

## A Study of Cu(II) Complexes of Isoniazid Schiff Bases: DNA/BSA Binding and Cytotoxicity



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### Abstract

A series of isonicotinoyl hydrazones have been synthesized via template method and were complexes to Cu(II). The ligands are coordinated to Cu(II) ion through the enolic oxygen and azomethine nitrogen resulting in a square planar geometry. The CT-DNA and bovine serum albumin binding propensities of the compounds were determined spectrophotometrically, the results of which indicate good binding propensity of complexes to DNA and BSA with high binding constant values. Furthermore, the compounds have been investigated for their cytotoxicities on A549 human lung cancer cell. Also the mode of cell death was examined employing various staining techniques and was found to be apoptotic.

### Introduction

A wide range of Schiff's bases with their reactive azomethine linkage show interesting inhibitory activity against tumor cells. Schiff's bases could be hydrolyzed selectively by the tumor cells to act as alkylating agents; at the same time the active amine becomes free to act as antimetabolite. Studies show that the metal complexes of Schiff's base ligands have better antimicrobial and anticancer activities as compared to Schiff's bases.

Copper is well known as a bioessential element and its complexes have proven to be excellent candidates for biological applications due to their binding ability and positive redox potential. Cu(II) complexes containing heterocyclic bases have been extensively explored in virtue of their strong interactions with DNA and cytotoxic activity. They can act as chemical nucleases and their cytotoxicity has been proposed to be caused by their ability to bind and cleave DNA that leads to cell cycle arrest and apoptosis or generation of reactive oxygen species (ROS) that in turn leads to cell death. Literature has shown that Cu(II)-based complexes exhibit antineoplastic potency towards human ovarian carcinoma (CH1), murine leukemia (L1210), and various cervicouterine carcinomas which is comparable to or even higher than that of cisplatin. Fei et al. in their article have reported the in vitro cytotoxicity of two copper-2,2'-bipyridine complexes towards five selected tumor cell lines HepG-2, HeLa, NCI-H460, MCF-7, and HL-6 with IC<sub>50</sub> value at a concentration of 50 μM. Cu(II) complexes of Schiff bases can show their antibacterial and antiproliferative activities because of the properties of the metal center or the coordinated ligands alone, as well as the structural and electronic properties which is ascribed to the coordination. Several Schiff base ligands and their Cu(II) complexes have been investigated to show apoptotic activity against various cancer cell lines. Oxoquinoline based Schiff base copper complexes have been synthesized and investigated upon and their IC<sub>50</sub> values were found to be in the range of 8–19 μM against HeLa and HL-60 cells.

INH is a first generation antitubercular drug whose derivatives and their metal complexes have been subjected to a lot of research for their antitubercular activities but their potentials as anticancer agents have

remained largely unexplored. Their potency as anticancer agent is reflected from the little work that has been published so far wherein a few researchers have synthesized hydrazones of INH and their iron complexes and evaluated them for their antitumoral activity suggesting a mechanism of inhibition of ribonucleotide reductase.

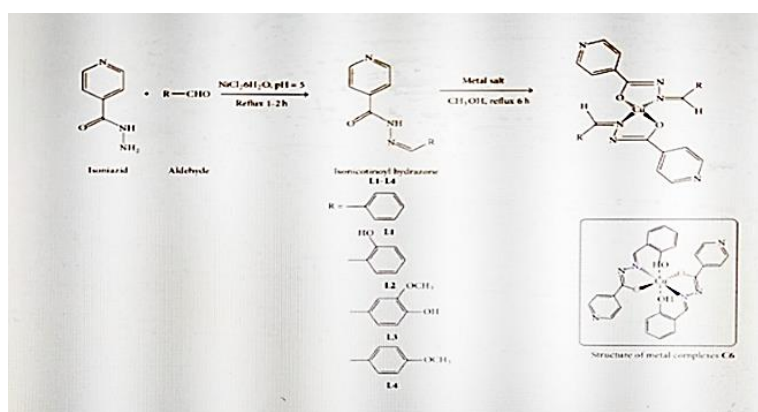
The research interest in the present work stems from the aforementioned points. With this view point we herein report Ni(II) mediated synthesis and characterization of four Schiff's bases of isonicotinoyl hydrazine (INH) by its condensation with four different aromatic aldehydes and their Cu(II) complexes. The synthesized isonicotinoyl hydrazones and their Cu(II) complexes were studied for their interactions with DNA and BSA. The results were indicative of better binding efficiencies of the copper bound ligands compared to the ligands themselves. Based on the results of their binding studies the ligands and their copper complexes were evaluated for their anticancer activity against human lung carcinoma A549 cell lines. Furthermore the mode of interaction of these complexes with A549 cells has been evaluated employing various staining techniques to assess the mechanism of cell death, via excessive ROS generation and/or nuclear condensation.

## Results and Discussion

### Synthesis and Characterization

In a general sense, transition metal-based catalysis can be viewed as template reactions where reactants coordinate to adjacent sites on the metal ion and, owing to their adjacency, the two reactants interconnect (insert or couple) either directly or via the action of another reagent. Template effects may arise from stereochemistry imposed by metal ion coordination of some of the reactants, promoting a series of controlled steps and characteristically providing routes to products not formed in the absence of the metal ion. Appreciable amount of work has been done on one pot multicomponent synthesis of Schiff bases using transition metal ions as templates. Following this insight we carried out the Ni(II) mediated Schiff base synthesis (Figure 1) and found that the reaction time had reduced and product yields had increased markedly.

The IR spectra of complexes **C5–C8** lack the strong secondary amide carbonyl absorption at 1650–1700  $\text{cm}^{-1}$  that is typically seen in the spectra of the free ligands **L1–L4**. In all the complexes, the band is shifted to lower frequency between 1570 and 1610  $\text{cm}^{-1}$  indicating coordination of the Schiff bases through the azomethine nitrogen. Moreover, the enolate structure of the coordinated ligand is supported by a band at 1059–1060  $\text{cm}^{-1}$  due to the enolic C–O stretching. The N–H stretching bands in the range 3150–3250  $\text{cm}^{-1}$  found in the IR spectra of **L1–L4** are completely lost in the spectra of their **C5–C8** which is also indicative of the enolization and deprotonation on coordination.



The UV spectra of the ligands and their complexes show bands in the wavelength range 200–350 nm. In the UV spectra of the ligands (Figure 2) the first band appearing within 200–270 nm region can be assigned to the medium energy transition of the aromatic rings. The second band observed within 280–340 nm region is due to the excitation of the electrons of the azomethine group which corresponds to an intraligand transition. In case of **L2** (Figure S1 in the Supplementary Material) there is a third band located at 326 nm that can be ascribed to charge transfer within the entire Schiff base molecule. This band is commonly observed in o-hydroxyl Schiff bases and is based on strong intramolecular hydrogen bonding between the hydroxyl group of the salicylidene and the azomethine nitrogen. The UV spectra of complexes (Figure 2) show the first band at nearly the same wave length as in the spectra of their corresponding ligands which reveal that these () transitions are not significantly affected by chelation. On the other hand the band corresponding to azomethine showed broadening and a shift to longer wavelength on going from ligand to complex, indicating coordination of ligand to metal through the azomethine moiety.

## Conclusions

The synthesis and characterization of four isonicotinoyl Schiff bases and their Cu(II) complexes (**L1–C8**) have been realized with physicochemical and spectroscopic methods. In all the complexes, the Schiff bases are bound to the metal via the enolic oxygen and the azomethine nitrogen adapting a square planar geometry around the metal centre. The interaction of the complexes with CT DNA and BSA is supported by the following observations: (i) high values of  $\epsilon$  in the range of  $10^4$ – $10^5$  L mol<sup>-1</sup> cm<sup>-1</sup> and hypochromism of the intraligand absorption bands of the compounds **L1–C8**, (ii) high values of  $K_{\text{b}}$  in the range of  $10^4$ – $10^5$  M<sup>-1</sup>, and (iii) efficient cleavage of CT-DNA. Cytotoxicity studies show that the compounds are less toxic than the clinically used drug cis-platin. The metal complexes of ligands **L1**, **L3** and **L4** show greater cytotoxicity as compared to the ligands. Moreover the results obtained from staining techniques indicate that the mode of cell death is essentially apoptosis, probably via ROS generation.

## Reference

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