Metal complexes of isonicotinylhydrazide and their antitubercular activity

Mohsin Ali[1], Mansoor Ahmed[2*], Saleem Hafiz[3], Mustafa Kamal[4], Majid Mumtaz[1], Muhammad Hanif[2] and Khalid Mohammed Khan[5]

[1]Department of Chemistry, University of Karachi, Karachi, Pakistan
[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Karachi, Pakistan
[3]Department of Microbiology, Sindh Institute of Urology and Transplantation (SIUT), Karachi, Pakistan
[4]Department of Biotechnology, University of Karachi, Karachi, Pakistan
[5]HEJ Research Institute of Chemistry International Center for Chemical and Biological Sciences, University of Karachi, Karachi, Pakistan

Abstract: The development and spreading of Multi Drug Resistant TB strains is hampering endeavours for the control and administration of tuberculosis (TB). The expansion episodes of multi-medication safe strains of *Mycobacterium tuberculosis* against first and second line antituberculosis drugs on one side and the unfavourable effects of these drugs on the other side has led the enthusiasm of researcher towards the synthesis of metal complexes of various medication. This approach is born with the expectation of finding new antituberculous operators without or least reactions as well as being active against the resistant strains of *Mycobacterium tuberculosis*. This study concentrates on the screening of five metal complexes of isoniazid (INH) against five *Mycobacterium tuberculosis* strains. These strains have been confirmed by WHO being active and even proliferating safely even in the presence of pyrazinamide, isoniazid (INH), ethambutol and rifampicin. In this work INH was taken as reference medication. All synthesized complexes and INH were subjected for a month and a half in BACTEC MGIT 960 technique. INH and its Fe (II) complex restrained the development of all bacterial strains for merely two weeks, while the Fe(III), Cu(II), Co (II) and Mn (II) complexes repressed the development five strains for three weeks. Conclusively, the strains utilized in this study were discovered to be more susceptible to the later four complexes than the ligand (INH) drug and its Fe (II) complex. Furthermore, elemental analysis and atomic absorption of all complexes were conducted for the determination of metal to ligand ratio.

Keywords: INH, Metal complex and *Mycobacterium tuberculosis.*

INTRODUCTION

Tuberculosis, an infectious ailment influencing numerous organs of the body is an insidious granulomatous disease bringing about dismalness and mortality in human eras for centuries. According to World Health Organization (WHO) roughly 2 billion people can be the potential bearers of TB, out of these people with inert TB contamination (Latent TB Infection- LTBI) around 200 million are inclined to suffer from dynamic TB amid their lifetime (Organization 2010). In another report of WHO, a TB patient gets by for an average of 2 years during which time he is likely to spread the bacteria to more than 20 others (Rieder 1999). Added to this is the wretchedness that most patients in high frequency settings are analyzed utilizing direct spread microscopy of un-concentrated sputum specimens which can identify the patients just with cutting edge disease (Enarson *et al*. 2010).

Isoniazid, otherwise called isonicotinylhydrazide (INH), an antibiotic is among the principal line treatment pharmacological agents for the treatment of both inert and active tuberculosis (Control 2016). It is active against not only mycobacteria, particularly *Mycobacterium tuberculosis* but is likewise effective against some atypical mycobacteria, such as *M. kansasii* and *M. xenopi*. Isoniazid is unique in that it goes about as a bactericidal agent against quickly dividing mycobacteria, while it is also bacteriostatic for the gradually developing mycobacteria making it a first line medication to battle bacteria which are otherwise hard to overcome by other common anti-infection agents because of their walling actions (Suarez *et al*. 2009). Isoniazid being a prodrug must be enacted by a bacterial catalase-peroxidase protein in *Mycobacterium tuberculosis* called KatG (Timmins *et al*. 2004).

In the current years, issues on multidrug safe microorganism have been drawn closer at alarming level the world over. A very number of *Mycobacterium tuberculosis* strain end up plainly impervious to isoniazid and other first line medication of tuberculosis (Sriram *et al*., 2005).

The science of metal coordination plays a noteworthy part in the field of biology and is used to investigate the metal complexes of natural drug molecules with the prospect of expanding their pharmacological actions and beating the resistance. The metallic combinations of thiosemi-

**Corresponding author:* e-mail: cando@cyber.net .pk

carbazones are accounted for more cytotoxic than free ligand (West *et al*. 1993, Beraldo *et al*. 2004). As research has demonstrated the important role of inorganic metal particles in different organic processes, recently "Elemental Medicine" (the utilization of inorganic science to medicine) has turned into a quickly creating field, bringing about the revelations and combinations of novel restorative and indicative metal –drug combinations which are exhibiting their huge effect on therapeutic practice (Sun *et al*., 1997).

Literature study uncovers that during the most recent decade there has been sublime consideration towards the designing and synthesis of metal complexes of various medications as well as study of their biological activities. The target of such tasks is to enhance the effectiveness of newer compounds as compared to the parent medication while bringing down the untoward reactions. Many research papers have portrayed the antimicrobial activity of metal combinations of isoniazid and its derivatives (Donia *et al*. 1989, Narang *et al*. 1996). It was built up that the combination of metal particles with anti-infection agents has the potential to actually change the organic action of ligand molecule. Subsequently on this reality grounds it was considered beneficial to combine metal particles with medications already utilized against tuberculosis since this malady needs long term treatment and is joined by different collateral (de Souza *et al*. 2005). Cognizance of this fact has brought about the synthesis of INH-metal ions compounds and their screening to evaluate the efficacy against various diseases has been carried out (Allan *et al*. 1984, Kriza *et al*. 2009, Kriza *et al*. 2010, Kriza *et al*. 2010). The blend of first Cu(II)- INH was accounted for in 1981 (Hanson *et al*. 1981). These and the Fe(II) complexes of INH have been found to possess antituberculous as well as antitumor activities. In vivo inhibitory impact of INH against *Mycobacterium tuberculosis* H37Rv had also been reported additionally (Sah *et al*. 1954, Gürsoy *et al*. 1997, Bedia *et al*. 2006, Sharma *et al*. 2006). In an exploratory work the copper complexes of INH subsidiaries were screened and observed to be active against bacteria (Prasanna *et al*. 2013). Moreover, metal of INH derivatives were tried against different Gram-positive and Gram-negative microbes and found to have higher antibacterial activities (Abou-Melha 2008).

Considering the essential role of these metal ions in the biochemical processes of living organisms, we have integrated the copper (II), Iron (II), Iron (III), cobalt (II) and nickel (II) compounds of isoniazid to find new lead molecules with more potential to kill the *Mycobacterium tuberculosis* when compared to the parent compound. In this review, five resistant strains of *Mycobacterium tuberculosis* were assessed against the synthesized derivatives.

MATERIALS AND METHODS

Preparation of complexes (INH)

Equimolar quantities of INH and metal salts (cobalt chloride) were weighed accurately on an analytical balance. Both were dissolved separately in the volumetric flask in methanol; each was introduced into a round-bottommed flask. The mixture was refluxed on a water bath and allowed to heat for 4 hours with occasional stirring. After refluxed the mixture was allowed to cool at room temperature upon cooling crystals were formed at the bottom of the flask were separated out by filtration. The crystals were dried at 60°C in an 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.

Antimycobacterial activity

Five reference multidrug resistant (i.e., resistant to isoniazid, pyrazinamide, ethambutol and rifampin) strains were used in this study for INH susceptibility test using the MGIT 960 system as per the manufacturer's instructions (Becton 1999), according to WHO protocol (Sotgiu *et al*. 2012). Since the BACTEC MGIT 960 system is routinely used in laboratory for primary isolation, 500μL from a positive MGIT tube was sub-cultured in vials for susceptibility test. The drug solutions were prepared by reconstitution of the provided lyophilized drugs with distilled water. The tests with the automated BACTEC MGIT 960 instrumentation were performed with MGIT cultures that were tested positive at least 1.

Each 7.0mL MGIT tubes were supplemented with 0.8mL of growth supplement OADS. The lyophilized drugs were rehydrated in accordance with the recommended procedure; 100µL of antibiotic solution (the recommended critical concentration of INH was 0.1μg/mL) was added to a labeled MGIT tube for each drug.

For the drug-free control, the culture was diluted 1:100 in distilled water before addition to the control tube. The tubes were placed in the proper MGIT rack in a fixed sequence; the rack was incubated in the cabinet drawer and left there until the conclusion of the test was signaled by the instrument. A single concentration of drug (i.e., critical concentration) was tested.

After bar code scanning all the inoculated tubes were loaded in the instrument and incubated at a temperature of 37°C. An un-inoculated tube was used as negative control, and incubated for six weeks (42 days).

The MIGIT 960 System flags the completion of a DST when the growth unit (GU) of the growth control reaches 400 and reports S for susceptible or R for resistant, as well as a GU value for each drug containing MGIT tube

Table 1: Comparison of percent elemental composition

Compound	%C	%H	%N	%Metal
	Theoretical / Practical	Theoretical /Practical	Theoretical /Practical	Theoretical / Practical
[a][Cu $(INH)_2Cl_2$].$4H_2O$	29.94/30.23	4.57/4.42	17.47/17.16	13.21/ 3.53
[b][Co $(INH)_2Cl_2$].$8H_2O$	26.26/26.03	5.47/5.38	15.32/14.96	10.96/11.11
[c][Fe(II) $(INH)_2Cl_2$].$4H_2O$	30.45/29.81	4.61/4.55	17.76/17.61	11.74/11.82
[d][Fe(III)$(INH)_2Cl_2$]Cl.$4H_2O$	28.32/28.43	4.32/4.28	16.52/16.59	10.92/11.05
[e][Ni $(INH)_2Cl_2$].$4H_2O$	30.25/29.88	4.62/4.52	17.65/15.91	12.33/12.32

M. mass: [a] 480.82 g/mole, [b] 548.21 g/mole, [c] 472.83 g/mole, [d] 508.33 g/mole and [e] 475.97 g/mole

Table 2: Sensitivity of *Mycobacterium Tuberculosis*

Strains	INH (GA)	Fe(II)-INH (GA)	Fe(III)-INH (GA)	Cu(II)-INH (GA)	Co(II)-INH (GA)	Ni(II)-INH (GA)	Control (GA)
1195	Positive (2 weeks)	Positive (2 weeks)	Positive (3 weeks)	Positive (3 weeks)	Positive (3 weeks)	Positive (3 weeks)	Positive (1 weeks)
3029	Positive (2 weeks)	Positive (2 weeks)	Positive (3 weeks)	Positive (3 weeks)	Positive (3 weeks)	Positive (3 weeks)	Positive (1 weeks)
1289	Positive (2 weeks)	Positive (2 weeks)	Positive (3 weeks)	Positive (3 weeks)	Positive (3 weeks)	Positive (3 weeks)	Positive (1 weeks)
1375	Positive (2 weeks)	Positive (2 weeks)	Positive (3 weeks)	Positive (3 weeks)	Positive (3 weeks)	Positive (3 weeks)	Positive (1 weeks)
2029	Positive (2 weeks)	Positive (2 weeks)	Positive (3 weeks)	Positive (3 weeks)	Positive (3 weeks)	Positive (3 weeks)	Positive (1 weeks)

GA = Growth Appeared

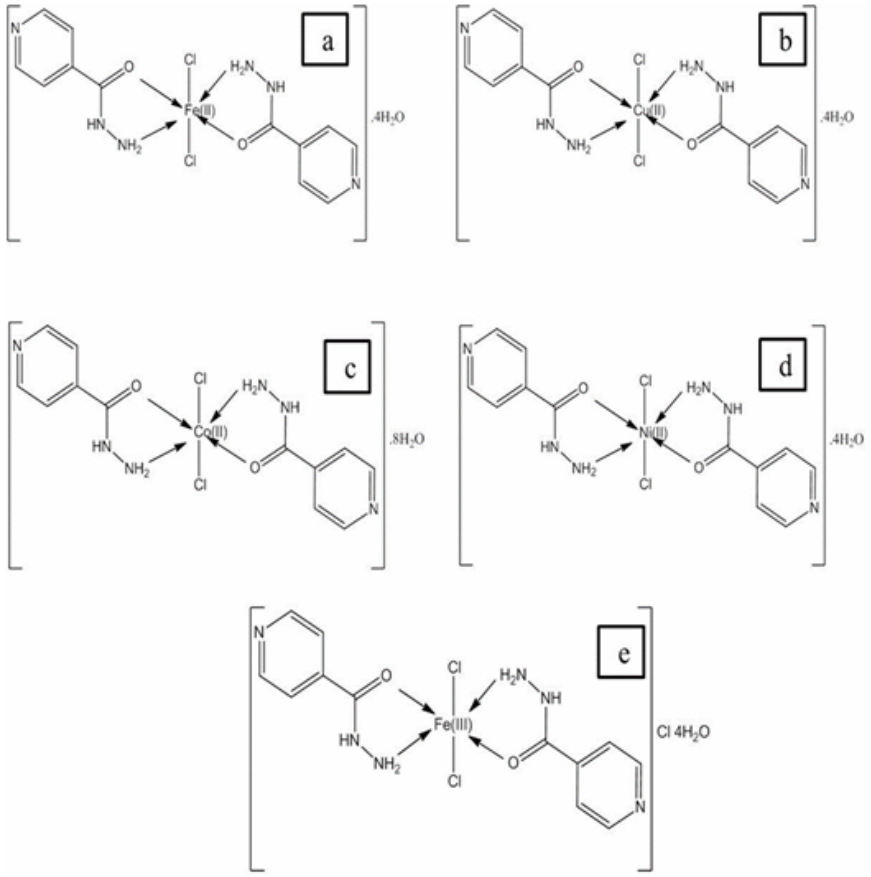

Fig. 1: Structures of Metal complexes of INH

on the printout. An isolate was interpreted to be susceptible when the GU of a drug containing MGIT tube was equal to or less than 100 or as resistant when the GU was greater than 100.

If an isolate was interpreted to be resistant, a smear was made and stained by ZN method to prove the presence of AFB with morphology compatible with that of *Mycobacterium tuberculosis* and the absence of contaminants.

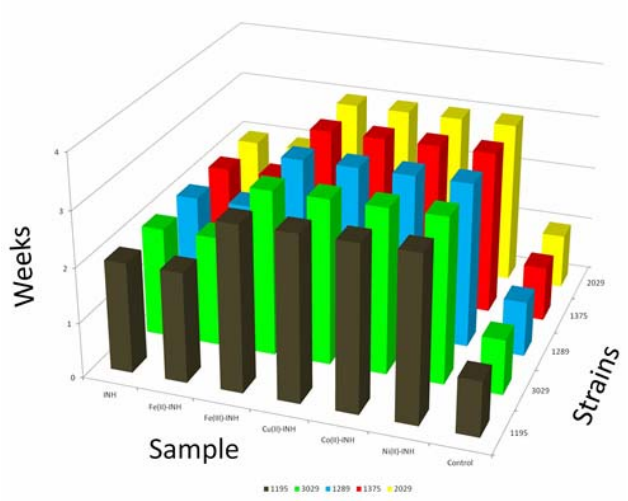

Fig. 2: Graphical representation of bacterial growth with respect to time

RESULTS

CHN and metal analysis of INH and INH- metal complexes

The quantitative estimation of elements (carbon, hydrogen and nitrogen) in the complexes of INH was estimated by elemental analyzer, Perkin Elmer 2400 Series II, whereas the percentage of metal in complexes was determined on an atomic absorption spectrophotometer (PerkinElmer AAnalyst 700). The results found are in agreement with the theoretical values. The metal to ligand ratio found to be 1:2. The percentage of elemental composition, molecular weight, proposed molecular formulas are tabulated in table 1, proposed structures are shown in fig. 1.

DISCUSSION

In this study, *in vitro* antimycobacterial activity of parent compound (INH) and its five metal complexes (Fe(II)-INH, Fe(III)-INH, Cu(II)-INH, Co(II)-INH, and Mn(II)-INH) were performed against five reference strains of *Mycobacterium tuberculosis* by using BACTEC MGIT 960 method. When parent drug and its Fe(II)-INH complex were tested for antimycobacterial activity they were found to resist the growth up to 2 weeks. The valuable results were obtained with complexes (Fe(III)-INH, Cu(II)-INH, Co(II)-INH, and Mn(II)-INH) in which growth of all five strains were retarded for 3 weeks. This showed that these complexes have the capability to resist the growth of mycobacteria and revealed that various derivatives of INH possess moderate activity against five strains as compared to INH and other first line anti TB drug (pyrazinamide, rifampicin and ethambutol) resistant strains of *Mycobacterium tuberculosis* for longer period of time. Out of these five complexes four complexes Fe(III)-INH, Cu(II)-INH, Co(II)-INH and Mn(II)-INH emerged with antitubercular activity for 3 weeks instead of just two weeks for parent drug. The promising activity of Fe(III)-INH, Cu(II)-INH, Co(II)-INH and Mn(II)-INH showed that they can be used to design further novel derivatives with better antitubercular activity.

Similar studies have been conducted by various groups indicating that metal complexes increases the antibacterial as well as antitubercular activity of such complexes of isoniazid (Sharma *et al*. 2010) indicated that the antimycobacterial activity of the ligands and their metal complexes against *Mycobacterium smegmatis*, showed clear enhancement in antitubercular activity upon metal complexation with Schiff bases.

CONCLUSION

This study identified new lead molecules and further modification and refining on these molecules may result molecules with potent antimycobacterial activity.

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