Received on 28/01/2016

Article Revised on 17/02/2017

Article Accepted on 09/03/2017

### ABSTRACT

New coordination complexes of Co, Ni and Zn with Schiff base bis (4-phenylpyrimidin-2-yl)(2-thioxo-1,2-dihydroquinolin-3-yl)methylene)hydrazono)methoxy) have been synthesized and characterized by several techniques using elemental analysis (C, H, N), IR spectra and <sup>1</sup>HNMR spectra. The new Schiff base has been synthesized by the reaction of 4-phenylpyrimidine-2-carbohydrazide and 2-thioxo-1,2-dihydro quinoline-3-carbaldehyde. The Schiff base behaves as tridentate ONS donor ligand and exhibited octahedral geometry. The Schiff base ligand and complexes were tested for their antibacterial activity against Staphylococcus aureus, Escherichia coli and Proteus vulgaris to assess their inhibiting potential. In screening medium was nutrient agar and biological screening were performed by employing cup plate method. Antibacterial activity of the ligand and its metal complexes is compared with the standard drug ciprofloxacin. In this series Co (II) complexes showed high antibacterial activity and the other complexes showed moderate antibacterial activity against different bacteria.

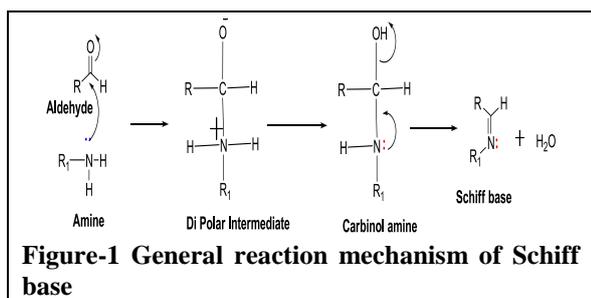
**KEYWORDS:** Schiff base, Metal complexes, antibacterial activity, ciprofloxacin.

### INTRODUCTION

Schiff base ligand are derived by the condensation of active carbonyl group with primary amine containing the azomethine (CH=N) group in which the carbonyl group of aldehyde or ketone is replaced by azomethine group under specific condition and it was named by Hugo Schiff (1864). Schiff base was invented by Hugo Schiff, which is named after him. Basically there are compounds with a functional group those possess a carbon-nitrogen double bond with the nitrogen atom connected to an aryl or alkyl group, not with hydrogen. In general sense Schiff bases, could be represented as the general formula  $R_1R_2C=NR_3$ , where R is an organic side chain. Some of them are restricted to the secondary aldimines (like azomethines as the carbon is attached with a hydrogen atom) with the general formula  $RCH=NR'$ .<sup>[1-3]</sup> Carbon-nitrogen double bond provides a significant contribution in various progresses of chemical sciences. Schiff-base compounds have been potentially used as fine chemicals and medical substrates. Number of methods are been applied for synthesis of Schiff base. In traditional method

of synthesis also known as organic synthesis, which commonly involve the removal solvents from the reaction mixture or liquid extraction especially in the case of aprotic dipolar solvent with high boiling point, or product isolation through liquid-liquid extraction.<sup>[4]</sup> Microwave-assisted reactions have been intensively investigated as mentioned in previous study. Microwave methods have been popularly used in Schiff base synthesis by organic synthesis as it has several benefits like better atom economy, environmental friendly, less hazardous, etc.<sup>[5-8]</sup>

In general mechanism of synthesis of Schiff base, an aromatic amine reacts with a carbonyl compound by nucleophilic addition. This forms a hemiaminal, followed by a dehydration to produce imines. Schiff bases obtained from aromatic aldehydes and aromatic amines have a shown number of applications in many fields including pharmaceutical, life sciences and chemical science including inorganic and analytical chemistry.<sup>[9-12]</sup>

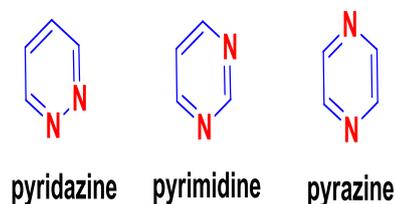


The chemistry of coordination compounds with heterocyclic ligands containing oxygen and nitrogen as donor atoms has attracted the attention of chemists in recent years. It is well known that such ligands coordinate to metal atom in different ways in different media. Transition metal complexes with Schiff base have played an important role in much biological system in nature.<sup>[13-15]</sup> The chelating properties of Schiff base display manifold applications in medicine and industry. Pyridine moieties have been reported to possess antimicrobial,<sup>[16]</sup> anti-inflammatory,<sup>[17]</sup> and anticonvulsant.<sup>[18]</sup> etc.

Schiff base metal complexes based research works have been widely carried out from 1930 because of their biological and industrial application.<sup>[19-22]</sup> In organic chemistry Schiff bases are most widely used as pigments and dyes, catalysts, intermediate in organic synthesis, and as a polymer stabilizer.<sup>[23]</sup> Schiff bases and their metal complexes are also well known to have pronounced biological activity.<sup>[24-25]</sup> and form an important class of compounds in medicine and pharmaceutical field also showed some degree of antibacterial, antifungal, antitumor, anticancer and anti-inflammatory activity.<sup>[26-34]</sup> the azo-methine linkage are responsible for the biological activity of Schiff bases.

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.<sup>[35,36]</sup> Hence, they have attracted considerable attention in the design of biologically active molecules<sup>[37,38]</sup> and advanced organic chemistry.<sup>[39,40]</sup> Also 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.<sup>[41]</sup> A totally unsaturated six membered -ring containing nitrogen is known as azine.<sup>[42]</sup> or pyridine (1); with two nitrogen atoms it is known as diazine.<sup>[43]</sup> and with a nitrogen at 1,2-position, it is known as pyridazine, at 1,3-position as Pyrimidine and at 1,4-position as

pyrazine (**Figure2**). However, the current review focus on the significance of Pyrimidines class of antimicrobial agents along with clinical and in vitro applications of pyrimidine derivatives to facilitate the development of more potent as well as effective antimicrobial agents.



**Fig 2 Structures of Pyridazine, Pyrimidine, Pyrazine**

Pyrimidines.<sup>[44]</sup> 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.<sup>[45, 46]</sup> Substituted purines and pyrimidines occur very widely in living organisms and were some of the first compounds studied by the organic chemists.<sup>[47]</sup>

The aim of this study was to make Schiff bases of 4-phenyl pyrimidine-2-carbohydrazide and their complexes, followed by characterization and antimicrobial activity determination of the schiff bases and metal complexes.

In view of these findings we have synthesized some metal complexes with a new Schiff base bis ((4-phenylpyrimidin-2-yl)((2-thioxo-1,2-dihydroquinolin-3-yl)methylene)hydrazono)methoxy) which was prepared by the reaction between 4-phenyl pyrimidine-2-carbohydrazide and 2-thioxo-1,2-dihydroquinoline-3-carbaldehyde.

## MATERIALS AND METHODS

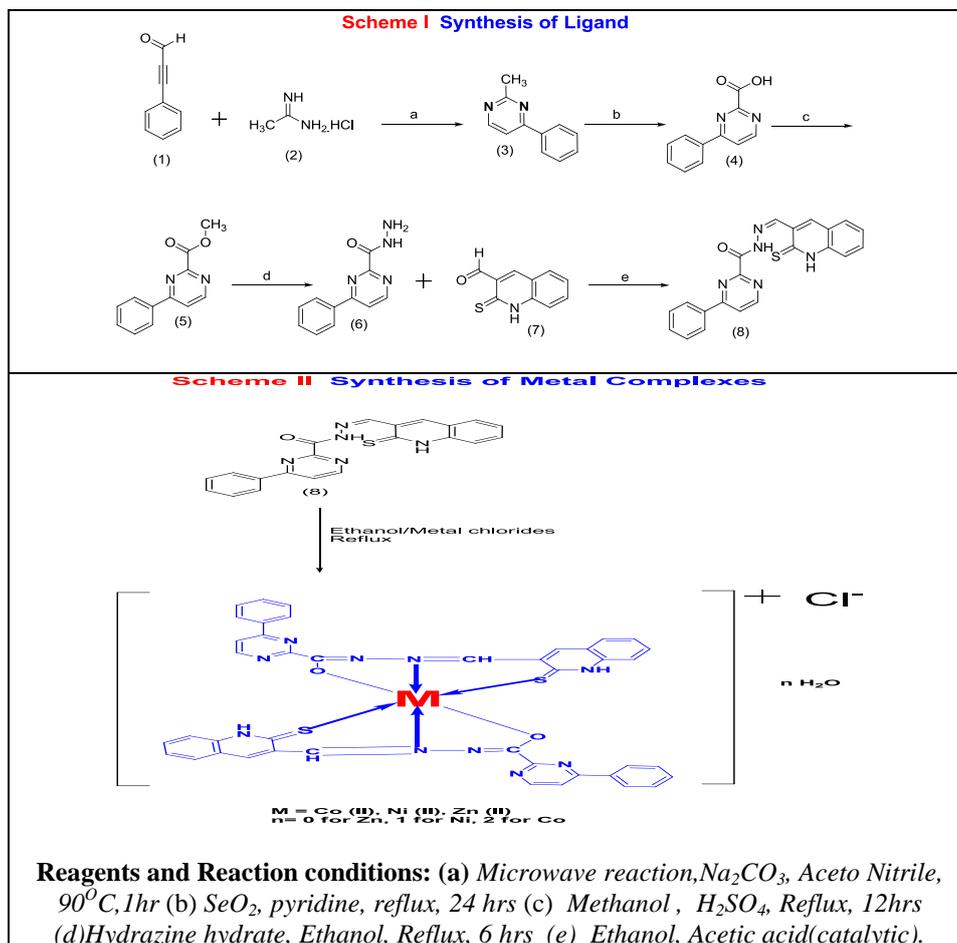
All the chemicals used were of analytical grade and were used without further purification.

## EXPERIMENTAL Section

All reagents and solvents used in this work were analytical grade and used directly. The melting points were determined in open glass capillaries tubes. Purity of the compounds was checked by thin layer chromatography (TLC) on silica gel G plates and spots were located by using iodine chamber. Elemental analysis (C, H, N) of all the synthesized compounds were

determined by perkin-Elmer 2400 elemental analyzer. The IR spectra were recorded on a Beckman Acculab-10 spectrometer ( $\nu_{\max}$  in  $\text{cm}^{-1}$ ) and the  $^1\text{H}$  NMR spectra

were recorded by Bruker DPX300 MHz using  $\text{CDCl}_3$  as solvent.



## Synthesis

### Synthesis of 2-methyl-4-phenyl pyrimidine(3)

A mixture of 3-phenylpropionaldehyde (0.01 mol) and acetimidine hydro chloride (0.01 mol) was stirred in dry aceto nitrile (10 ml) and dry  $\text{Na}_2\text{CO}_3$  (0.02 mol) was added to it. The stirring was continued for 0.5 hr under Micro Wave conditions at  $90^\circ\text{C}$ . Reaction progress was monitored by TLC. After completion of reaction cool to RT. Then concentrated under reduced pressure by using rota evaporator & Purified by column chromatography (100-200 mesh size silica) with elution of 10% Ethyl acetate to get pure yellow solid yield: 47 % mp:  $130^\circ\text{C}$ - $132^\circ\text{C}$ .

### Synthesis of 4-phenylpyrimidine-2-carboxylic acid (4)

A mixture of compound (3) (0.01 mol), selenium dioxide (0.05 mol), and pyridine (10 ml) was refluxed for 2 hours. Reaction progress was monitored by TLC. After completion of compound 3, concentrated under reduced pressure, then added water (10 ml), acidified with

Conc. HCl, white solid was formed, filter off, dried, to get 75 % yield. M.P.:  $187$ - $189^\circ\text{C}$ .

### Synthesis of Schiff base (8)

The Schiff base (ligand) was prepared by the reaction of 4-phenylpyrimidine-2-carbohydrazide (0.6 gm, 0.00280 mol) and 2-thioxo-1, 2-dihydroquinoline-3-carbaldehyde (0.530 gm, 0.0028 mol) in the presence of catalytic of glacial acetic acid (1-3 drops) under reflux for 7-8 h on the water bath. The reaction mixture was cooled at room temperature. The precipitate was then removed from the reaction mixture by filtration, washed with ethanol, dried and recrystallized from appropriate solvent. The synthesis of Schiff base have been shown in scheme 1.

### Synthesis of complexes

Schiff base (Z)-4-phenyl-N'-((2-thioxo-1,2-dihydroquinolin-3-yl)methylene)pyrimidine-2-carbohydrazide (0.77 gm, 0.002 mol) in ethanol (30 ml) and stirred gently for one hour to give homogeneous solution and added Ethanolic solution (10 ml) of

respective metal chlorides (0.002 mol). The resulting solution was refluxed for 5 h on steam bath and then sodium acetate (0.5 gm) was added to it and refluxed for 2 h. It was poured into distilled water. The solid complexes were collected by filtration, washed with distilled water and dried in a vacuum over anhydrous calcium chloride in a desiccator. The synthesis of metal complexes is given in scheme 2 and physical analytical data of ligand and its complexes are given in table 1.

### IR, <sup>1</sup>HNMR Spectra of Ligand

The IR spectrum of ligand showed peak at 1635 cm<sup>-1</sup> due to imine group and three absorption band at 3115 and 3060 cm<sup>-1</sup> due to amide NH and NH of quinolone moiety. The bands observed at 1635, 1562 and 1161 cm<sup>-1</sup> are due to carbonyl C=O, azomethene C=N and C=S function at 2-position of quinoline moiety respectively.

### IR Spectral data of the complexes of ligand

IR spectral data of ligand and its complexes are given in table 2. The IR spectrum of ligand was compared with those of the complexes of metal ions Co (II), Ni (II) and Zn (II) in order to study the binding mode of schiff base

to the metal ions. In the IR spectra of ligand the band appeared at 1635 cm<sup>-1</sup> assigned to carbonyl group (C=O) has disappeared in its complexes the above metal ions suggesting the involvement of carbonyl oxygen atom in coordination by the deprotonation after its enolization. All the complexes except Zn (II) complex of the ligand under the present study displayed broad bands in the region 3425-3410 cm<sup>-1</sup> indicating the presence of lattice or coordinated water molecules. The peak observed at 3060 cm<sup>-1</sup> due to quinolone NH has appeared at about the same region 3020-3075 cm<sup>-1</sup> in all the complexes indicates the non-involvement of quinolone NH in complexation. Absorption band at 1562 cm<sup>-1</sup> in case of ligand due to C=N of azomethine function has been found to be shifted towards the lower frequency side 1536-1515 cm<sup>-1</sup> in all the complexes indicates the metal ions have coordinated to azomethine nitrogen. A band at of all the complexes indicating the coordination of metal ions with sulfur of C=S function. The bands of weak intensity in the region 545-505 cm<sup>-1</sup> in case of all the complexes of the ligand are assigned M-O vibrations and the bands in the region 457-410 cm<sup>-1</sup> to M-N vibration.

**Table 1: Physical and analytical data of ligand and its complexes**

Compounds	Molecular formula	Mol.wt	M.P.(°C)	Elemental analysis (%) Calculated (Found)			
				M	C	H	N
Ligand	C <sub>15</sub> H <sub>11</sub> N <sub>5</sub> OS	308.442	342-346	---	65.44	3.92	18.17
Co- complex	Co[C <sub>42</sub> H <sub>26</sub> N <sub>8</sub> O <sub>2</sub> S <sub>2</sub> ] <sub>2</sub> H <sub>2</sub> O	863.285	364-366	6.82 (6.80)	58.40 (58.35)	3.73 (3.77)	16.21 (16.26)
Ni- complex	Ni[C <sub>42</sub> H <sub>30</sub> N <sub>10</sub> O <sub>2</sub> S <sub>2</sub> ] <sub>2</sub> H <sub>2</sub> O	845.591	370-372	6.80 (6.78)	58.41 (58.34)	3.73 (3.71)	16.22 (16.20)
Zn- complex	Zn[C <sub>34</sub> H <sub>28</sub> N <sub>8</sub> O <sub>2</sub> S <sub>2</sub> ]	832.113	346-349	7.84 (7.82)	60.47 (60.45)	3.38 (3.35)	16.79 (16.74)

**Table 2: IR data of ligand and its complexes**

Compound	ν <sub>H<sub>2</sub>O</sub>	Amide NH	Quinoline NH	ν <sub>C=O</sub>	ν <sub>C=S</sub>	ν <sub>C=N</sub>	ν <sub>M-O</sub>	ν <sub>M-N</sub>
Ligand	---	3112	3045	1655	1160	1562	---	---
Co- complex	3410	---	3020	---	1135	1536	515	467
Ni- complex	3425	----	3028	---	1145	1542	526	465
Zn- complex	---	---	3075	---	1164	1515	534	430

### Pharmacological Studies

#### Antibacterial Activity

All the newly synthesized metal complexes were tested for their antibacterial activity. Antibacterial activity was determined by agar cup plate method<sup>[48]</sup> against the following organism- Staphylococcus aureus, Escherichia coli and Proteus vulgaris. The zone of inhibition of each strain was recorded. Antibacterial activity of the ligand and its metal complexes was compared with the standard drug ciprofloxacin. The variation in the activity of different metal complexes against different micro-organism depends on their impermeability of the cell or

the differences in ribosomes in microbial cell. The lipid membrane surrounding the cell favours the passage of any lipid soluble materials and it is known that lipo solubility is an important factor controlling antibacterial activity.

**Table 3: Antibacterial activity of ligand and its complexes**

Compounds	Bacterial inhibition zone/mm		
	S. aureus	E. coli	P. vulgaris
Ligand	13	16	14
Co- complex	19	20	19
Ni- complex	14	13	18
Zn- complex	12	18	19
Ciprofloxacin	20	22	20

## RESULTS AND DISCUSSION

### Synthesis

2-methyl-4-phenyl Pyrimidine (3) was synthesized according to the reported procedure.<sup>[49]</sup> The reaction of 2-methyl- 4-phenyl pyrimidine with selenium di oxide in pyridine to afford the corresponding 4-phenyl pyrimidine-2-carboxylic acid(4) as per the reported procedure.<sup>[50]</sup>, which was reacted with methanol as per the reported procedure.<sup>[51]</sup> to afford methyl 4-phenylpyrimidine-2-carboxylate (5), which was reacted with hydrazine hydrate in Ethanol as per the reported procedure.<sup>[51]</sup> to afford 4-phenylpyrimidine-2-carbohydrazide (6). Compound (6) reacts with 2-thioxo-1,2-dihydroquinoline-3-carbaldehyde (6) to form (Z)-4-phenyl-N'-((2-thioxo-1,2-dihydroquinolin-3-yl)methylene)pyrimidine-2-carbohydrazide [Schiff base] (8) as per the reported procedure.<sup>[52]</sup>

The results showed (table-3) that ligand exhibits moderate activity against all tested bacteria. Co (II) complex showed high antibacterial activity against S. aureus, E. coli and P. vulgaris. Ni complex exhibited moderate activity against S. aureus and E. coli but highly effective against P. vulgaris. Zn complex exhibited moderate activity against S. aureus but showed good activity against E. coli and P. vulgaris microorganism. Antibacterial activity of the ligand and its metal complexes is compared with the standard drug ciprofloxacin. The variation in the activity of different metal complexes against different micro-organism depends on their impermeability of the cell.

### CONCLUSION

Based on overall study it was concluded that metal complexes of schiff bases are more efficient antimicrobial agent than its native form. However the antimicrobial potentiality of metal complex is highly dependent in the metal ion used for formation of metal complex. A new ligand (Z)-4-phenyl-N'-((2-thioxo-1,2-dihydroquinolin-3-yl)methylene)pyrimidine-2-carbohydrazide and its complexes have been synthesized and characterized by IR and <sup>1</sup>HNMR spectral data which includes that all the complexes exhibited octahedral

geometry. Some of these complexes have exhibited good antibacterial activity.

### REFERENCES

- Anand P., Patil V.M., Sharma V.K., Khosa R.L. and Masand N., Schiff bases: A Review on Biological Insights Biological activities of Schiff bases, *Int. J. Drug Des. Discov.*, 2012; 3: 851–866.
- Yang Z. and Sun P., Compare of three ways of synthesis of simple Schiff base., *Molbank*, 2006; 12–14.
- Savalia R.V, Patel A.P., Trivedi P.T., Gohel H.R. and Khetani D.B., Rapid and Economic Synthesis of Schiff Base of Salicylaldehyde by Microwave Irradiation., *Res. J. Chem. Sci.*, 2013; 3: 97–99
- Kumar N. and Sharma P., Synthesis of New Schiff-Base Complexes and Their Applications, *Int. J. Appl. Res. study*, 2013; 2: 1–6
- Somani R. et al., Optimization of Microwave Assisted synthesis of some Schiff 's bases, *Int. J. Chem Tech Res.*, 2010; 2: 172–179
- Kulshrestha A. and Baluja S., Microwave Promoted Synthesis of Some Schiff Bases, *Sch. Res. Libr*, 2010; 2: 221– 224
- Mohanambal D. and S A.A., Synthesis , Characterization and Antimicrobial activity of some novel schiff Base 3d Transition Metal Complexes Derived from Di hydro pyrimidinone and 4- Amino antipyrine, *Res. J. Chem. Sci.*, 2014; 4: 11–17
- Girgaonkar, M. V and Shirodkar, S. G. Synthesis, characterization and Biological studies of Cu (II) and Ni(II) complexes with New Bidentate Schiff's base ligands as 4-hydroxy-3-(1-(acrylimino)ethyl)chromen-2-one. *Res. J. Chem. Sci*, 2012; 1: 110–116
- Bader, N. R. applications of Schiff 's bases chelates in quantitative analysis : a review *RASAYAN J. Chem*, 2010; 3: 660–670
- Satyanarayana, V. S. V, Sreevani, P., Sivakumar, A. and Vijayakumar, V. Synthesis and antimicrobial activity of new Schiff bases containing coumarin moiety and their spectral characterization. *Arxivoc* 2008; 221–233.
- Patel, V., Trivedi, P., Gohel, H. and Khetani, D. Synthesis and Characterization of Schiff Base of p -chloro aniline and their Metal Complexes and their evaluation for Antibacterial Activity. *Int. J. Adv. Pharmacy, Biol. Chem.* 2014; 3: 999–1003
- Ashraf, M. A., Mahmood, K., Wajid, A., Maah, M. J. and Yusoff, I. Synthesis, Characterization and Biological Activity of Schiff Bases. *Int. Conf. Chem. adn Chem. Process* 2011; 10: 1–7.

13. Mohmoud WH, Mohamed GG and ElDessouky MMI. Synthesis, characterization and in vitro biological activity of mixed transition metal complexes of lornoxicam with 1,10phenanthroline. *Inter J Electrochem Sci.* 2014; 9: 1415-1438.
14. Eissa HH. Synthesis, characterization and antibacterial activity of macrocyclic Schiff bases based on 1,3-docarbonyl phenyl dehydrazide 1,4-docarbonyl phenyl dehydrazide. *Organic Chemistry Current Research.* 2013; 2(3): 1-8.
15. Bhagat SM and Deshpande MM. Synthesis and antimicrobial activity of some transition metal ion chelates of 2-(cinnamyl)-4-bromo-6-methyl benzothiazolyl hydrazones. *Journal of Chemical and Pharmaceutical Research.* 2012; 4(3): 1454-1456.
16. Patel NB, Agraval SN and Shaikh FM. Synthesis and antimicrobial activity of new pyridine derivatives. *Med. Chem. Research.* 2011; 20: 1033-1041.
17. Al-Omar MA, Amr A-G and Al-Salahi RA. Antiinflammatory, analgesic, anticonvulsant and antiparkinsonian activities of some pyridine derivatives using 2,6-disubstituted isonicotinic acid hyrazides. *Arch Pharm.* 2010; 343(11-12): 648-656.
18. Pattan SR, Dighe NS, Musmade DS, Tambe SK, Kale SH, Craware VM and Chavan PA. Synthesis and evaluation of some new substituted 1,4 dihydropyridine derivatives and their anticonvulsant activity. *J Chem Parm Res.* 2010; 2(1): 246-252.
19. M.A.Ali, S.E. Livingstone and D.J. Philips, *Inorganic Chimica Acta.*, 1973; 7: 179.
20. M.A. Ali and R. N. Bose, *Polyhedron*, 1984; 3(35): 517.
21. S. Kumar, D.N. Dhar, and P. N. Saxena, Applications of metal complexes of Schiff bases a review, *Journal of Scientific and Industrial Research.*, 2009; 68(3): 181-187.
22. T. Punniyamurthy, S. J. S. Kalra and J. Iqbal., *Tetrahedron Letters*, 1995; 36: 8497.
23. D.N. Dhar, C.L. Taploo, Schiff bases and their application, *J.Sci. Ind. Res.* 1982; 41(8): 501.
24. L. Messori, F.Abbate, G. Marcon et al, *Journal of Medicinal Chemistry.*, 2000; 43(19): 3541.
25. Md.Kudrat-E-Zahan and M. S. Islam, *Russain Journal of General Chemistry.*, 2015; 85(4): 979.
26. Dr.A. Xavier, N. Srividhya, 2014; 2278-5736, 7, 11, 06-15.
27. R.R Gupta, Kumar, V. Gupta, *Heterocyclic chemistry I*, 1998.
28. D.Worku, M Negussie, V. J.T. Raju, S. Theodros, J.A. Jonson, *Bull. Chem. Soc. Ethiop.* 2002; 29.
29. D.Worku, M Negussie, V. J.T. Raju, R. Negussie, *Bull. Chem. Soc. Ethiop.* 2002; 17: 30.
30. D.N. Dhar, C.L. Taploo, Schiff bases and their applications. *J Sci Ind Res* 1982; 41(8): 501.
31. G.Bringmann, M. Dreyer, J.H. Faber, Dalsgaard, D Pw, Staerk, J.W. Jaroszewski, et al. Ancistrotanzanine C and related 5,1/Jaroszewski JW, et al. Ancistrotanzanine C and related 5,1/ and 7,3/ coupled naphthylisoquinoline alkaloids from *Ancistrocladus tanzaniensis*. *J Nat prod* 2004; 67(5): 743.
32. P.Przybylaki, A.Huczynaki, K. Pyta B. Brzenzinski, F. Bartl, Biological properties of Schiff bases and azo derivatives of phenols, *Curr. Org. Chem.* 2009; 13(2): 124-48.
33. A.O. de Souza, F.C.S0 Galetti, C.L. Silva, B. Bicalho, M.M.Pharma, S.F. Fonseca, et al. Antimycobacterial and cytotoxicity activity and natural compounds. *Quim Nova* 2007; 30(7): 1563-6.
34. Z. Guo, R. Xing, S. Liu, Z. Zhong, X. Ji, L. Wang et al. Antifungal properties of Schiff bases of chitosan, N-substituted chitosan and quaternized chitosan. *Carbohydrate Res* 2007; 342(10): 1329-32.
35. Y. Ju and R. S. Varma, "Aqueous Nheterocyclization of primary amines and hydrazines with dihalides: microwaveassisted syntheses of N-azacycloalkanes, isoindole, pyrazole, pyrazolidine, and phthalazine derivatives," *Journal of Organic Chemistry*, 2006; 71(1): 135-141.
36. 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,
37. P. D. Lokhande, B. Y. Waghmare, and S. S. Sakate, "Regioselective one-pot synthesis of 3,5-diarylpyrazoles," *Indian Journal of Chemistry B*, 2005; 44(11): 2338-2342.
38. 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.
39. C. A. Zificsak and D. J. Hlasta, "Current methods for the synthesis of 2-substituted azoles," *Tetrahedron*, 2004; (60)41: 8991- 9016,
40. 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): 112277- 2279.

41. M. García-Valverde and T. Torroba, "Special issue: sulfur-nitrogen heterocycles," *Molecules*, 2005; (10)2: 318–320.
42. 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.
43. A. Manlove and M. P. Groziak, "Six-membered ring systems: diazines and benzo derivatives," *Progress in Heterocyclic Chemistry*, 2009; 21: 375–414.
44. A. D. Russell, "Mechanisms of bacterial insusceptibility to biocides," *The American Journal of Infection Control*, 2001; 29(4): 259–261.
45. D. J. Brown, *Comprehensive Heterocyclic Chemistry*, vol. 14, Pergamon Press, Oxford, UK, 1984.
46. R. C. Elderfield, *Heterocyclic Compounds*, vol. 6, John Wiley & Sons, New York, NY, USA, 1957.
47. P. Y. Bruice, *Organic Chemistry*, Pearson Education, Singapore, 3<sup>rd</sup> edition, 2007.
48. Smith QE. In pharmacological screening tests progress in medicinal chemistry. Butterworths London. 1960; 1: 1-33.
49. Bagley, Mark C.; Hughes, David D.; Taylor, Paul H. *Synlett*, 2003 , # 2 p. 259 – 261 [50]. Sakamoto; Sakasai; Yamanaka *Chemical and Pharmaceutical Bulletin*, 1980; 28(2): 571 – 577.
50. Wu, Jiahui; Zhang, Dengyou; Chen, Lei; Li, Jianneng; Wang, Jianling; Ning, Chengqing; Yu, Niefang; Zhao, Fei; Chen, Dongying; Chen, Xiaoyan; Chen, Kaixian; Jiang, Hualiang; Liu, Hong; Liu, Dongxiang *Journal of Medicinal Chemistry*, 2013; (56)3: 761 – 780.
51. Li, Huan-Qiu; Li, Dong-Dong; Lu, Xiang; Xu, Yun-Yun; Zhu, Hai-Liang *Bioorganic and Medicinal Chemistry*, 2012; (20)1: 317-323.