Synthesis, Characterization and Anticancer Activity of Isonicotinylhydrazide Metal Complexes

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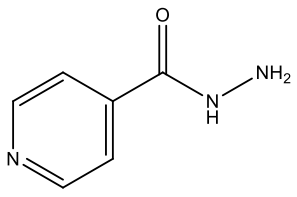
Summary: This study focuses characterization of iron (II), iron (III), cobalt (II), copper (II) and nickel (II) complexes of Isoniazid (INH) and studying their spectroscopic as well as physiochemical properties. FTIR studies showed that INH binds the metal from oxygen of carbonyl group and nitrogen of amino group. The proton NMR spectra of the metal complexes confirmed the conversion of ligand molecules into their respective metal complexes. However, pattern of splitting and shapes of peaks was observed but the protons resonated in the expected region. XRD patterns may be concluded that the complexes are mostly comprised of nano-sized particles behaving like amorphous materials. Scanning electron microscopy (SEM) revealed marked changes in the morphology of complexes, and their degradation at higher temperature strengthens the hypothesis of the successful formation of complexes. The MTT cytotoxicity assay was used for the screening these complexes against four human cell lines but the results did not prove significant.

Keywords: Drug Metal Complexes, Isoniazid, Scanning Electron Microscopy, X-Ray Diffraction and Cytotoxicity.

Introduction

Heterocyclic compounds consisting of hydrazides and hydrazones have gained great interest in medical sciences owing to their significance in biological processes and clinical applications [1, 2]. Although the drug has been in clinical practice for treating Tuberculosis for the last two decades, the research on its cytotoxic activity is only scarcely available [3]. Kumar and coworkers have demonstrated the promising potential of Isoniazid (Scheme-1) in this respect by different analogues of the drug [4] Rollas *et al* evaluated the cytotoxic activity of hydrazones of INH and their iron complexes and proposed a mechanism of inhibition of ribonucleotide reductase [5].



isoniazid

Scheme-1: INH ligand.

The discovery and subsequent successful use of Platinum based compounds in treating malignant neoplastic conditions has embolden the scientific society worldwide to peruse the transition metal characteristics while seeking new chemotherapeutic agents. The resulting field of medicinal chemistry has progressed over the years producing various transition metal complexes of medicinal significance. Copper is a well-known bio essential element and the redox potential and binding capacities of its complexes have made it suitable for biological applications [6-9]. Copper complexes have been explored and it was revealed that these complexes show cytotoxic activity because of their capability to bind and cleave DNA leading to cell cycle halting or they may generate reactive oxygen species that ensues in cell death [10] heterocyclic bases complexes with copper(II) have the potential to act as chemical nucleases [11, 12] on DNA molecules and have demonstrated antineoplastic activity against human ovarian carcinoma, murine leukemia and different cervical and uterine malignant conditions [13, 14].

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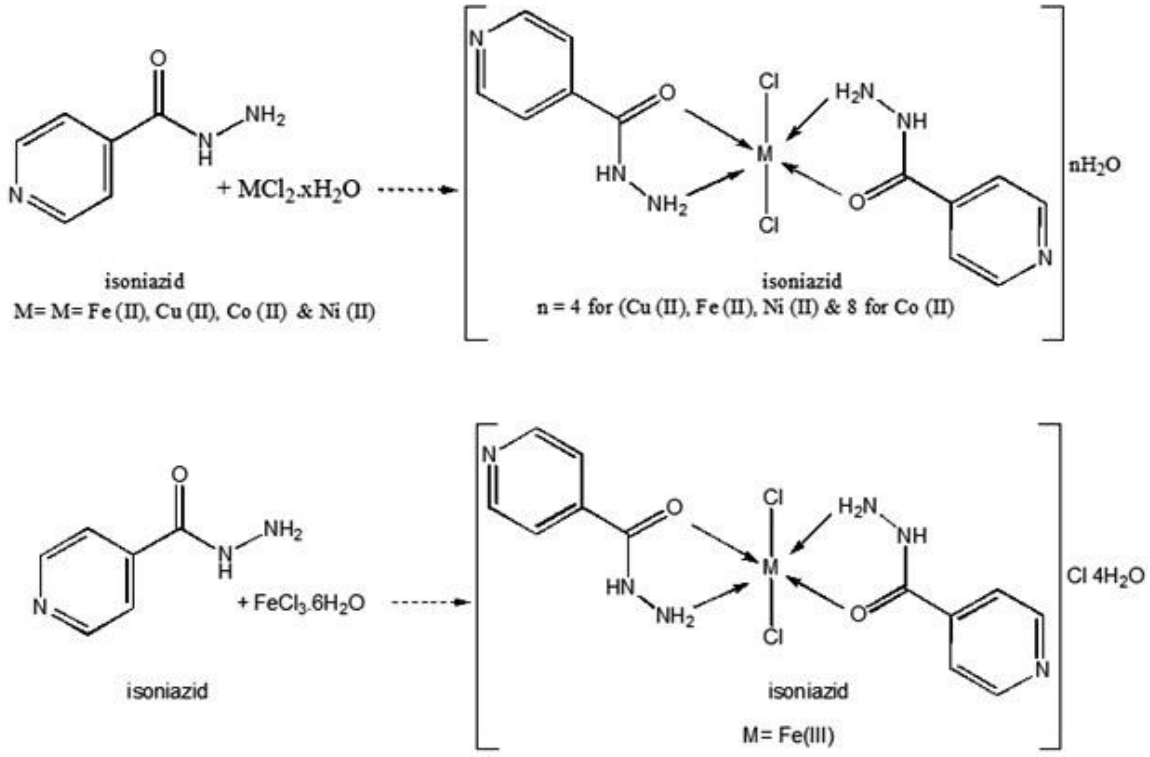


Scheme-2: Predicted structures for complexes.

As research has proven extremely invaluable role of inorganic metal ions in various biological processes, recently the application of inorganic chemistry to medicine ("Elemental Medicine") has become a rapidly developing field. Literature survey reveals that in the last decade, there has been marvelous attention towards the design, synthesis and biological studies on metal complexes of drugs. The objective of such projects is to improve / maintain the efficiency than the parent drug and lowers the side effects. Many research paper have been contributed which described the antimicrobial activity of metal complexes of isoniazid and its derivatives [15, 16]. It was established that the interaction of metal ions with antibiotic can change the biological activity of ligand molecule. Thus, considering this fact, it become worthwhile to incorporate metal ions into drugs used against tuberculosis because this disease needs long treatment and is accompanied by various collateral [17]. Since it was recognized, the interest in the preparation of INH coordination compounds with transition metals and their screening against biological activities have been carried out [18-21]. Metal complexes (Fe (II) and Cu (II)) of INH were reported to possess anti-tumor and antitubercular activities. The synthesis of first Cu (II)-INH was reported in 1981 [22]. In-vivo inhibitory effect of INH against M. tuberculosis H37Rv was also reported [23-26]. In an exploratory work the copper complexes of INH derivatives were screened and found to be active against bacteria [27]. Furthermore, metal complexes of INH derivatives were tested against various gram positive and gram negative bacteria and reported to possess higher anti-bacterial activity [28].

Keeping in view the important roles of these elements in living organisms' biochemical processes we have synthesized the copper (II), iron (II), iron (III), cobalt (II) and nickel (II) complexes of isoniazid as an attempt to make newer compounds with more therapeutic effects. These metal complexes were evaluated for cytotoxicity against human astrocytoma SNB-19, human Dukes' type C colorectal adenocarcinoma HCT-15, human Dukes' type D colorectal adenocarcinoma COLO-205 and human cervix carcinoma KB-3-1 cancer cell lines.

Experimental

Materials

Reference drug of Isoniazid (INH) was obtained as a gift from Wyeth Pakistan Ltd., Karachi. The chlorides of iron (II), iron (III), manganese (II), cobalt (II) and copper (II) were all obtained from

Sigma Aldrich and used without further purification. Solvents methanol (Sigma Aldrich) and water were freshly distilled just prior to use. Dulbecco's modified Eagle's Medium (DMEM), fetal bovine serum (FBS), penicillin/streptomycin and trypsin 0.25% were purchased from Hyclone (GE Healthcare Life Science, Pittsburgh, PA). Phosphate buffered saline (PBS) was purchased from Invitrogen GIBCO (Grand Island, NY). Dimethyl sulfoxide (DMSO) and 3-(4, 5-dimethylthiazole-2-yl)-2, 5-biphenyl tetrazolium bromide (MTT) were purchased from Sigma Chemical Co (St. Louis, MO).

Preparation of Complexes (INH)

Equimolar quantities of INH and metal salts (cobalt chloride) were weighed accurately on analytical balance. Both were dissolved separately in the volumetric flask; each was introduced into a round bottom flask. The mixture was refluxed on water bath and allowed to heat for 3-4 hours while stirring time to time. After refluxed the mixture was allowed to cool at room temperature upon cooling crystals were formed at the bottom of the round bottom flask were separated out by filtration. The crystals were dried at 60ºC in oven for 30 minutes. The same procedure was followed for the synthesis of Cu (II), Fe (II), Fe (III) and Ni (II) complexes of INH detailed of preparation was already reported in earlier research paper.

Characterizations

The FTIR spectra were noted in KBr pellets using Shimadzu Prestige-21 spectrophotometer in the range of 4000-400 cm^{-1}. The ^1H NMR spectra of INH and its metal complexes were recorded in DMSO-d_6 on an Avance AV-400 and 500 MHz spectrometer. Morphological studies were performed on a Scanning Electron Microscope, Jeol Japan model no. JSM6380A with auto-coater Jeol Japan model no. JFC1500. XRD data were obtained at D8 Advance XRD, Bruker, elemental (CHN), metal and physico chemical analysis are reported in earlier papers.

Cell Lines and Cell Culture

Four cancer cell lines were selected to access the cytotoxicity of the compounds. The human astrocytoma SNB-19 cell line, human Dukes' type C colorectal adenocarcinoma HCT-15 cell line, human Dukes' type D colorectal adenocarcinoma COLO-205 cell line, and human cervix carcinoma KB-3-1 cell line were purchased from the American Type Culture Collection (ATCC, Manassas, VA). All the cell lines were cultured in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin in a humidified incubator at 37 °C with 5% CO_2.

Cytotoxicity Assay

A modified MTT assay was conducted to access the cytotoxicity of INH and five metal complexes of INH to cultured cancer cells. The assay assesses cell viability by detecting the formazan product formed from the reduction of 3-(4,5-dimethylthiazole-2-yl)-2,5-biphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase of metabolically active cells [29]. In brief, all the four cell lines were seeded in 96-well plates at a final density of 5 x 10^3 cells/well and allowed to adhere and grow for 24 hours. After then, various concentrations of the six compounds were added respectively to the cells for 72-hour continuous drug incubation. At the end of the 68th hour of incubation, 20 µl MTT (4 mg/ml) was added to each well. The plates were incubated at 37°C for another 4 hours. Subsequently, the medium was aspirated carefully and 100 µl of DMSO were added to dissolve the formazan crystals. After shaking the plates for 5 minutes, the absorbance at 570 nm was measured using the accuSkan™ GO UV/Vis Microplate Spectrophotometer (Fisher Sci., Fair Lawn, NJ). The cytotoxicity of the compounds was represented by the calculated IC50 (concentration that inhibited the survival of cells by 50%) values.

Result and Discussion

FTIR Spectra

The structure of INH reveals that molecule have three potential donor sites namely as nitrogen of pyridine ring, nitrogen of amides and oxygen of carbonyl. Each atom can donate a pair of electron for coordination with metal ion. IR spectroscopy is used widely to distinguish the changes appears in the spectrum of unreacted ligand molecule and product obtained after complexation upon comparing both spectra generally intensity of the peaks, their shapes and shifting of peaks are observed in the spectrum of pure ligand and its corresponding complex. This investigation helps in the diagnosis of coordination / interaction of metal ion with ligand molecule.

The prominent bands at 1662 cm^{-1}, 3302 cm^{-1}, 3313 cm^{-1} and 1633 cm^{-1} were observed in the IR spectrum of free INH. These were assigned to (C=O), (NH asymmetric & symmetric) and (NH_2). The former strong bands were assigned (N-H) and other band where to (C=O). The region of the other bands and their frequencies are reported in Table **3**.

Interestingly the spectra of INH complexes also displayed the bands which have been shifted in another region. The band of (C=O) was shifted to lower frequency at 1649-1651in the spectra of Co(II), Ni(II) & Fe(II) whereas this band was displayed at higher frequencies 1697 & 1704 in the complexes of Fe (III) and Copper; band of (NH2) has migrated at 1620, 1622 & 1654 in the spectra of Fe(III), Co(II) and Cu(II). While band expected in the same region was disappeared in the complexes of Fe (II) and Ni (II) thus giving an indication for the participation of oxygen of (C=O) and nitrogen of (NH_2) in coordination with metal ions the FTIR date incorporated in Table-1

^{1}H-NMR Spectra

The proton NMR spectra of INH and its corresponding complexes were recorded in deuterium oxide and were compared for the confirmation of transformation of ligand molecule into its respective metal complex. Obviously, number of protons in the complex molecules is same as the ligand possessed; thus, integration of each proton was found equal in both unreacted ligand and its ligand metal complex. However, a change pattern of splitting / shape of peaks were observed.

The four ring protons two sharp doublets in the spectrum of INH were changed in the spectrums of complexes as broad doublet at 7.8 – 8.6 ppm. The two sharp doublets associated with (NH_2) in the INH were resonate in the down field region at 3.25 ppm and the singlet at 3.88 ppm were attributed to amide protons which were not appeared in spectra of complex that might be due to the exchange of nitrogen proton with deuterium of deuterium oxide.

Morphological Studies

The morphologies of Isoniazid (INH) and its metal complexes with cobalt (Co), copper (Cu), iron (Fe (II), nickel (Ni) and Iron Fe (III) were explored by scanning electron microscopy (SEM). Fig. 1a shows the scanning electron micrographs for the pure drug Isoniazid (INH). The morphology appears as cylindrical rods with variable length in the range of 20 to 50 microns. The thickness of the rods is around 15 microns. The surface is relatively smooth which indicates the drug is likely in a homogeneous phase.

Fig. 1b shows the morphology of the Co(II)-INH complex. A change in the morphological features and a marked reduction in the particle sizes are noticeable. The morphology appeared as large and small rock-like crystalline particles of rather irregular shapes and a broad size distribution. Interestingly, agglomerate of small crystals with the size ranges in nanometers can be seen distributed on the surface larger crystals.

The morphology of Cu(II)-INH complex particles is depicted in Fig. 1c where a drastic reduction in the particle size is visible. The morphology of complex appeared as aggregates of small particles of relatively narrow size distribution and the particle diameter in the nanometer range. The smaller average particle diameters observed for the complex suggest likely polycrystalline morphology with nano-sized grains.

As depicted in Fig. 1d, SEM revealed a non-spherical, platelet-shaped morphology for Fe(II)-INH complex. Particles are of rather irregular shape with a broad size distribution. Few small crystals with the size ranges in nanometers can also be seen. The morphology of the Ni(II)-INH complex is depicted in Fig. 1e which appeared as large rock-like irregular shaped crystalline particles. Interestingly, compared to the pristine Isoniazid and its Co(II) and Fe(II) complexes, the surface of the Ni(II)-INH complex in rather uneven. This may likely caused by the fact that the large crystals are formed by the agglomeration of small particles.

The morphology of Fe(III)-INH complex particles is depicted in Fig. 1f. Compared to pristine ligand, a drastic change in the morphology is visible which is likely caused by the complex formation. The particles display a rock-like morphology with an irregular shape and a broad size distribution. Compared to pure INH (Fig. 1a), a marked reduction in particle size is also visible.

Table-1: FTIR Data of INH and Metal Complexes in cm-$^{1.}$

Compound	C=O	N-H	NH_2	Ring	N-N	C-N	H_2O	O-H
			Frequency (cm^{-1})					
INH	1662	3302* / 3113**	1633	1602/1556	1192 / 889	1058	-	-
Cu(II)-INH	1705	3099	1654	1560	1134 / 858	1062	910	3448
Co(II)-INH	1651	3136	1622	1544	1141 / 856	1066	900	3369
Fe(II)-INH	1649	3118	X	1559	1128 / 856	1066	896	3414
Fe(III)-INH	1697	3136	1620	1554	1058 / 862	1058	904	3433
Ni(II)-INH	1649	3118	X	1554	1143 / 852	1068	904	3412

* Asymmetric stretching and ** symmetric stretching.

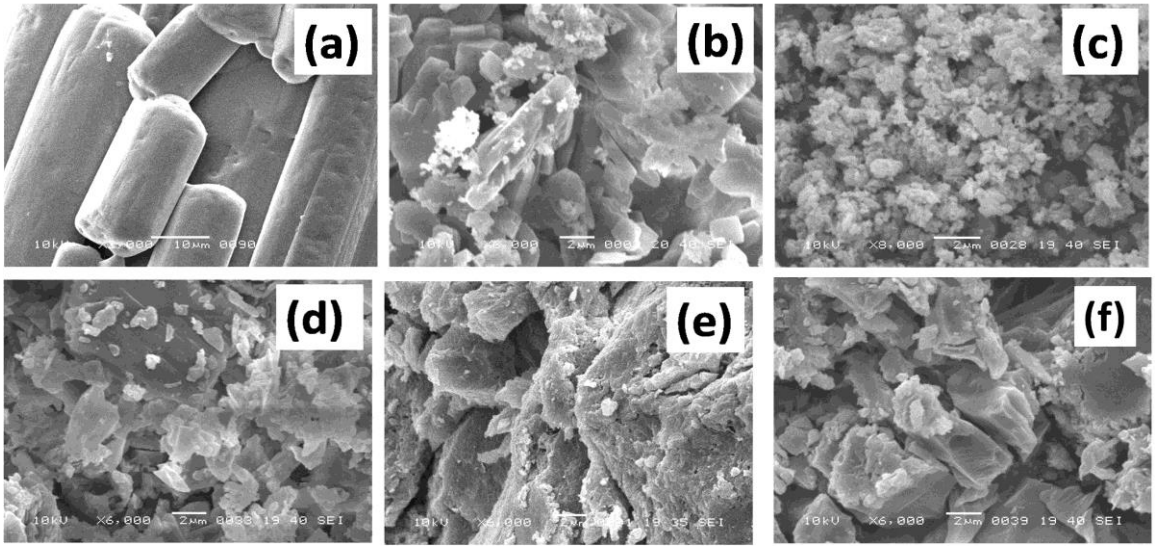


Fig. 1: SEM micrographs of (a) pure Isoniazid, (b) Co(II)-INH, (c)Cu(II)-INH, (d) Fe(II)-INH (e) Ni(II)-INH (f) Fe (III)-INH complex particles.

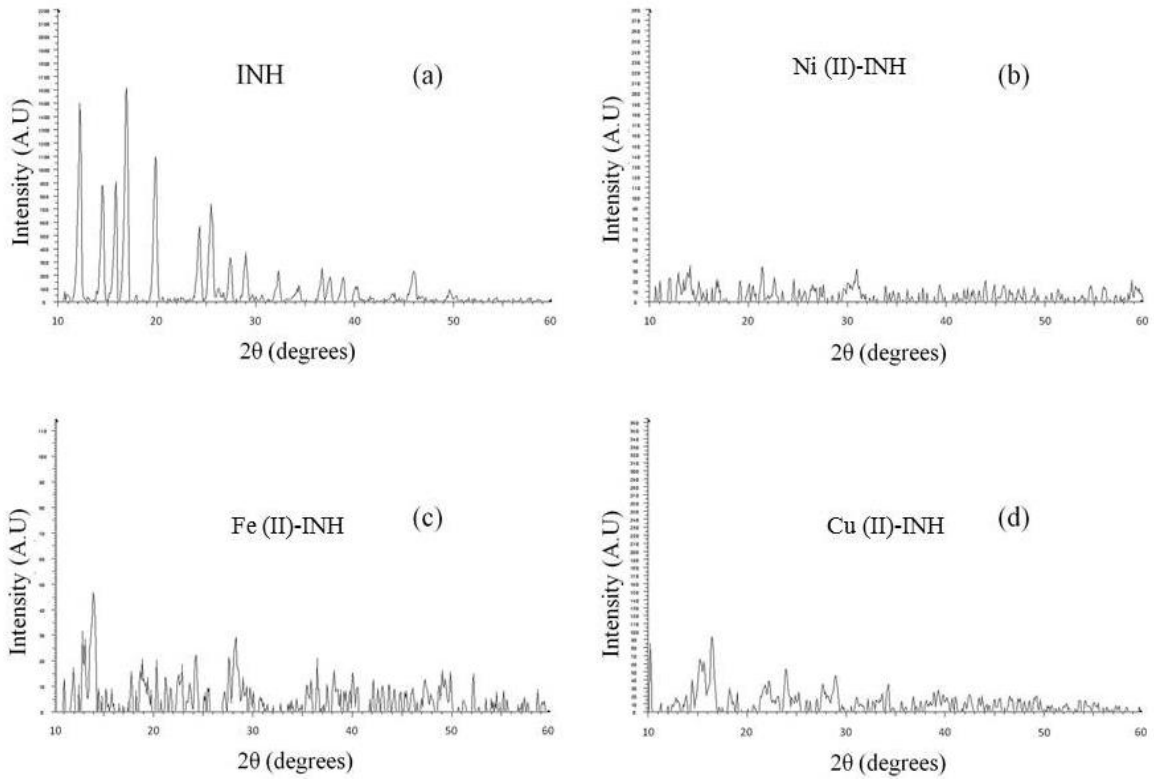


Fig. 2: X-ray diffractograms of (a) pure Isoniazid (INH), (b) Ni(II)-INH, (c) Fe(II)-INH and (d) Cu(II)-INH complexes.

X-Ray Diffraction

To probe the structural changes accompanying the complexation process, the crystalinity of pristine INH and its metal complexes was examined by X-ray powder diffraction. Pure drug Isoniazid (INH) is reported to form somewhat rod-like cylindrical crystals [30, 31]. As revealed by the X-ray diffractograms of pure Isoniazid shown in Fig. 1a, the drug exhibit multiple sharp peaks in the

range of 2θ angles explored (10–60°) indicating the presence of crystallinity. The characteristic diffraction peaks at 2θ values of around 12.2°; 14.6°; 15.7°; 16.9°; 19.8°; 24.2°; 25.6°; 27.4°, 29.0°, 32.4°, 36.5°, 37.8° and 38.9° were obtained with the maximum intensity peak was observed at 16.8°. Similar diffractograms for Isoniazid are reported by Fukuoka *et al* [32] and Nibedita *et al* [33]. It can be concluded that in its pure form, Isoniazid is polycrystalline in nature and this observation is further supported by SEM characterizations (Fig. **1**). The diffractograms obtained for the metal complexes Ni(II)-INH, Fe(II)-INH and Cu(II)-INH are given in Fig. **2b**, **c** and **d**. Although the pure isoniazid exhibited sharp Bragg reflections, typical of crystalline materials, the XRD patterns recorded for its metal complexes showed no appreciable crystalline peaks. From the XRD patterns of the complexes, it may be concluded that the complexes are mostly comprises of nano-sized particles behaving like amorphous materials in XRD characterizations. This result correspond well with the results obtained with SEM and indicates the molecular dispersion of drug in the complexes [34].

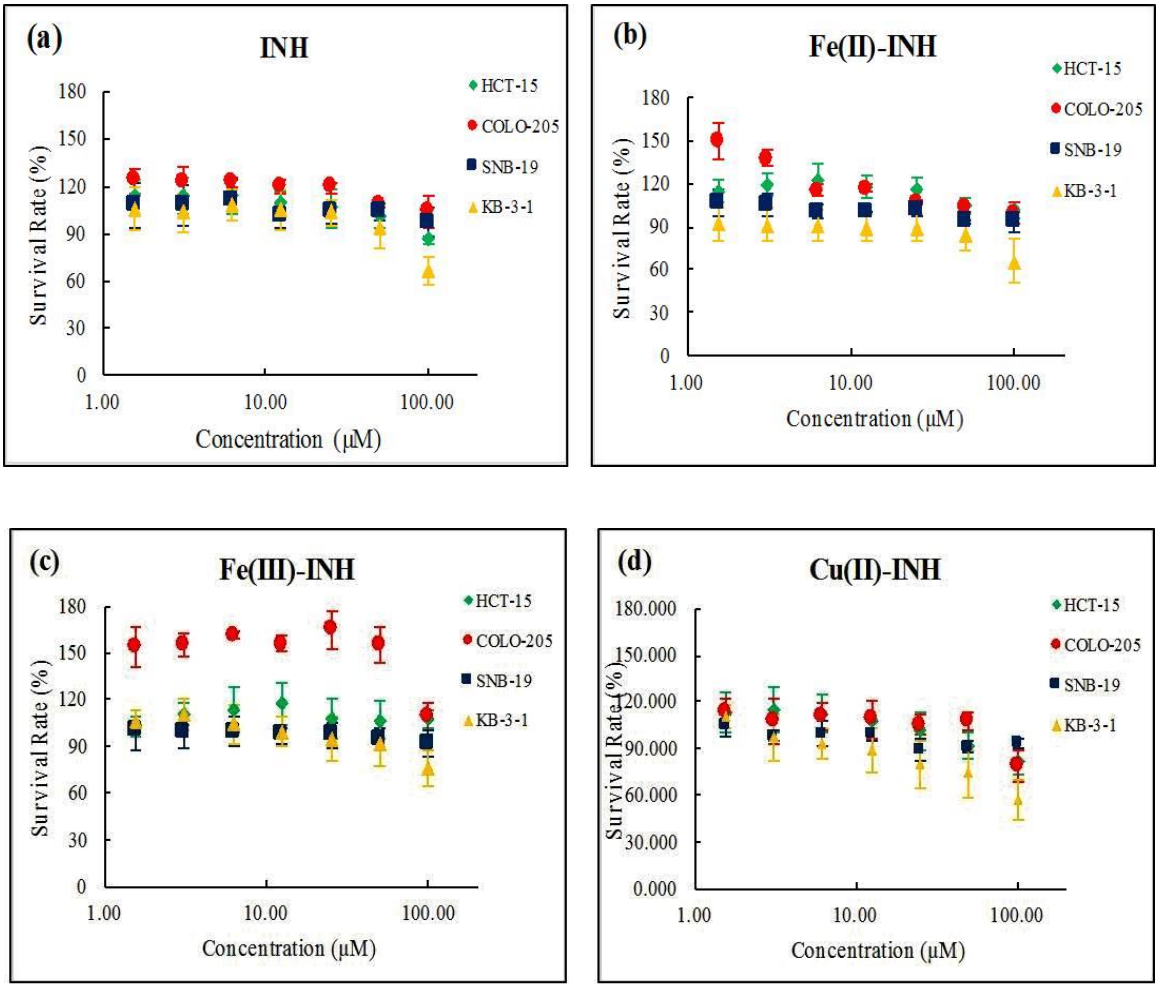
Cytotoxicity

The IC_{50} values of pure INH and five INH metal complexes on the cancer cells used are summarized in Table-2. All the compounds showed an IC_{50} of greater than 100 µM on the four cell lines tested shown in Fig. **3**.

Table-2: Cytotoxicity of MA Series on Four Human Cancer Cell Lines.

	IC_{50} ± STD (µM)			
Drug #	**SNB-19**	**HCT-15**	**COLO-205**	**KB-3-1**
INH	**>100**	**>100**	**>100**	**>100**
Fe(II)-INH	**>100**	**>100**	**>100**	**>100**
Fe(III)-INH	**>100**	**>100**	**>100**	**>100**
Cu(II)-INH	**>100**	**>100**	**>100**	**>100**
Co(II)-INH	**>100**	**>100**	**>100**	**>100**
Ni(II)-INH	**>100**	**>100**	**>100**	**>100**

Data represents the mean IC50 values for each cell line ± SD obtained from three independent sets of experiments.



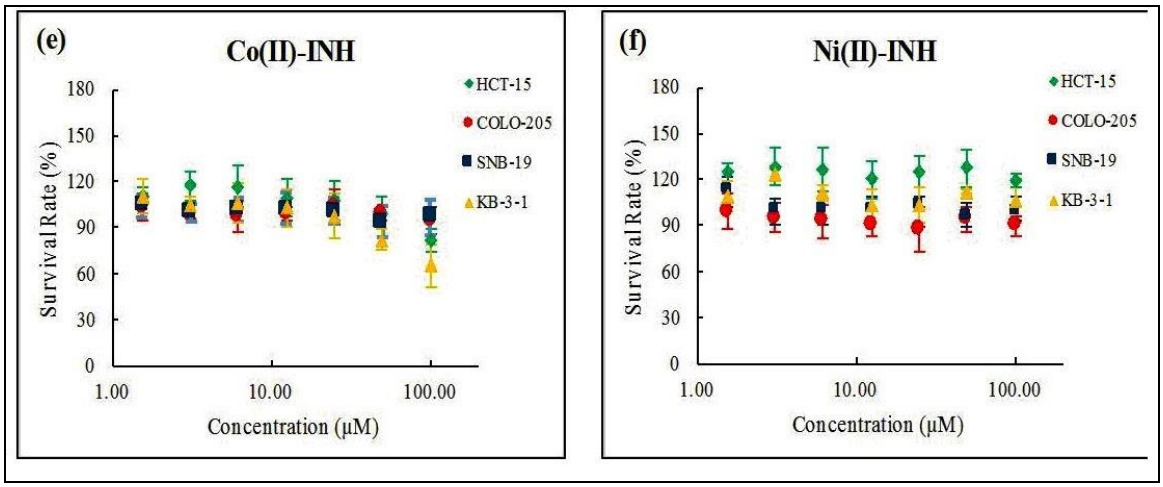


Fig. 3: The cytotoxic effects of (a) pure INH, and its metal complexes with (b) Fe (II), (c) Fe (III), (d) Cu (II), (e) Co (II), (f) Ni (II), in the HCT-15, COLO-205, SNB-19 and KB-3-1 cell lines as determined by MTT assays. The mean±SD of survival rate were calculated from three independent experiments.

Conclusion

This study focuses on spectroscopic and morphological characterizations and biological activities of metal complexes of INH. FTIR data showed measure shifting in the peaks of carbonyl and amino group in metal complexes as compared to pure INH. Changes in the shapes of the NMR and XRD spectra and changes in morphology strengthen the hypothesis of successful formation of complexes.

In order to understand the cellular effects of these compounds, their cytotoxicity was determined on four cancer cell lines. Since, all the compounds showed a significantly high IC_{50} values (>100 µM), these compounds are not cytotoxic and have less potential of being anti-cancer drug candidates [35, 36]. Further study is needed to explore more cancer cell lines or to make more derivatives of INH.

Conflict of Intrest

There is no conflict of interest.

Reference

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