



## “Anti-microbial Study and Synthesis of Schiff bases of 3-actyl 4-hydroxy Quinolin-2-one”

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### ABSTRACT

Quinoline based new Schiff bases were synthesized from 3-Actyl 4-Hydroxy Quinolin-2-(1H)-one and screened their antibacterial and antifungal activity. The Schiff bases 4-hydroxy-3-(1-((4-picolin-2-yl)imino)ethyl)quinolin-2-(1H)-one(L<sub>1</sub>), 4-hydroxy-3-(1-((5-picolin-2-yl)imino)ethyl)quinolin-2-(1H)-one(L<sub>2</sub>), 4-hydroxy-3-(1-((6-picolin-2-yl)imino)ethyl)quinolin-2-(1H)-one(L<sub>3</sub>) and 4-hydroxy-3-(1-((3-nitro-4-picolin-2-yl)imino)ethyl)quinolin-2-(1H)-one(L<sub>4</sub>) were prepared from 3-Acetyl 4-Hydroxy Quinolin-2-One with 2-amino picolines. The structures of Schiff bases were confirmed by Infrared, mass, proton-NMR and <sup>13</sup>CNMR spectral analysis. *In vitro* studies of these Schiff bases were carried out for their antibacterial activity by Agar contact method and antifungal activity by the poison plate method. The bacterial species used were *B. subtilis*, *E. coli*, *S. typhi* and *S. aureus*. Fungal species used were *F. moniliforme*, *A. niger*, *A. flavus*, and *P. chrysogenum*.

**Keywords:** 3-Actyl 4-Hydroxy Quinolin-2-One, Amino picoline, Spectral study, Schiff bases, Biological study.

### INTRODUCTION

Study of nitrogen containing heterocyclic moiety is being very popular area of interest for researchers as these possess pharmacological properties. Quinoline is an important fused heterocyclic aromatic compound. 4-hydroxyquinolin-2(1H)-one, the essential moiety of major interest of the many research as it has importance due to its synthetic, medicinal values.<sup>1,2</sup> The importance of this structural moiety is due to its presence in many naturally occurring organic heterocyclic compounds. Bucharidine (I) and foliosidine (II) is quinoline

alkaloid, extracted from *Haplophyllum bucharicum* and *Haplophyllum foliosum* respectively. Both have estrogenic action.<sup>3</sup> Viral-RNA polymerase Inhibitory action of few 4-hydroxyquinoline-2-one derivatives such as Compounds (III) and (IV) strongly prevent the replication of the Hepacivirus C.<sup>4-7</sup>

In addition to this 4-Hydroxy-2(1H)-quinolinones along with their derivatives are among the group of valuable heterocyclic compounds associated with many pharmacological,<sup>8-13</sup> various medicinal values such as analgesic,<sup>14</sup> anti-



  
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inflammatory,<sup>15</sup> diuretic,<sup>16</sup> antiallergenic,<sup>17</sup> orally active antagonists,<sup>18</sup> cardiovascular agents,<sup>19</sup> anticonvulsant,<sup>20</sup> antimicrobial (antibacterial and antifungal),<sup>21-23</sup> antitubercular,<sup>24</sup> dye-stuffs,<sup>25</sup>

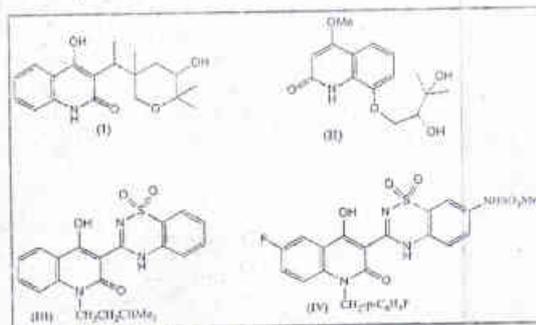


Fig. 1. Biologically active Quinoline derivatives

The condensation reaction of carbonyl moiety with primary amines forms azomethine group also referred as Schiff's base. The researcher have observed their wide spread pharma-ceutical applications such as, anti-oxidant,<sup>26</sup> anti-cancer,<sup>27</sup> antimicrobial,<sup>28</sup> anti-inflammatory.<sup>29</sup>

Contemplating the above evidences and their increasing significance of medicinal and biological values initiated to synthesize few new Schiff bases with substituted amino picolines and 3-acetyl-4-hydroxyquinolin-2-one and study their biological activity. All these Schiff bases were subjected to antibacterial and antifungal assessment competing with standard drugs.

## EXPERIMENTAL

### Synthesis of 3-acetyl-4-hydroxyquinolin-2-one

3-acetyl-4-hydroxyquinolin-2-one is prepared by refluxing methyl 2-aminobenzoate (48 mL) and ethyl 3-oxobutanoate (42 mL) with catalytic amount of sodium (8.7 g) in absolute ethanol (125 mL) for six hours. After completion of reaction, mixture was poured over ice and acidify with acetic acid, white colored solid formed was filtered and washed with water. Glacial acetic acid is used for the recrystallization of product.

### Synthesis of schiff bases of 3-acetyl-4-hydroxyquinolin-2-one

100 mL of pure ethyl alcohol is combined with 0.05 moles of 3-acetyl-4-hydroxyquinolin-2-one (I), 0.05 moles of aromatic amine (IIa-d), and 0.2 g of N,N-dimethylpyridine as a catalyst. Over a

heating mantle, the reaction mixture in the alcohol is heated for three hours at refluxing temperature. The mixture is cooled three hours later. The solid Schiff base is vacuum filtered after being washed with ethanol. The Schiff base is dried and recrystallized from ethanol. The purity of the Schiff bases was checked by m.p. and TLC.

## Biological activity

### Antibacterial activity

Anti-bacterial activity was performed by agar contact method.<sup>30</sup> *B. subtilis* and *S. typhi* were gram+ve bacteria that were utilized as test organisms, whereas *S. aureus* and *E. coli* were gram-ve microorganisms. Mueller Hinton Agar for bacteria was used for all tests for antibacterial activity. Ampicillin was used as positive control for bacteria. The solvent and positive control used was DMSO. Antibiotics and dehydrated media powder were brought from Hi-Media, India. Using sterile wire-loop, test organisms were aseptically added to sterile MH broth before being incubated at 37°C for 18 hours. This suspension was utilized as an inoculant. Wells in the media plates with a 10mm diameter were made using a sterile cork borer for the addition of compound solutions and controls. With the aid of a micropipette, 100 µL of the compound solution was aseptically poured to the wells to reach a ultimate strength of 10 g of compound in each well. As controls, the same quantity of DMSO and ampicillin solution were introduced. The plates were cooled for 30 min to allow solutions to diffuse through the agar substrate. Further, Plates were incubated at 37°C for a period of 24 hours. The zone margin should be regarded as the region that does not clearly display any expansion that the unaided eye can see. With a measuring scale in millimetres, the clean zone was measured.

### Antifungal activity

The poison plate approach was used to provide antifungal activity.<sup>31</sup> For the evaluation of antifungal activity, Potato Dextrose Agar (PDA) media was utilized as a culture. The sterilization of the medium was archived by autoclaving at 120-125°C for 25-30 min under 15 psi of pressure. 20 mL of sterilized, melted PDA was added to sterilized petri plates with 2 mL of each component, and the mixture was then gently stirred in a circular motion to get homogenized. With positive Neomycin and negative DMSO controls, the identical process

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was followed. *A. niger*, *A. flavus*, *F. moniliforme*, and *P. chrysogenum* were chosen to assess the antifungal activities. The fungal spores from the slant culture were transferred to a test tube containing sterile saline and thoroughly mixed with a sterile wire loop. As an inoculant, this spore solution was employed. The plates were kept for incubation for 100 h at room temperature. Further, the growth of the infected fungi was monitored on the plates. The outcomes were noted.

**RESULT AND DISCUSSION**

All reactions were conducted using standard procedures. In the presence of sodium ethoxide, methyl anthranilate and ethylacetoacetate were refluxed to produce the intermediate 3-acetyl-4-hydroxy-quinolin-2(1H)-one (I) needed for the synthesis of Schiff bases. The purity of the intermediate product (I) was assessed by TLC after it was recrystallized in ethanol. Various substituted 4-hydroxy-3-(1-(heteroarylimino)ethyl)quinolin-2-one (L<sub>1</sub>-L<sub>4</sub>) were prepared carrying out reaction in ethanol for 4 hours.

In the analytical results as detailed above, the significance of the peaks identified in the IR, <sup>1</sup>HNMR, and <sup>13</sup>CNMR spectra of the compounds (L<sub>1</sub>-L<sub>4</sub>) is clarified. The compound (L<sub>1</sub>-L<sub>4</sub>) IR spectra have shown a prominent band at 3507-3498 cm<sup>-1</sup> and is given to the (-OH) vibration, confirming the presence of enolic -OH group present in Schiff bases.<sup>32</sup> 1614-1607 cm<sup>-1</sup> is predicted for the (C=N) vibration, confirming the formation of Schiff bases.<sup>33</sup> The two bands at 1570-1504 cm<sup>-1</sup> and 1470-1422 cm<sup>-1</sup> are designated to the aromatic ring. Strong band between 1668 and 1658 cm<sup>-1</sup> is assigned for lactam carbonyl.

Each of the (L<sub>1</sub>-L<sub>4</sub>) <sup>1</sup>H NMR spectra showed a singlet(3H) in the range 2.23-2.38 ppm that was attributed to an methyl hydrogen bonded to imine group. A singlet (3H) in the region 2.22-2.65 ppm is given to picoline's methyl substituent. The peaks observed in the region 8.2 and 7.0 ppm were ascribed for aromatic Hydrogen atoms. The existence of the 4-hydroxyl group is confirmed by a wide singlet at 15.63-15.92 ppm. The peak observed between 10.50 - 10.64 ppm reveals the presence of secondary amino group.<sup>34</sup> Lactam carbon revealed peaks in the range of 165-161 ppm, while imine carbon showed peaks in the range of 176-175 ppm. The explanation provided for other peaks found in <sup>1</sup>HNMR, <sup>13</sup>CNMR,

and mass spectra, as well as molecular ion peaks, supports the structures of compounds (L<sub>1</sub>-L<sub>4</sub>).

The synthesized Schiff's bases were investigated for anti-bacterial with *Bacillus subtilis* and *Salmonella typhi* (Gram-positive bacteria) while *Staphylococcus aureus* and *Escherichia coli* (Gram-negative bacteria). The results are reported in Table 1. All compounds have displayed good antibacterial activity with all bacterial species in the range of 10-14mm diameter of zone of inhibition but lesser, except L<sub>4</sub> which shown maximum zone of inhibition within range of 16-18mm of diameter than reference used. The enhanced activity observed in L<sub>4</sub> might be due to presence of nitro group in the moiety. The screening test for antifungal activity against *F. moniliforme*, *A. niger*, *A. flavus*, and *P. chrysogenum* fungi revealed that (L<sub>1</sub>-L<sub>4</sub>) exhibit significant activity, especially L<sub>4</sub> have shown minimum growth of all fungi.

**Reaction scheme**

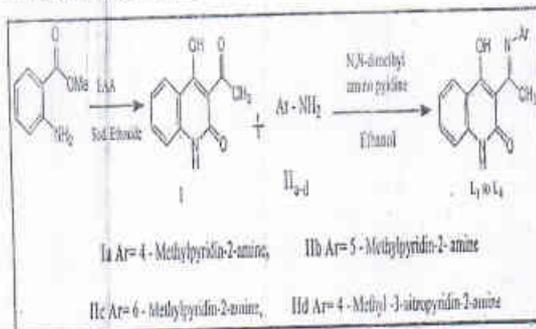


Fig. 2. Synthesis of Schiff bases L<sub>1</sub> to L<sub>4</sub>

Table 1: Anti-bacterial activity

Synthesized Schiff base	Zone of inhibition (diameter measured in mm)			
	Gram-positive		Gram-negative	
	S. typhi	B. subtilis	E. coli	S. aureus
Ampicillin (Reference)	19	16	18	17
L1	13	11	12	11
L2	14	12	13	14
L3	13	10	14	10
L4	17	16	18	18

Spectral data for the synthesized compound is given as below

L<sub>1</sub>: 4-hydroxy-3-(1-((4-picolin-2-yl)imino)ethyl)quinolin-2(1H)-one

Yield: 72%; Colour: Yellow; Melting Point: 234-236°C IR (KBr, cm<sup>-1</sup>): 3500 Broad -O-H and enolic, 3402 Broad and weak >N-H, 1668(>C=O)

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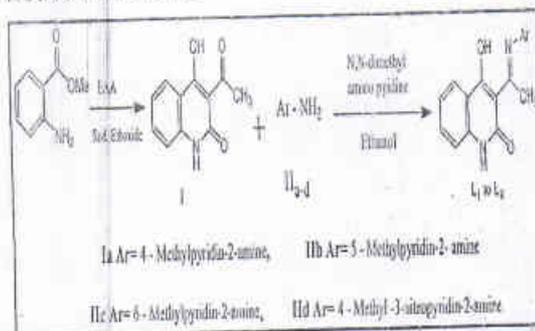


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$L_2$	14	12	13	14
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Spectral data for the synthesized compound is given as below

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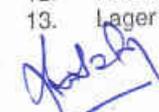
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