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METAL ION-DRUG INTERACTION: PHYSICO-CHEMICAL CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF METAL COMPLEXES DERIVED FROM ESTABLISHED DRUGS

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ABSTRACT

In the present work isoniazid, pyrazinamide, fluconazole, metformin and losartan potassium were chosen and transformed into complexes upon their interaction with metal salts. Physico-chemical properties of the resultant complexes and antibacterial activity against 5 gram positive and 2 gram negative species are determined. Some of our newly synthesized complexes demonstrated higher activity as compared to their respective ligand.

Key-words: PZ, INH, FCZ, DMBG, LS-K, metal complexes, Anti-bacterial activity, physico-chemical characters.

Abbreviations:
Isoniazid (INH), Pyrazinamide (PZ), Fluconazole (FCZ), losartan potassium (LS-K), Metformin (DMBG).

INTRODUCTION

The recent advances in the field of bioinorganic chemistry have increased the interest in heterocyclic complexes. It has been established that many of these complexes have potential to be used as models for biologically important species. Heterocyclic pharmaceutical compounds play an important role in several biological processes because of their various significant properties, one of these being their metal coordination ability. Literature survey reveals that over the last few decades much attention has been paid towards studies on metal complex formation using various drugs as ligand. It has been reported that metal complexes are more potent than the pure drugs(Sigel, 1973;Mukherjee*et al.*, 1955).The prime objective of such type of work is to decrease the toxicity while maintaining the same or achieving greater efficacy than the parent drug.

The present study is aimed to investigate the physico-chemical characteristics and determining the anti-bacterial activity of drug metal complexes. It deals with the evaluation of metal complexes of five widely prescribed drugs. The bivalent and trivalent metal ions were chosen for the complex formation with isoniazid (INH), pyrazinamide (PZ), fluconazole (FCZ), losartan potassium (LS-K) and metformin (DMBG) as chelating agents.

PZ and INH are the first line treatment drugs used for the cure of tuberculosis (Bedia *et al*., 2006, Gürsoy *et al*., 1997, Sah and Peoples, 1954; WHO, 2002). Complexes of PZ have been reported in literature for their anti-mycobacterial properties (Katzung, 1989, Opletalova *et al*., 2002). Tewari reported anti-bacterial activity of PZ complexes towards *E. coli, streptocoli* and *mycobacteria* (Tiwari, 2012). The emergence of resistant mycobacterium strain against PZ has been declared as an important public health problem, as this drug is capable of shortening the tuberculosis therapy from 9–12 months to a period of 6 months (Somoskovi *et al*., 2004). Pd (II) and Pt (II) complexes of antibiotics belonging to tetracycline family have been proven more potent against *E. coli HB101/pBR322*, a tetracycline resistant bacterial strain. This finding strengthens the thought that metal's coordination with biologically active molecules can effectively be used as a means to enhance their activity and overcome resistance. PZ has previously been reported to form stable metal complexes several of which more antitubercular activity (Akyuz, 2003; Akyuz *et al*., 2007; Budhani *et al*., 2010; Lavini *et al*., 2001; Rao and Kumar, 1994).

In an exploratory work the copper complexes of INH derivatives were screened and found to be active against bacteria(Prasanna and Kumar, 2013). Furthermore, metal complexes of INH derivatives were tested against various gram positive and gram negative bacteria and reported to possess higher anti-bacterial activity (Abou-Melha, 2008).

Fluconazole, the globally used antifungal has a significantly better clinical and mycological cure rate as compared to it raconazole in oropharyngeal candidiasis with a more rapid symptomatic response (Oude Lashof *et al*., 2004), because of its good adhesion to the oral mucosal surface (Epstein *et al*., 2002; Goins *et al*., 2002; Lefebvre and Domenge, 2002; Sholapurkar *et al*., 2009; Taillandier *et al*., 2000).

Oral hypoglycemic agent Metformin, the leading biguanide has been chosen for the synthesis of transition metal complexes and its Cr (IV) and Fe(III) complexes have been synthesized and screened against *E. coli and B. megaterium.* Both complexes showed promising activity (Sharma*et al*., 2010), while Cr (III) complex of this drug exhibit activity against gram positive and gram negative bacterial strains (Adam *et al*., 2015).

Losartan selectively inhibits the rennin angiotensin system by specifically targeting the angiotensin II AT1 receptor and is widely used for the treatment of hypertension in humans (Khairnar *et al*., 2012; Wong *et al*., 1991).

Losartan complex with Copper has been studied previously for its effects on cell proliferation using osteoblast like cells from rats. The study shows that the complex had greater antiproliferative effect on cells as compared to the both compounds acting separately. The study also reported the effect of this compound on cell morphology with a decrease in the number of the surviving cells by effecting the intercellular connections (Etcheverry *et al.,* 2007).

MATERIALS AND METHODS

Preparation of metal complexes: The solution of PZ (0.1 M) in 20.0 mL methanol was added, under stirring, into a round bottom flask, containing 10 mL of 0.1 M solution of copper chloride. The resulting mixture was kept stirring for few minutes at room temperature followed by refluxing on a water bath at 80ºC for 3 to 4 h. The formed solid material was filtered using whatman filter paper and washed by hot methanol to furnish Cu complex of PZ. Same procedure was adopted for the synthesis of complexes of other metal (II) with PZ and other ligands listed in Table 1 (Ali *et al*., 2016; Ali *et al*., 2017; Ali *et al*., 2018a, b; Ahmed *et al*., 2018; Ahmed *et al*., 2019).

Cytotoxicity or Antibacterial Activity Assay: Antibacterial activity of pyrazinamide, isoniazid, fluconazole, metformin and losartan potassium and their complexes against 5 gram positive and 2 gram negative bacterial strains were determined by 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), cells inhibition assay or cytotoxicity assay, freshly harvested bacterial cells isolated from tryptone soya agar were seeded at 10^6 cells in each well of 96 wells plate (Piaru *et al*., 2012). Eight Different concentrations of drugs and its metal complexes ranged from 500 to 5 µg/mL were serially diluted in Muller Hinton broth, then 200µL of each concentration was added in duplicate well and the plate were incubated for 18-24 h at 35.5 ± 2ºC. After incubation 20 µL of tetrazolium solution having a concentration of 5mg/mL was added in each well and the plate was incubated at 37 ºC for 4 h. The absorbance was measured at 570nm with a reference wavelength of 650nm DMSO and % inhibition was calculated.

$$\% \, Inhibition = \left\{\frac{(O.D.in\ Control - O.D.of\ test)}{O.D.in\ control}\right\} \times 100$$

RESULTS AND DISCUSSION

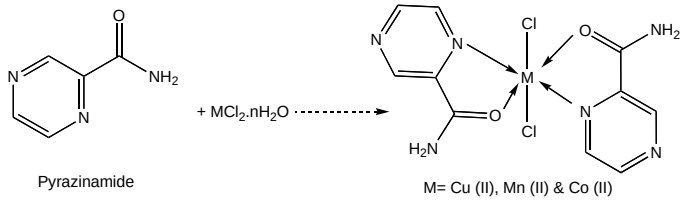
Chemistry

All complexes of PZ and INH were found to decompose in the range of 180 to 315°C which were higher than the melting points of the ligands. This indicates the successful formation of new compounds. All complexes are soluble in polar solvent indicating their polar nature. For all the complexes, pH values were found to be in the acidic range except Co (II)-PZ which is close to neutral compound while Mn(II)-PZ was slightly acidic. Iron (II) and (III) and copper complexes of INH exhibit more acidic nature. Magnetic susceptibility (BM) values were very close to the theoretical values of unpaired electron of metal's d-system. Physico-chemical data are listed in Table 1. Physico-chemical studies of iron (II) and (III) complexes of PZ, metal complexes of FCZ, LS-K and DMBG have been reported earlier(Ali *et al*., 2016; Ali *et al*., 2017).

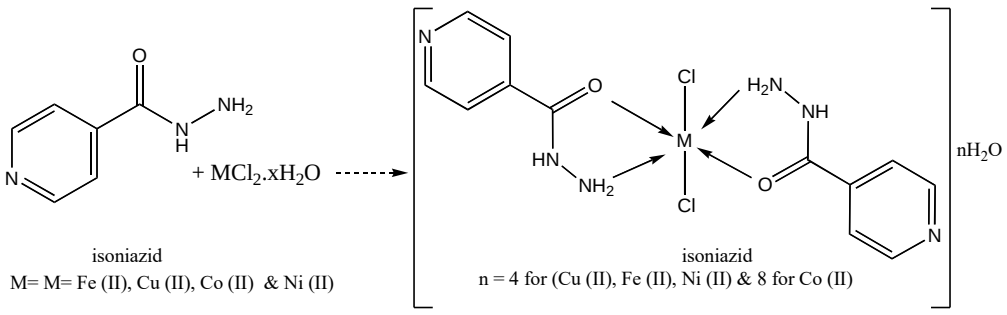
Table 1. Physico-chemical data of Isoniazid (INH) and Pyrazinamide (PZ) and their metal complexes.

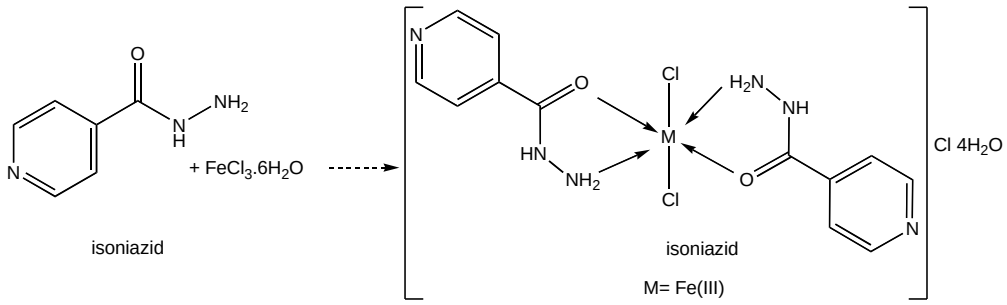
Compound	Color	M.P (^{O}C)	BM	pH0.1%
Isoniazid (INH)	White	172 ± 3	-	-
Fe (II)-INH	Golden brown	275D ± 3	4.14	2.82
Fe(III)-INH	Golden brown	315D ± 3	5.31	2.52
Cu(II)-INH	Sea green	228D ± 3	1.88	2.79
Co(II)-INH	Purple	280D ± 3	3.42	3.49
Ni(II)-INH	Light blue	180D ± 3	2.61	3.21
PZ	White	190 ± 2	-	-
Cu(II)-PZ	Green	245 D ± 2	1.73	2.46
Mn(II)-PZ	Light yellow	272 D± 2	5.71	5.92
Co(II)-PZ	Purple	225 D± 2	3.88	6.11

Scheme 1

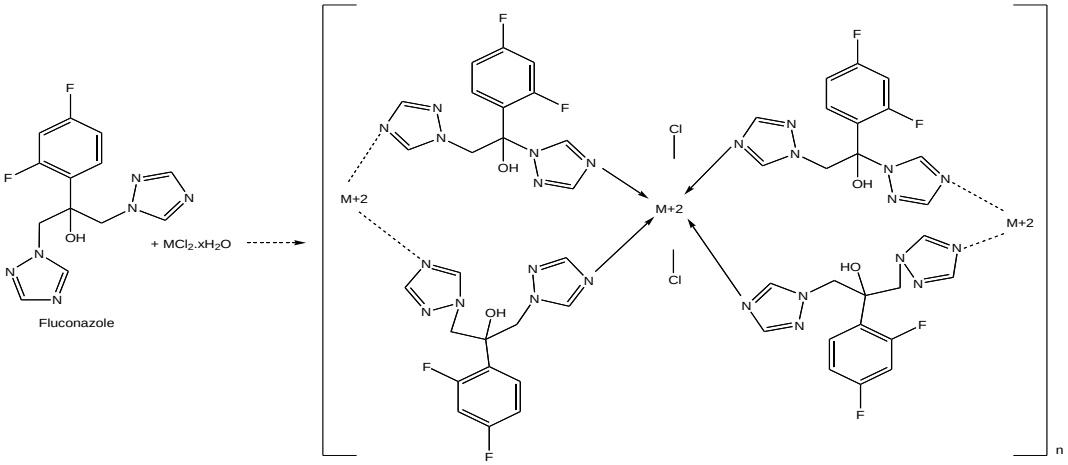


Scheme 2



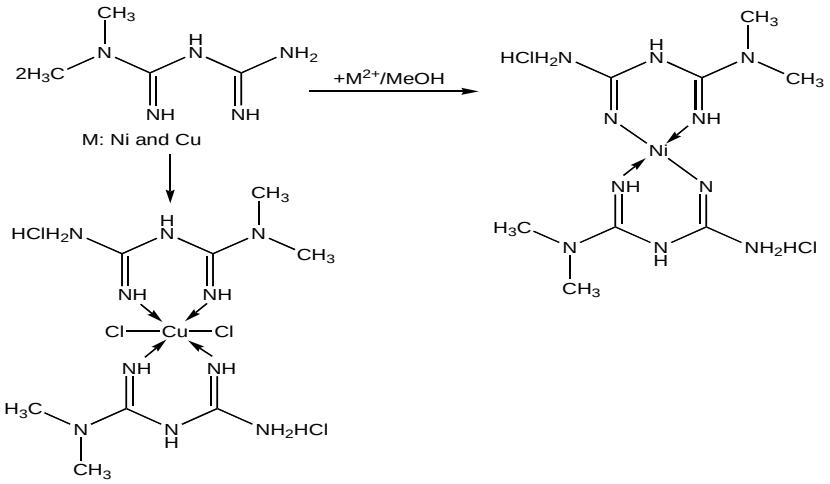


Scheme 3

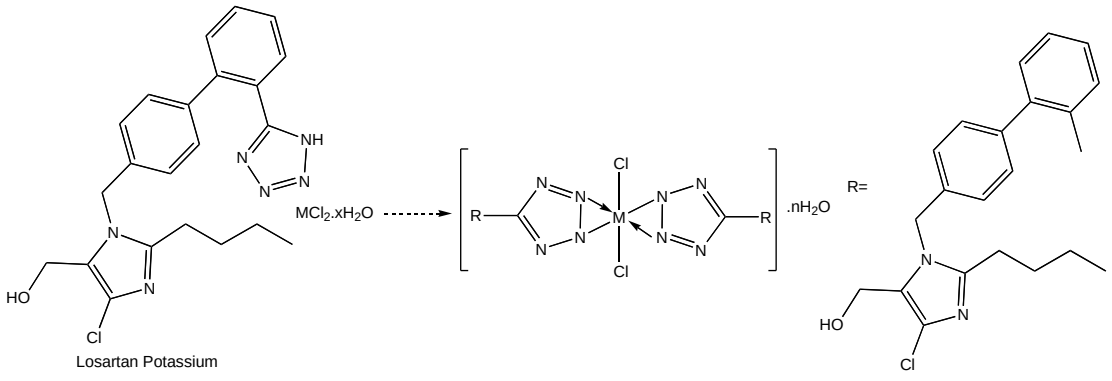


M = {Cd(II), Cu(II), Fe(II), Co(II), Mn(II) & Ni(II)}

Scheme 4



Scheme 5



M= Cu(II) & Ni(II) and n = 6 for Cu(II) and 1 for Ni(II)

Microbiology

Antibacterial activity of PZ and its Metal Complexes

The *in vitro* antibacterial screening results of metal complexes derived from PZ are depicted in Table 2. For this study 5 gram positive bacterial strains including *S. aureus* (9144, 11632 and 25923), *M. luteus* 10240, and *S. epidermidis* 13518 and 2 gram negative pathogens inclusive of *E. coli* (8739 and 10536), were used to test the ligand and its metal complexes. The observations showed that Cu(II)-PZ complex was more microbial toxic demonstrating higher activity toward the microbes than its ligand. While pure PZ did not exhibit activity against any of the gram positive and negative bacteria, the Cu(II)-PZ complex showed 2% activity toward *S. aureus* 25923 as well as against *E. coli* 8739. On the other hand, none of the other complexes synthesized in this study proved active against our targeted gram positive and negative bacterial strains. It implies that only Cu(II)-PZ complex is endowed with an anti-bacterial activity against two microorganisms *E. coli* 8739 and *S. aureus* 25923.

Table 2. Anti-Microbial activity of drugs and their metal complexes against Gram Positive and Negative Bacteria at 500 µg/mL.

Antibacterial activity of INH and its Metal Complexes

	Gram Positive Bacteria					Gram Negative Bacteria	
Compound	***S. aureus* 9144 % inhibition**	***S. aureus* 11632 % inhibition**	***S. aureus* 25923 % inhibition**	***M. luteus* 10240 % inhibition**	***S.epidermidis* 13518 % inhibition**	***E. coli* 8739 % inhibition**	***E.coli* 10536 % inhibition**
PZ	-	-	-	-	-	-	-
Fe(II)-PZ	-	-	-	-	-	-	-
Fe(III)-PZ	-	-	-	-	-	-	-
Cu-PZ	-	-	2%	-	-	0.5%	-
Mn-PZ	-	-	-		-	-	-
Co-PZ	-	-	-	-	-	-	-
INH	-	-	-	-	-	-	-
Fe(II)-INH	-	-	-	-	-	-	-
Fe(III)-INH	-	-	-	-	-	-	-
Cu(II)-INH	2.5%	3.5%	0.9%	1.7%	-	4%	5.5%
Co(II)-INH	-	-	-		-	-	-
Ni(II)-INH	-	-	-	-	-	-	-
FCZ	-	-	1%	1.5%	2%	-	-
Fe(II)-FCZ	-	-	-	-	-	-	-
Mn(II)-FCZ	-	0.3%	0.5%	-	0.7%	-	-
Cu(II)-FCZ	-	-	-	-	-	5%	10%
Co(II)-FCZ	-	-	-		-	-	-
Cd(II)-FCZ	0.6%	0.3%	1.6%	4%	3%	2%	3%
Ni(II)-FCZ	-	-	-	0.5%	0.9%	-	-
DMBG	-	-	-	-	-	-	-
Cu(II)-DMBG	-	-	-	-	-	4%	5.5%
Ni(II)-DMBG	-	-	-	-	-	-	-
LS-K	-	-	-	-	-	-	-
Cu(II)- LS-K	-	-	-	-	-	0.2%	2.6%
Ni(II)- LS-K	-	-	-	-	-	-	-

Keeping in view the functional importance of INH complexes, in the present work we have screened the INH complexes for their antibacterial activity. 5 gram positive and 2 gram negative pathogens already mentioned above were used to evaluate these metal complexes. The tests were carried out by MIC method for concentration of 5, 25, 50, 100, 200, 300, 400 and 500 µg / mL in DMSO. The results of the screening test are tabulated in Table 2. None of the complex and the reference drug was found active against any gram positive and gram negative bacterial strain

over the entire concentration range except Cu(II)-INH complex showed antibacterial activity against two gram positive bacterial strains including *S. aureus* (9144, 11632 and 25923), *M. luteus* 10240 and also active against gram negative bacteria (8739 and 10536). The compounds reported in this work revealed that the ligand and its complexes have no promising antibacterial activity.

Antibacterial activity of FCZ and its Metal Complexes

Six metal complexes of FCZ were screened against previously specified 5 gram positive and 2 gram negative bacterial strain for their antibacterial activity. The FCZ was set as reference for comparing the level of activity of its metal complexes. The minimal inhibitory concentrations of compounds were determined by MTT assay method. Eight different dilutions of concentration ranging from 5 to 500 µg / mL referred in the section of INH were prepared to test the compounds. The antibacterial susceptibility testing of compounds demonstrated that FCZ did not show any positive results toward gram negative bacteria but was found active against three gram positive strains named *S. aureus* 25923, *M. luteus* 10240 and *S. epidermidis* 13518. While its complexes showed activity against *E. coli* (8739 and 10536), *S. aureus* (9144, 11632 and 25923), *M. luteus* 10240 and *S. epidermidis* 13518. Fe(II)-FCZ, Mn(II)-FCZ, Co(II)-FCZ and Ni(II)-FCZ did not harm gram negative bacteria whereas Cu(II)-FCZ showed 5 and 10 % activity against *E. coli* 8739 and 10536 respectively. Anti-bacterial activity was also observed in Cd (II)-FCZ against *E. coli* 8739 and 10536. Cd (II)-FCZ and Mn (II)-FCZ also showed negligible activity against gram positive organisms which other complexes were found inactive. It is noteworthy that the complexes of FCZ were more active as compared to pure FCZ. The results of antibacterial are tabulated in Table 2.

Antibacterial activity of DMBG and its Metal Complexes

Antibacterial evaluation of DMBG free ligand and its Cu(II) and Ni(II) complexes were performed by MIC method using eight different concentrations ranges (5, 25, 50, 100, 200, 300, 400 and 500 µg / mL). These compounds were subjected against seven different grams positive and negative bacterial strains mentioned in earlier section. The DMBG free ligand was not found to be effective towards any microbial strain. Whereas Cu(II)-DMBG showed 4% and 5.5% activity at 0.5 mg / mL against *E. coli* (8739 and 10536), respectively. This study reveals that moderate activity is recorded for Cu (II)-DMBG complexes as compared to DMBG free ligand and Ni(II)-DMBG complex as computed in Table 2.

Antibacterial activity of LS-K and its Metal Complexes

Antibacterial screening of LS-K and its two transition metal complexes were determined against the same strain of gram positive and negative bacteria and same concentrations ranges as previously reported, results are depicted in Table 2.

Neither the LS-K nor its Cu(II) and Ni(II) complexes showed any activity against both the gram positive and gram negative bacterial strain.

Conclusion

The contemporary work in the field of inorganic chemistry has produced meaningful results and transition metal complexes are gaining significant consideration as a therapeutic avenue for the treatment of various diseases. The rising incidence of numerous bacterial infections particularly in under developed countries along with the simultaneous increase in drug resistance as a result of their frequent use provides basis for the search of newer antibacterial agents .Our earlier study showed that Co (II)-PZ complex was found active against all five strains(1195, 3029, 1289, 1375 and 20290) whereas Mn (II)-PZ complex was active against two stains (Ali *et al.*, 2018).In comparison to PZ two of our synthesized complex displayed a significant antibacterial activity, thus suggesting that these two compounds based on the PZ can provide a good starting point for the discovery of newer potential compound against tuberculosis. Metal complexes of INH revealed that various derivatives of INH possess moderate activity against five strains of *Mycobacterium tuberculosis* (1195, 3029, 1289, 1375 and 2029) (Ali *et al.*, 2017). In present study Cd (II)-FCZ and Cu (II)-INH showed very mild activity. Metal complexes of the DMBG and LS-K drugs used in this study showed no promising results for antibacterial activity though some parent compounds were devoid of antibacterial attributes combining these with transition metals gives them this potential albeit moderate to weak in strength. These findings are encouraging for future work with transition metals on some other pharmacological agents that may unveil some competent combination and produce novel antibacterial agent.

Conflict of Interest:

We hereby confirm that there is no conflict of interest regarding the publication of this research work.

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