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# Spectroscopic, magnetic and thermal studies of Co(II), Ni(II), Cu(II) and Zn(II) complexes of 3-acetylcoumarin–isonicotinoylhydrazone and their antimicrobial and anti-tubercular activity evaluation

Rekha S. Hunoor[a], Basavaraj R. Patil[a], Dayananda S. Badiger[a], Ramesh S. Vadavi[a], Kalagouda B. Gudasi[a,*], V.M. Chandrashekhar[b], I.S. Muchchandi[b]

[a] Department of Studies in Chemistry, Karnatak University, Pavate Nagar, Dharwad 580 003, Karnataka, India
[b] H.S.K. College of Pharmacy, Bagalkot 587 101, Karnataka, India

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ABSTRACT

Co(II), Ni(II), Cu(II) and Zn(II) complexes with a new heterocyclic Schiff base derived by the condensation of isonicotinoylhydrazide and 3-acetylcoumarin have been synthesized. $^{1}H$, $^{13}C$ and 2D HETCOR NMR analyses confirm the formation of title compound and existence of the same in two isomeric forms. The metal complexes were characterized on the basis of various spectroscopic techniques like electronic, EPR, IR, $^{1}H$ and $^{13}C$ NMR studies, elemental analysis, magnetic properties and thermogravimetric analysis, and also by the aid of molar conductivity measurements. It is found that the Schiff base behaves as a monobasic tridentate ligand coordinating in the imidol form with 1:1 metal to ligand stoichiometry. Trigonal bipyramidal geometry has been assigned for Ni(II) and Cu(II) complexes, while tetrahedral for Co(II) and Zn(II) complexes. The compounds were subjected to antimicrobial and anti-tubercular activity screening using serial broth dilution method and Minimum Inhibitory Concentration (MIC) is determined. Zn(II) complex has shown significant antifungal activity with an MIC of 6.25 μg/mL while Cu(II) complex is noticeable for antibacterial activity at the same concentration. Anti-TB activity of the ligand has enhanced on complexation with Co(II) and Ni(II) ions.



## 1. Introduction

Hydrazones belonging to azomethine class of compounds have attracted the attention of many chemists owing to their wide spectrum of pharmacological activity profile with structural flexibility and ligating behavior. Hydrazones distinguished by the presence of two interlinked nitrogen atoms $R_1C{=}N{-}NR_2$ are interesting, because they can function as antimicrobial, antitubercular and antitumor agents [1–3]. The structural flexibility of hydrazones allows them to exhibit amido-imidol tautomerism and they can also exist in different isomeric forms arising due to free rotation around C–C or N–N single bonds around azomethine framework [4–6]. Chemistry of transition metal complexes with such multidentate Schiff base ligands is fascinating because these metal ions exhibit different oxidation states [7]. Such complexes with different oxidation states have strong role in bioinorganic chemistry and redox enzyme systems [8]. They also find applications as catalysts in oxidation and epoxidation reactions [9].

Isonicotinoylhydrazide (Isoniazid: INH) is one of the most potent anti-TB drugs, used to kill the *Mycobacterium tuberculosis*. Isonicotinoylhydrazone derivatives containing heterocyclic moiety have found to exhibit better anti-tubercular activity [10]. This prompted us to prepare the hydrazone of isonicotinoylhydrazide with a heterocyclic moiety possessing interesting pharmacological properties. In this perspective, Coumarins (1,2-benzopyron) are attractive heterocyclic compounds because of their anticancer, anticoagulant, spasmolytic antibacterial and also anti-HIV activity [11,12]. Thus keeping in mind all the above factors, we aimed to synthesize a potential ligand containing both, isonicotinoylhydrazide and 3-acetylcoumarin moieties, linked through azomethine group. Thus, the present investigation throw light on synthesis, characterization, antimicrobial and anti-tubercular activity of novel Schiff base ligand viz., 3-acetylcoumarin–isonicotinoylhydrazone and its transition metal complexes.

* Corresponding author. Tel.: +91 836 2215286x28; fax: +91 836 2771275.
*E-mail address:* kbgudasi@gmail.com (K.B. Gudasi).

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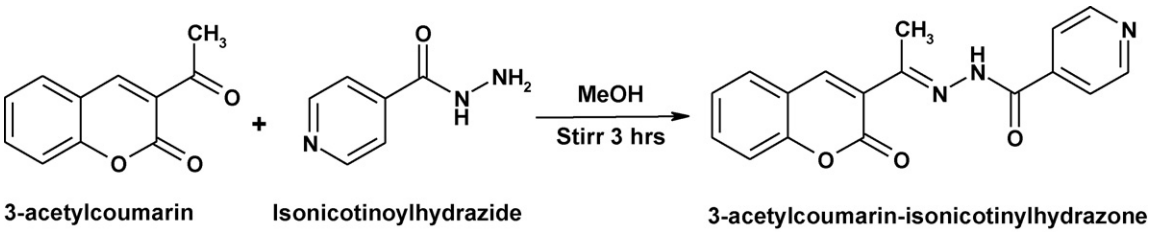


**Scheme 1.** Synthetic route for the preparation of ACINH.

## 2. Experimental

### 2.1. Physical measurements

Isonicotinoylhydrazide and 3-acetylcoumarin were obtained from Himedia and Aldrich, respectively. All the chemicals used were of AR grade and used without further purification. Methanol, ethanol and chloroform were distilled before use. After wet ashing with HCl and $HClO_4$, cobalt, nickel and copper were determined gravimetrically while zinc was determined volumetrically [13]. Chloride content was determined as silver chloride after decomposition with dilute $HNO_3$ [13]. The elemental analysis (C, H, N) was carried on a TruSpec CHN/CHNS analyzer. The mass spectrum of the ligand was obtained on SHIMADZU GCMS-QP2010S. Magnetic susceptibility measurements were made at room temperature on a Gouy balance using $Hg[Co(SCN)_4]$ as the calibrant and diamagnetic corrections were made using Pascal's constants. Electronic spectra were recorded using VARIAN CARY 50 Bio UV–visible spectrophotometer in DMF. The IR spectra of ligand and complexes were recorded as KBr pellets in the region 4000–400 $cm^{-1}$ on Nicolet 170SX FT-IR spectrometer. $^1H$, $^{13}C$ NMR of ligand and its Zn(II) complex and 2D HETCOR of ligand were recorded in DMSO-$d_6$ on Bruker Avance 400 MHz spectrometer using TMS as the internal standard. Conductivity measurements of $10^{-3}$ M solutions of complexes in DMF were made using ELICO-CM82 Conductivity Bridge provided with a cell having cell constant of 0.51. Simultaneous thermogravimetric (TG) and differential thermal analysis (DTA) curves were recorded on Perkin Elmer TGA7 ANALYSER at a heating rate of 10 °C per minute and maximum temperature of 1000 °C in $N_2$ atmosphere. The EPR spectrum of a polycrystalline Cu(II) complex was recorded at room temperature and liquid nitrogen temperature on a Varian E-4 X-band spectrometer using TCNE (tetracyanoethylene) as the calibrant.

### 2.2. Synthesis of 3-acetylcoumarin–isonicotinoylhydrazone (ACINH) ligand

3-Acetylcoumarin (1.88 g, 0.01 mol) was added to a solution of isonicotinoylhydrazide (1.37 g, 0.01 mol) in methanol (30 mL) and stirred for 3 h (Scheme 1). The pale yellowish solid separated was filtered, washed repeatedly with methanol, dried in air and recrystallized from ethanol. The purity of the compound was checked by TLC on pre-coated silica-gel plates Attempts to grow crystals of ACINH suitable for single crystal X-ray analysis were unsuccessful. Yield: 90%, m.p. = 270–275 °C.

### 2.3. Synthesis of complexes

3-Acetylcoumarin–isonicotinoylhydrazone (ACINH) (0.307 g, 1 mmol) dissolved in 20 mL chloroform was mixed with equimolar quantities of ethanolic solution (5 mL) of corresponding metal (II) chlorides (Co, Ni, Cu and Zn) and stirred for 2 h at room temperature. The precipitates thus obtained were filtered, washed repeatedly with hot chloroform and then with ethanol and dried in air. The complexes melt at a temperature higher than 300 °C.

### 2.4. Antimicrobial activity

Peptone (5 g), sodium chloride (5 g), beef extract (1.5 g) were suspended in 1000 mL distilled water. The solution was boiled to dissolve all the ingredients completely. The pH of the solution at 25 °C was adjusted to 7.4 ± 0.2 and sterilized by autoclaving at 15 *lb* pressure (121 °C) for 15 min. One day prior to the test, bacterial and fungal strains were made in the sterile nutrient broth and incubated at 37 °C overnight. Sample solutions were prepared by dissolving 1 mg of sample in 10 mL of 2% DMSO to give the concentration 100 μg/mL. The standard solutions of Ciprofloxacin (antibacterial drug) and Flucinozole (antifungal drug) were prepared in 2% DMSO to give concentration of 100 μg/mL.

Serial broth microdilution was adopted as a reference method. Serial dilutions of test compounds were made in broth, after which a standardized microorganism suspension was added (10 test tubes). Quantities of test compounds were serially diluted to attain the final concentrations of 100, 50, 25, 12.5, 6.25, 3.125, 1.6, 0.8, 0.4 and 0.2 μg/mL. One of the test tubes was kept as control. Each of the 10 test tubes was inoculated with a suspension of microorganism to be tested and incubated at 35 °C for 18 h. At the end of the incubation period, the tubes were visually examined for the turbidity. Cloudiness in the test tubes indicated that microorganism growth has not inhibited by the antibiotic contained in the medium at the test concentration.

### 2.5. Anti-tubercular activity

Test compounds were evaluated for *in vitro* antimycobacterial activity. The MICs were determined and interpreted for *M. tuberculosis* $H_{37}Rv$ according to the procedure of the approved microdilution reference method of antimicrobial susceptibility testing [14]. Compounds were taken at concentrations of 100, 50 and 25 μg/mL in 2% DMF. *M. tuberculosis* $H_{37}Rv$ strain was used in Middle brook 7H-9 broth which was inoculated with standard as well as test compounds and incubated at 37 °C for 4 weeks. The bottles were inspected for growth twice a week for a period of 3 weeks. Readings were taken at the end of fourth week. The appearance of turbidity was considered as bacterial growth and indicates resistance to the compound. The growth was confirmed by making a smear from each bottle and performing a ZN stain. Test compounds were compared to reference drugs Isoniazid (MIC = 0.025 μg/mL) and Ciprofloxacin (MIC = 10 μg/mL).

The antimicrobial and anti-tubercular activity tests were run in triplicate.

## 3. Results and discussion

The elemental analysis of the ligand is in consistence with the molecular formula $C_{17}H_{13}N_3O_3$. Mass spectrum show the molecular ion peak at *m*/*z* 307, which corresponds to the molecular weight of the ligand. The elemental analyses of the complexes suggest 1:1 metal to ligand stoichiometry. All the complexes are colored and stable even on prolonged exposure to air. They are