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Synthesis, characterization and antimicrobial activities of schiff base ligand and its metal complexes

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ABSTRACT

A new Schiff base, (E)-N'-(1-(1-ethyl-4-hydroxy-2-oxo-1,2-dihydroquinolin-3-yl)ethylidene)nicotinohydrazide and its Cu(II), Ni(II), Co(III), Mn(II) and Fe(III) complexes have been prepared. The characterization of ligand was processed by elemental analysis and spectroscopic techniques like IR, ¹H NMR and mass spectroscopy. The structure of the complexes have been investigated by elemental analysis, magnetic susceptibility, molar conductance, UV-Visible, infrared, mass spectroscopy. From analytical data, the stoichiometry of the complexes was found to be 1:2 (metal:ligand). On the basis of magnetic susceptibility and spectral data, octahedral geometry has been assigned to all metal complexes. Antibacterial activity of ligand and its complexes were studied against Gram-positive bacteria, *Staphylococcus aureus*, *Bacillus subtilis* and Gram-negative bacteria *Salmonella typhi*, *Escherichia coli* by agar cup method. Their antifungal activity was also tested against *Aspergillus niger*, *Penicillium chrysogenum*, *Fusarium moniliforme* and *Aspergillus flavus* by poison plate method using potato dextrose agar medium at one percent concentration.

Key words: Tridentate ligand, Aroyl hydrazone, Antimicrobial activity.

INTRODUCTION

Schiff bases, which are potential chelating ligands in coordination chemistry show promising applications in medicine as anti-oxidant, anti-microbial and anti-inflammatory agents (1,2). Tridentate Schiff bases with ONO donor atoms are well known to coordinate with various metal ions. In recent years, metal complexes have received much attention in biochemistry and pharmacy as promising compounds for the creation of novel drugs (1,3). These studies revealed that complexation of metal ions and different Schiff base ligands improves the biopotential of the ligands. It is well accepted that metal ions can modify both magnitude and direction of the pharmacological activity of the parent ligand as a result of changes in their size, shape, charge density distribution and redox potential (4). The use of metal complexes as pharmaceutical drugs has shown promise in recent years. Hence the recent research has been centered on the search of new and more efficacious metal based drugs with novel chemotherapeutic properties. Due to rich coordination chemistry of tridentate Schiff bases, it has attracted great deal of interest. Hydrazones and aroyl-hydrazones show various biological activities like, anticonvulsant, antidepressant, analgesic, antimicrobial, antitumor, antiplatelet and antiviral (3). Similarly 4-hydroxy-2-quinolone is well known to possess various medicinal applications, like, antitubercular, anti-inflammatory, larvicidal, antitumor, antidepressant and antioxidant (5-8). In view of the potential values of biological activities of hydrazones and 4-hydroxy-2-quinolone prompted us to develop a new Schiff base by combining these two moieties with a hope to get their cooperative

effects to improve the biological activities of newly synthesized ligand and its metal complexes. In continuation of our earlier similar work (9-16), we report herein the synthesis, characterization and *in vitro* studies of Schiff base ligand (E)-N'-(1-(1-ethyl-4-hydroxy-2-oxo-1,2-dihydroquinolin-3-yl)ethylidene)isonicotinohydrazide derived by the condensation of 2-quinolone with nicotinic hydrazide and its Cu(II), Ni(II), Co(III), Mn(II) and Fe(III) complexes. Ligand coordinates to the metal ion as a tridentate Schiff base via quinolone carbonyl, azomethine nitrogen and amide oxygen in all the complexes. A search of literature reveals that no work has been done on the transition metal complexes of Schiff base hydrazone derived from nicotinic hydrazide and quinolone.

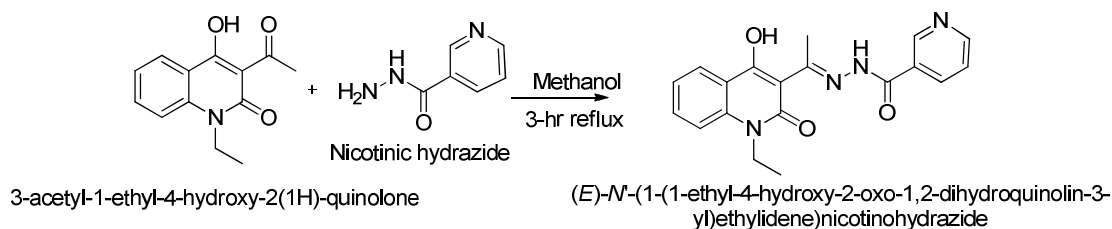
MATERIALS AND METHODS

All chemicals used were of analytical grade and used without purification. All metal salts were purchased from SD fine chemicals. 3-acetyl-1-ethyl-4-hydroxy-2(1H) quinolone was prepared by reported method (17). Elemental analysis (C,H,N,O) were performed on Perkin Elmer-2400, IR spectra were recorded on FTIR spectrophotometer model RZXC Perkin-Elmer in the range (4000-400 cm^{-1}), ^1H NMR spectra were recorded on Bruker Avance II at 400 MHz using tetramethylsilane as an internal standard. Electronic spectra was recorded on Shimadzu 1800 spectrophotometer using DMSO as solvent. Mass spectra was recorded on Waters, Q-TOF Micro Mass (LC-MS). Solution conductivity was measured by using 10^{-3} M solution in DMSO on Elico cm-180 conductometer. Magnetic data at room temperature were collected on Guoys balance. Mercury (II) tetrathiocyanato cobalt acetate was used as a calibrant. Diamagnetic contributions were calculated for each compound by Pascal's constants.

Synthesis of Ligand :

3-acetyl-1-ethyl-4-hydroxy-2(1H) quinolone was prepared by reported method (17) and reacted with nicotinic acid hydrazide under reflux condition by using methanol as solvent. Initially quinolone (10g) was taken and heated upto dissolution. nicotinic hydrazide (5.93g) was also heated upto dissolution in methanol. quinolone-INH ratio was kept in 1:1 molar ratio. Both contents were mixed in round bottomed flask in 1:1 molar ratio and refluxed with stirring for 2 hours. Orange product, (E)-N'-(1-(1-ethyl-4-hydroxy-2-oxo-1,2-dihydroquinolin-3-yl)ethylidene)isonicotinohydrazide was formed. It was then filtered, washed with methanol and dried under vacuum. On the basis of elemental analysis, FTIR, ^1H NMR and mass spectra the following structure have been proposed for the present ligand.

Fig-1 Reaction of the ligand formation



Synthesis of Metal Complexes :

The complexes were synthesized by reaction of metal salt and the ligand, in amounts equal to metal : ligand molar ratio of 1 : 2. The complex was prepared by adding methanolic solution of metal salt to the methanolic solution of ligand subsequently raising the pH of the mixture gradually to 8.0 by adding 10% ammonia solution in methanol. The reaction mixture was refluxed with constant stirring for 4 to 6 hrs to get solid colored precipitate of the complex. The precipitate was filtered, washed several times with methanol and dried in a desiccator to constant weight. Co(II) acetate was used as the metal salt but the complex was obtained as Co(III) complex due to air oxidation (18-19).

RESULTS AND DISCUSSION

The complexes are colored solids, stable in air and insoluble in water and nonpolar solvents but soluble in polar solvents like DMF and DMSO. The analytical data of the ligand and its metal complexes, molar conductance in DMSO (10^{-3} M) and magnetic moments are listed in Table-1. The molar conductivities are in the range that indicates the non-electrolytic nature of all the complexes. The elemental analysis show 1 : 2 (metal : ligand) stoichiometry in the complexes.

FTIR Spectra :

The IR spectra of the free ligand and the metal complexes were compared and assigned on the basis of careful comparison. A broad band in the ligand centered at 3429 cm^{-1} attributed to 4-hydroxy group of quinolone is also present in the range $3410\text{-}3420\text{ cm}^{-1}$ in all of the metal complexes indicating noninvolvement of -OH group in coordination. The peak exhibited at 1629 cm^{-1} due to (C=O) vibration of quinolone in the ligand was shifted to lower frequency $1594\text{-}1599\text{ cm}^{-1}$ in the complexes. This shift to lower frequency by $30\text{-}35\text{ cm}^{-1}$ confirms that this group forms coordinate bond with the metal. The band appeared at 1602 cm^{-1} in the ligand attributed to amide >C=O vibration was found to be absent in all complexes suggesting the coordination of ligand through amide carbonyl group. The band appeared at 1587 cm^{-1} due to azomethine >C=N group in the ligand shifts by $15\text{-}25\text{ cm}^{-1}$ and appeared in the region $1562\text{-}1572\text{ cm}^{-1}$ indicative of the coordination of azomethine nitrogen atom to the metal (20). This is further supported by the small red shift in the N-N stretching frequency in the metal complexes (21). This coordination behavior of the ligand is also proved by the appearance of IR bands in the complexes due to M-O And M-N vibrations in the range $474\text{-}502\text{ cm}^{-1}$ and $429\text{-}456\text{ cm}^{-1}$ respectively.

The above discussion reveals that the ligand coordinates to the metal ion as a tridentate via quinolone carbonyl, azomethine nitrogen and amide oxygen in all the complexes (22,12).

Mass and $^1\text{H-NMR}$ Spectra of the Ligand:

In the mass spectrum of the ligand molecular ion peak is observed at m/z 351 a.m.u. corresponding to (M+1) consistent with the calculated molecular weight 350.

$^1\text{H-NMR}$ Spectra of ligand was recorded in DMSO. It shows signals at 1.22 δ ppm (t, 3H $\text{N-CH}_2\text{-CH}_3$), 4.22 δ ppm (q, 2H, $\text{N-CH}_2\text{-CH}_3$), 2.89 δ ppm (s,3H, N=C-CH_3 azomethine CH_3), 7.12-8.15 δ ppm (m, 8H, H_{arom}), 8.81 δ ppm (s,N-H), 16.63ppm (s, 1H, $\text{OH}_{\text{enolic}}$) in agreement with the proposed ligand structure.

Electronic Spectra and Magnetic Studies:

The electronic spectra of the ligand show series of bands within the range 224-238 nm, 252-256 nm and 358-423 nm, attributed to $n\text{-}\pi^*$ and $\pi\text{-}\pi^*$ transitions (23-24).

The Cu(II) complex showed a band in the region, 536-559 nm expected for d-d transition (25) and a second band in the region 334-398 nm which is assigned to the charge transfer transition (26). A magnetic moment of 1.91 BM which is higher than the spin only value (1.73) BM expected for one unpaired electron confirmed the octahedral geometry of the complex (27). Ni(II) complexes are diamagnetic and show bands in the region 327-388 nm due to charge transfer (28-29). The absence of band below 100 nm indicates octahedral geometry. The diamagnetic nature and green color of Ni(II) complex give additional support to its octahedral geometry. The electronic spectra of Co(III) complex exhibits weak bands at 414 nm, 390 nm and 382 nm are assigned to LMCT transitions and other bands appeared at 557-527 nm are due to d-d transitions. The diamagnetic nature and the observed electronic spectra confirms the low spin octahedral geometry of Co(III) complex (30). Mn(II) complex show three weak bands at 714-527 nm, 413-389 nm and 365-348 nm which may be assigned to the spin and parity forbidden transitions $^6\text{A}_{1g} \rightarrow ^4\text{T}_{1g}$ (G), $^6\text{A}_{1g} \rightarrow ^4\text{T}_{2g}$ (G), and $^6\text{A}_{1g} \rightarrow ^4\text{A}_{1g}$, $^4\text{E}_g$ (G) respectively indicating octahedral geometry (31-32). The octahedral geometry is also supported by the observed magnetic moment 5.46 BM. The electronic spectra of Fe(III) complex is characterized by three weak bands at 713-701 nm, 552-531 nm and 324-317 nm assigned to spin forbidden $^6\text{A}_{1g} \rightarrow ^4\text{T}_{2g}$ (D), $^6\text{A}_{1g} \rightarrow ^4\text{T}_{1g}$ and $^6\text{A}_{1g} \rightarrow ^4\text{T}_{2g}$ transitions respectively (33-34). The effective magnetic moment 5.30 B.M. obtained for Fe(III) complex further confirms its octahedral geometry. On the basis of physico-chemical and spectral study, Following structure may be proposed for the complexes.

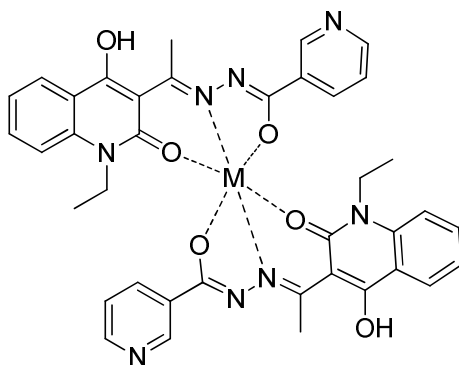


Fig-2 Proposed structure for the complexes

Table- 1: Physical and Analytical Data of Ligand and its Metal Complexes

| Comp-ound | Mol.formula | Colour | Mol.wt | M.P | C% Found (calc) | H% Found (calc) | N% Found (calc) | O% Found (calc) | Metal% | μ_{eff} .B.M. | Molar Condu. $\text{Ohm}^{-1}\text{cm}^2\text{mol}^{-1}$ |
|--------------------|---|----------------|--------|---------|-----------------|-----------------|-----------------|-----------------|--------|--------------------------|--|
| L | $\text{C}_{19}\text{H}_{18}\text{O}_3\text{N}_4$ | Orange | 350 | 185°C | 64.43 (65.1) | 5.26 (5.14) | 12.27 (13.01) | 15.68 (14.74) | NA | NA | |
| Co(L) ₂ | $\text{Co}[\text{C}_{38}\text{H}_{34}\text{O}_6\text{N}_8]$ | Brown | 756.93 | > 300°C | 59.16 (60.02) | 4.31 (4.49) | 8.99 (9.25) | 13.39 (12.98) | 7.78 | Dia-magnetic | 9.4 |
| Mn(L) ₂ | $\text{Mn}[\text{C}_{38}\text{H}_{34}\text{O}_6\text{N}_8]$ | Light Brown | 752.93 | > 300°C | 58.66 (59.87) | 4.23 (4.31) | 9.12 (9.32) | 12.88 (12.62) | 7.29 | 5.46 | 10.3 |
| Cu(L) ₂ | $\text{Cu}[\text{C}_{38}\text{H}_{34}\text{O}_6\text{N}_8]$ | Blackish brown | 761.54 | > 300°C | 60.33 (59.98) | 4.06 (3.91) | 9.81 (9.69) | 12.63 (12.54) | 8.34 | 1.91 | 6.8 |
| Fe(L) ₂ | $\text{Fe}[\text{C}_{38}\text{H}_{34}\text{O}_6\text{N}_8]$ | Black | 753.84 | > 300°C | 58.94 (58.74) | 4.21 (4.14) | 9.06 (9.17) | 12.77 (12.65) | 7.40 | 5.30 | 5.5 |
| Ni(L) ₂ | $\text{Ni}[\text{C}_{38}\text{H}_{34}\text{O}_6\text{N}_8]$ | Greenish | 756.70 | > 300°C | 59.96 (59.77) | 4.29 (4.35) | 8.91 (8.86) | 13.32 (12.94) | 7.75 | Dia-magnetic | 7.1 |

Microbial Activity of Schiff Base Ligand and its Metal Complexes:

The ligand and its metal complexes have been screened *invitro* for their antibacterial and antifungal activities by agar- cup method using DMSO as a solvent and poison- plate method using potato dextrose agar medium in DMSO respectively.

In the study of antibacterial activity, *penicillium* was used as standard reference. The procedure used for the study was as follows: The tested compounds were dissolved in DMSO which does not have inhibition activity. All the compounds were tested at fixed concentrations (1%) in DMSO. The test was performed on nutrient agar cup of 10mm diameter bored in the agar plate with the help of sterile cork borer. The prepared solutions were added separately in to each bore. One cup with DMSO as a blank and other for standard reference *penicillium* were also placed on the seeded nutrient agar. The plates were incubated for 24 hours. The resultant activities measured in diameter (mm) are presented in Table-2.

From Table-2 it is found that the ligand and all the metal complexes do not show any activity against Gram –ve bacteria, *E. coli* and *S. typhi*, except Mn(II) complex which show highest activity against *E.coli* comparable to the standard, *penicillium*. On the other hand, the ligand show considerable activity against Gram +ve bacteria, *S. aureus* and remains inactive against *B. subtilis*. The most active is the Ni(II) complex which shows better inhibitory action against both gram positive bacterial species. Mn(II) and Cu(II) complexes also inhibit the growth. However Co(III) and Fe(III) complexes do not have significant effect against both the species. All these active metal complexes exhibited a more inhibitory effect compared to the ligand. This is because the chelation reduces the polarity of the metal ion because of partial sharing of its positive charge with the donor groups which results in to the increase of lipophilic nature of the complexes which in turn favors its permeation to the lipid layer of the membrane. Moreover, other factors, like stability constants, molar conductivity, solubility and magnetic moments also affect the antimicrobial activity of the complexes (35). The observed enhancement in the activity of ligand after complexation can be explained on basis of chelation theory (36) .

The antifungal activities were tested by the reported method (37).The fungal suspension was spot inoculated on the plates using compound with nicrome wire loop. *Gresiofulvin* was prepared as standard reference plate. The plates were incubated at room temperature for 48 hours. The results obtained are listed in Table- 3.

From the Table 3 it may be seen that the ligand acts as a very effective antifungal agent since it totally arrests the growth of tested fungi. Though the ligand exhibits the best antifungal activity against all of the four fungi used in the study, its metal complexes are found to be inactive against *A. Flavus*. However, the complexes show high activity against all other fungi. The best antifungal activity was obtained for Cu(II) and Fe(III) complexes. Second highest activity was observed for Co(III) complex. Nickel complex exhibits high activity against *Penicillium chrysogenum* and moderate activity against *Fusarium moniliforme* and remains inactive against other fungal species.

Table 2. Antibacterial Activity

Medium : Nutrient Agar
Dose of compound : 1%

Method : Agar Cup Method
Cup size : 10 mm

| Sr.No | Compound | Escherishia coli | Salmonella typhi | Staphylococcus aureus | Bacillus subtilis |
|-------|--------------------|------------------|------------------|-----------------------|-------------------|
| 1 | L | -ve | -ve | 16mm | -ve |
| 2 | Co(L) ₂ | -ve | -ve | -ve | -ve |
| 3 | Mn(L) ₂ | 14mm | -ve | 18mm | 12mm |
| 4 | Fe(L) ₂ | -ve | -ve | -ve | -ve |
| 5 | Cu(L) ₂ | -ve | -ve | 17mm | 13mm |
| 7 | Ni(L) ₂ | -ve | -ve | 21mm | 22mm |
| 8 | DMSO | -ve | -ve | -ve | -ve |
| 9 | Penicillin | 14mm | 20mm | 36mm | 28mm |

Legends : -ve - No Antibacterial Activity
Zone of inhibition : -- in mm

Table 3 Antifungal Activity

Medium : Potato Dextrose Agar
method

Method : Poison plate
Dose : 1%

| S.No | Compounds | Aspergillusniger | Penicilliumchrysogenum | Fusariummoneliforme | Aspergillusflavus |
|------|---------------------|------------------|------------------------|---------------------|-------------------|
| 1 | L | -ve | -ve | -ve | -ve |
| 2 | Co(L) ₂ | RG | RG | -ve | RG |
| 3 | Mn(L) ₂ | RG | +ve | +ve | +ve |
| 4 | Fe(L) ₂ | -ve | -ve | -ve | +ve |
| 5 | Cu(L) ₂ | -ve | -ve | -ve | RG |
| 7 | Ni(L) ₂ | +ve | -ve | RG | +ve |
| 8 | DMSO | +ve | +ve | +ve | +ve |
| 9 | <i>Griseofulvin</i> | -ve | -ve | -ve | -ve |

Legends : +ve –Growth (Antifungal Activity absent)
-ve – No Growth (More than 90% reduction in growth Antifungal Activity present)
RG – Reduced Growth (More than 50% and less than 90% reduction in growth observed)

CONCLUSION

A novel ligand (E)-N'-(1-(1-ethyl-4-hydroxy-2-oxo-1,2-dihydroquinolin-3-yl)ethylidene)isonicotinohydrazide and its Cu (II), Mn (III), Fe (III), Cu (II) And Ni (II) complexes were synthesized and characterized by elemental analysis, molar conductance, magnetic measurements, ¹H NMR, mass and UV-Vis spectra. The chemical analysis and spectral data indicate that the ligand acts as ONO tridentate Schiff base coordinating to the metal through quinolone carbonyl, azomethine nitrogen and amide oxygen in all the complexes. Antibacterial activity of the ligand and its complexes *in vitro* were studied against some Gram-positive and Gram-negative bacteria. Their antifungal activity was also tested against four fungal strains. The complexes were found to have more biological activity than their parent ligands.

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