



## SYNTHESIS, CHARACTERIZATION AND ANTI-MICROBIAL STUDY OF HETEROCYCLIC SCHIFF BASE LIGANDS OF 3-ACETYL 4-HYDROXY QUINOLIN-2-ONE

S. Anjanikar<sup>1</sup>, S. Chandole<sup>\*2</sup>

<sup>1</sup> Department of Chemistry, Sharadchandra College, Naigaon, District- Nanded MS- 431709,  
India,

(Affiliated to Swami Ramanand Teerth Marathwada University, Nanded)

<sup>\*2</sup> Department of Chemistry, S.G.B. College, Purna Jn., MS- 431511, India.

[\\*schandole@reddifmail.com](mailto:schandole@reddifmail.com)

### ABSTRACT:

Novel heterocyclic Schiff bases were synthesized from 3-Acetyl 4-Hydroxy Quinolin-2(1H)-one, 4-hydroxy-3-(1-(pyridin-2-ylimino)ethyl)quinolin-2(1H)-one, 4-hydroxy-3-(1-(pyridin-3-ylimino)ethyl)quinolin-2(1H)-one, 4-hydroxy-3-(1-(pyridin-4-ylimino)ethyl)quinolin-2(1H)-one and 3-(1-((5-chloropyridin-2-yl)imino)ethyl)-4-hydroxyquinolin-2(1H)-one. These were prepared by condensation of 3-Acetyl 4-Hydroxy Quinolin-2-one with substituted amino pyridine moiety. These Schiff base derivatives were characterized by IR, <sup>1</sup>HNMR, <sup>13</sup>CNMR and mass spectral analysis. In vitro biological screening effects of the synthesized compounds were tested for their antibacterial and antifungal activity. For antibacterial activity the bacterial species used were *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi*, and *Staphylococcus aureus* while fungal species used were, *Aspergillus flavus*, *Penicillium chrysogenum*, *Aspergillus niger* and *Fusarium moneliforme*. The antibacterial activity and antifungal activity was screened using agar well diffusion method.

**KEYWORDS:** 3-Acetyl 4-Hydroxy Quinolin-2-one, Schiff Bases, Amino Pyridine, Biological study.

### INTRODUCTION:

Heterocyclic compounds occur in a wide variety of natural and synthetic compounds and are essential to life in various ways. Due to excellent applications of Heterocyclic compounds, they have attracted significant interest from chemists and pharmacologists and a huge number of applications have been reported in the literature related to the biological activities of Heterocyclic compounds.<sup>i-iv</sup> The quinoline moiety is found in a large number of dyes, and pharmaceutical compounds and forms a very important class of heterocyclic compounds and several of these have medicinal values.<sup>v</sup> Quinolines and their derivatives have shown the broad range of biological applications such as antimalarial,<sup>vi-vii</sup> bactericidal,<sup>viii-ix</sup> and anti-inflammatory,<sup>x-xi</sup> antioxidant,<sup>xii</sup> anticancer,<sup>xiii</sup> and anti-inflammatory.<sup>xiv</sup>

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Schiff bases have gained considerable importance due to their synthetic flexibility, selectivity and sensitivity towards the central metal atom.<sup>xv</sup> The complexes formed by Schiff bases through chelation with oxygen, nitrogen donors have revealed wide variety of biological activities against bacteria, fungi, and certain type of tumors.<sup>xvi-xvii</sup> Imine or azomethine groups present in such compounds are responsible for enhancing their biological activities.<sup>xviii</sup> In view of the above facts and their increasing importance in pharmaceutical and biological field, it was considered of interest to synthesize some novel compounds integrating the two active pharmacophores in a single molecular frame work and to evaluate their biological activities. Hence an attempt was made towards the synthesis of Schiff bases with substituted 3-acetyl-1-amino-quinolin-2-one and to investigate the biological activity. Hence all the synthesized compounds were evaluated for their antimicrobial activity compared with standard drugs.

## EXPERIMENTAL:

### General Procedure for synthesis of 3-Acetyl 4-Hydroxy Quinolin-2(1H)-one:

To a clean and dry 500 ml three-necked round-bottomed flask is placed 8.7 gms freshly pieces of cut neat sodium metal. The central neck of this flask is firmly fixed with a glass plug, the right neck is attached to a separating funnel with 125 ml of anhydrous absolute alcohol. The left neck is fitted to a double surface condenser. The condenser as well as separating funnel is attached to their respective top with a guard tube containing anhydrous calcium chloride. The three-necked round-bottomed flask is shielded with a cloth soaked in water to control the vigor of the following reaction and placed in a large water bath with cold water. Absolute alcohol, (70 ml) is poured slowly with care into the flask over sodium. The forced reflux of alcohol in the condenser is controlled by running a stream of cold water over a wet cloth. Lastly, the remaining alcohol is added. A heavy stirrer is introduced from the central neck of the three-necked round-bottomed flask. From the central neck is added 48.75 gms (42 ml) of freshly purified ethyl acetoacetate and 56.5 gms (48 ml) of methyl anthranilate on stirring the solution. The reacting mixture is heated at reflux for 6hrs. The progression of reaction is observed by TLC. On completion of the reaction, the contents were cooled and poured over ice. On acidification with acetic acid, the white solid is formed is filtered, washed with water and dried. Glacial acetic acid is used for the recrystallization of product. Yield: 77% melting point: 162-164°C

### General Procedure for Synthesis of Schiff Bases of 3-Acetyl 4-Hydroxy Quinolin-2(1H)-one:

Accurately weighed 10.2 gms (0.05 moles) of 3-acetyl-4-hydroxy-quinolin-2(1H)-one (I), 0.05 moles aromatic amine (IIa-d) and 0.2 grams of N,N-dimethylpyridin-2-amine as a catalyst is mixed with 100ml of pure ethyl alcohol. The reaction mixture in alcohol is heated at refluxing temperature over a heating mantle for three hours. After three hours, the mixture is cooled. The solid Schiff base is washed with ethanol and filtered at suction. The Schiff base is dried and recrystallized from ethanol. The purity of the ligands was checked by m.p. and TLC.

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# REACTION SCHEME:

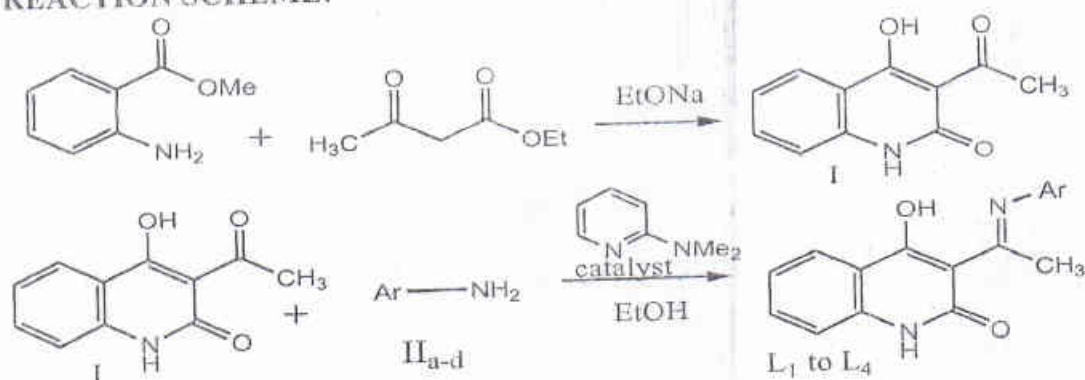


Fig. 1 Synthesis of ligand L<sub>1</sub> to L<sub>4</sub>

II a Ar = pyridin-2-amine,

II b Ar = pyridin-3-amine,

II c Ar = Pyridin-4-amine

II d Ar = 5-Chloropyridin-2-amine,

## BIOLOGICAL STUDY:

### ANTIBACTERIAL ACTIVITY

The antibacterial activity was measured by agar well diffusion method.<sup>xix</sup> The bacteria used as test organism were *Bacillus subtilis* and *Salmonella typhi* as a gram positive while *Staphylococcus aureus* and *Escherichia coli* as a gram-negative bacterial strains. All tests for antimicrobial activity were performed on Mueller Hinton Agar for bacteria. Ampicillin was used as positive control for bacteria. DMSO was used as solvent and positive control. The dehydrated media powder and antibiotics were purchased from Hi-Media, India. Sterile MH broth was inoculated with test organisms aseptically using sterile wire-loop and was incubated at 37°C for 18 hrs. This suspension was used as inoculants. With help of sterile cork borer wells of 10mm diameter were prepared in the media plates for the addition of compound solutions and controls. 100µl of the compound solution was added to the wells aseptically with the help of micropipette to achieve a final concentration of 10 µg of compound in each well. Same amount of DMSO and Ampicillin solution was added as controls. The plates were kept in refrigerator for half an hour for diffusion of solutions in agar media. Then plates were incubated at 37°C for 24 Hours. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. The clear zone was measured with measuring scale in mm.

### ANTIFUNGAL ACTIVITY

Antifungal activity was performed by agar well diffusion method.<sup>xx</sup> A culture of Potato Dextrose Agar (PDA) media was used for the test of antifungal activity. This medium was placed in Autoclave under 15 psi pressures, at 121°C for 25 min for sterilization of media. 2 ml of each compound was poured in sterilized Petri plates followed by the addition of 20mL of sterilized melted PDA and agitated gently in circular motion to become homogenized. The same procedure was done with positive Neomycin and negative DMSO controls. For testing the antifungal activity *Aspergillus niger*, *Aspergillus flavus*, *Fusarium moneliforme*, *Penicillium chrysogenum* were selected. Using a sterile wire-loop the fungal spores from slant culture was transferred to test tube containing sterile saline and mixed well. This spore solution was used as inoculant. The plates were incubated at room temperature for four days. After incubation plates were observed for the growth of inoculated fungi. Results were recorded.

## RESULT AND DISCUSSION:

All the reactions were carried out under conventional methods. 3-acetyl-4-hydroxy-quinolin-2(1H)-one (I) was the intermediate that required for preparing Schiff bases 4-hydroxy-3-(1-(arylimino)ethyl)quinolin-2(1H)-one (L<sub>1</sub>-L<sub>4</sub>). 3-acetyl-4-hydroxy-quinolin-2(1H)-one (I) was prepared from Methyl anthranilate and Ethyl acetoacetate in presence of sodium ethoxide. The reactions were carried out in a protective hood. The intermediate product (I) formed was recrystallized in ethanol and purity was tested by TLC. 4-Hydroxy-3-(1-(aryl imino)ethyl)quinolin-2(1H)-one (L<sub>1</sub>-L<sub>4</sub>) were obtained by refluxing in ethanol for 4 hrs. Increase in the time of refluxing did not improve the yield of product.

Assignment of significant peaks observed in IR, <sup>1</sup>H NMR, <sup>13</sup>C NMR spectra of the compounds (L<sub>1</sub>-L<sub>4</sub>) is clarified in the analytical data. The IR spectra of compound (L<sub>1</sub>-L<sub>4</sub>) showed high intensity band observed at 1600 cm<sup>-1</sup> is assigned to ν(C=N) vibration suggesting the formation of Schiff base.<sup>xxi</sup> Broad weak band around 3514- 3494 cm<sup>-1</sup> is assigned to H bonded -OH in the Schiff bases. The band at 1567-1480 cm<sup>-1</sup> is assigned to the combination of ν(C=C) of the aromatic ring. A high intensity band in the region 1224 - 1260 cm<sup>-1</sup> is assigned to enolic ν(O-H) vibration and 1654 -1662 cm<sup>-1</sup> for lactam (-NH-C=O).<sup>xxii</sup> Each one of the <sup>1</sup>H NMR spectra of (L<sub>1</sub>-L<sub>4</sub>) revealed singlet for 3H between 2.63 -2.72 ppm assigned to imino methyl group. Peaks between 8.2- 6.94 ppm are assigned to aromatic protons of quinolone and pyridine moiety. <sup>1</sup>H NMR spectra of compounds (L<sub>3</sub>) showed double doublet at 8.54 & 7.22 ppm confirming para substitution at aryl moiety bonded to imino nitrogen while in L<sub>4</sub>, singlet of one hydrogen of pyridine observed at 8.81 ppm indicates the presence of electronegative atoms adjacent to it. A broad singlet at 16.55-16.65 ppm confirms the presence of 4-hydroxyl group.<sup>xxiii</sup> <sup>13</sup>C NMR showed peaks between 165-160 ppm for lactam carbon, between 167-176 ppm for imine carbon. Assignment given to other peaks observed in <sup>1</sup>H NMR, <sup>13</sup>C NMR spectra and also molecular ion peaks in mass spectra justifies the structures of compounds (L<sub>1</sub>-L<sub>4</sub>).

The Schiff's bases synthesized were evaluated for anti-bacterial and anti-fungal activity with different strains of bacteria and fungi. Results are shown in Table-1 and Table-2. All imines except (L<sub>4</sub>) have shown lesser activity against *E. coli*, *S. aureus* and *B. subtilis* compared with Ampicillin taken as standard. All compounds have shown maximum zone of inhibition with *S. Typhi*. The activity of compound (L<sub>4</sub>) was maximum with all the bacteria. This may be due to the presence of halogen in the molecule. All Schiff's bases have shown lesser activity against *Aspergillus niger*, *Aspergillus flavus*, *Fusarium moneliforme*, *Penicillium chrysogenum* with Neomycin taken as standard. Antifungal activity observed against *Penicillium chrysogenum* species was encouraging in comparison with other fungi. However, compound (L<sub>4</sub>) reduced the growth to greater extent as compare to other compounds. The presence of Chlorine atom in the molecule (L<sub>4</sub>) might responsible for enhanced antibacterial and antifungal activity among the others (L<sub>1</sub>-L<sub>3</sub>).<sup>xxiv</sup>

Spectral data for the synthesized compound is given as below

3-Acetyl 4-hydroxy quinolin-2(1H)-one, IR (KBr, cm<sup>-1</sup>): 3500-2600, 1658, 1690, 1555, 1505, 1340; <sup>1</sup>H NMR (CDCl<sub>3</sub>, in ppm): δ 2.74 (s, 3H), 7.20-8.00 (m, Ar-H), 11.52 (bs, s, 1H), 17.21 (bs, s, 1H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, in ppm): δ 29.64, 104, 142-120, 164, 175, 210.41; Mass Spectra: [M<sup>+</sup>] = 204.; Colour: White; Yield: 73%; m.p. 162-164°C.

L<sub>1</sub>: 4-hydroxy-3-(1-(pyridin-2-ylimino)ethyl)quinolin-2(1H)-one, IR (KBr, in cm<sup>-1</sup>): 3494, 3349, 1658, 1612, 1600, 1575, 1480, 1369, 1224, 748 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, in ppm): δ 2.22 (s, 3H), 8.06-7.22 (m, 4H), 7.59-6.95 (m, 4H), 16.55 (s, 1H), 10.56 (s, 1H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, in ppm): δ 20, 85, 120, 151, 117-145, 165, 162, 174; Mass Spectra [M<sup>+</sup>]: 280.08; Yield: 74%; Colour: yellow; Melting Point: 203-205°C.

**L<sub>2</sub>: 4-hydroxy-3-(1-(pyridin-3-ylimino)ethyl)quinolin-2(1H)-one**, IR (KBr in cm<sup>-1</sup>): 3514, 3298, 1660, 1609, 1600, 1577, 1486, 1380, 1250, 755 cm<sup>-1</sup>; <sup>1</sup>HNMR (CDCl<sub>3</sub>, in ppm): 82.32 (S, 3H), 8.06-7.02 (m, 3H), 8.60 (S, 1H), 7.59-6.95 (m, 4H), 16.56 (S, 1H), 10.46 (S, 1H); <sup>13</sup>CNMR (CDCl<sub>3</sub>, in ppm): 21, 81, 119, 154, 116-148, 162, 165, 176; Mass Spectra[M<sup>+</sup>]<sup>+</sup>: 280.34; Yield, 72%; Colour: yellow; Melting Point: 210-212 °C.

**L<sub>3</sub>: 4-hydroxy-3-(1-(pyridin-4-ylimino)ethyl)quinolin-2(1H)-one**, IR (KBr in cm<sup>-1</sup>): 3504, 3334, 1654, 1610, 1600, 1574, 1476, 1366, 1226, 751; <sup>1</sup>HNMR (CDCl<sub>3</sub>, in ppm): 82.22 (S, 3H), 8.54 & 7.22 (dd, 4H), 8.06-7.24 (4H), 16.55 (S, 1H), 10.56 (S, 1H); <sup>13</sup>CNMR (CDCl<sub>3</sub>, in ppm): 24, 83, 114, 149, 114-140, 160, 163, 167; Mass Spectra[M<sup>+</sup>]<sup>+</sup>: 280.32; Yield: 75%; Colour: Pale Yellow; Melting Point: 214-216 °C.

**L<sub>4</sub>: 3-(1-((5-chloropyridin-2-yl)imino)ethyl)-4-hydroxyquinolin-2(1H)-one**. IR (KBr, in cm<sup>-1</sup>): 3509, 3339, 1662, 1612, 1600, 1514, 1469, 1260, 747; <sup>1</sup>HNMR (CDCl<sub>3</sub>, in ppm): 82.63 (S, 1H), 88.81 (S, 1H), 88.36-8.33 & 87.19-7.18 d(2H), 88.16-7.51 m (Ar-H), 816.65 (S, 1H), 810.68 (S, 1H); <sup>13</sup>CNMR (CDCl<sub>3</sub>, in ppm): 20, 884, 8155-115, 8161, 8162, 8176; Mass Spectra[M<sup>+</sup>]<sup>+</sup>: 314.52; Yield: 82%; Colour: Pale Green; Melting Point: 227-229 °C.

**Table No.1 Anti- Bacterial Activity**

Synthesised Qunoline Schiff Bases	Zone of Inhibition(diameter in mm)			
	<i>E. coli</i>	<i>S. typhi</i>	<i>S. aureus</i>	<i>B. subtilis</i>
Ampicillin (Reference)	18	19	17	16
L <sub>1</sub> : 4-hydroxy-3-(1-(pyridin-2-ylimino)ethyl)quinolin-2(1H)-one,	12	14	12	10
L <sub>2</sub> : 4-hydroxy-3-(1-(pyridin-3-ylimino)ethyl)quinolin-2(1H)-one	13	14	12	10
L <sub>3</sub> : 4-hydroxy-3-(1-(pyridin-3-ylimino)ethyl)quinolin-2(1H)-one	12	14	12	12
L <sub>4</sub> : 3-(1-((5-chloropyridin-2-yl)imino)ethyl)-4-hydroxyquinolin-2(1H)-one	17	18	18	17

**Table No.2 Anti- fungal Activity**

Synthesised Qunoline Schiff Bases	Growth of Fungi			
	<i>Aspergillus niger</i>	<i>Aspergillus flavus</i>	<i>Fusarium moniliforme</i>	<i>Penicillium chrysogenum</i>
Neomycin(Reference)	-	-	-	-
L <sub>1</sub> : 4-hydroxy-3-(1-(pyridin-2-ylimino)ethyl)quinolin-2(1H)-one,	++	+++	++	+++
L <sub>2</sub> : 4-hydroxy-3-(1-(pyridin-3-ylimino)ethyl)quinolin-2(1H)-one	+++	++	++	+
L <sub>3</sub> : 4-hydroxy-3-(1-(pyridin-3-ylimino)ethyl)quinolin-2(1H)-one	++	+	+	+

*S. Chandole*  
**Coordinator**  
**IQAC**



*S. Chandole*  
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L4:3-(1-((5-chloropyridin-2-yl)imino)ethyl)-4-hydroxyquinolin-2(1H)-one

Moderate growth (++), Reduced growth (+) and No growth (-) of fungi

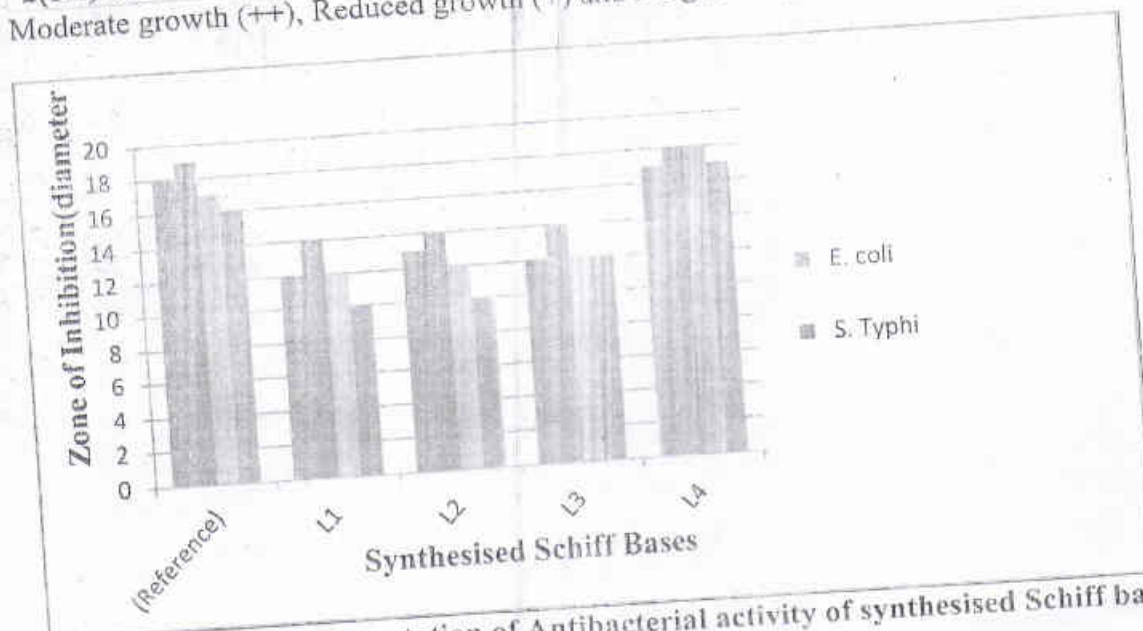


Fig.2 Graphical Representation of Antibacterial activity of synthesised Schiff bases.

## CONCLUSION:

In the present paper we have synthesized the 3-acetyl-4-hydroxy quinolin-2-(1H)-one and their Schiff base ligand by condensation with amino pyridine. Biological activities of synthesized compounds were tested against bacteria and fungi. The Antibacterial and antifungal study revealed that all compounds have shown good activity and compound L4 have found to be more active as compared to others.

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**Co-ordinator  
IQAC**

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**PRINCIPAL**  
Shri Guru Buddhiswami Mahavidyalaya  
Purna (Jn.) Dist. Pa.