



Preparation, structural elucidation and antimicrobial activity of complexes of some metal ions with 4-{1H-benzimidazol-2-ylethylideneamino}-6-methyl-3-thioxo-3,4-dihydro-1,2,4-triazine-5(2H)-one (Hbethtr)

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ABSTRACT: The complexes of Hbethtr with Mn^{II} , Ni^{II} , Co^{II} , Cu^{II} , Zn^{II} , Cd^{II} and Pd^{II} of composition $[M(bethtr)_2]$ ($M = Co^{II}$, Ni^{II} , Cu^{II} , Zn^{II} and Cd^{II}) and $M(Hbethtr)Cl_2$, ($M = Pd^{II}$, Cu^{II} or Ni^{II} and Co^{II}) have been prepared, characterised and their structures have been proposed from the studies of magnetic susceptibility, electronic absorption and i.r spectra. The antibacterial activity of complexes has been studied against *Escherichia Coli*, *Staphylococcus aureus*, *Salmonella typhi* and antifungal activity against *Aspergillus niger* and *Candida albicans*. Cobalt(II) and copper(II) complexes were found more active against *S.aureus* and *Candida albicans*.

KEY WORDS: Structure, antimicrobial activity, Benzimidazole thioxotriazine, Metal complexes.

I. INTRODUCTION

The studies on microbial activity of benzimidazole, thiazole, triazine nucleus containing ligand, picked up momentum due to their immense importance in medicinal potentiality, strong complexing ability, wide variety of microbial activity and their analytical applications^[1-10]. Our interest developed due to above facts and we are pursuing research work on coordination complexes of benzimidazole and triazine derivatives^[11-15]. In present communication we report the preparation, structure elucidation and antimicrobial activity of complexes of some metal ions with 4-{1H-benzimidazol-2-ylethylideneamino}-6-methyl-3-thioxo-3,4-dihydro-1,2,4-triazine-5(2H)-one (Hbethtr)

II. EXPERIMENTAL

A. Material and Methods

4-Amino-6-methyl-3-thioxo-3,4-dihydro-1,2,4-triazine-5(2H)-one (amtrz) was prepared by condensing thiocarbohydrazide ($NH_2-NH-CS-NH-NH_2$) with pyruvic acid in aqueous ethanol using acetic acid as catalyst^[16]. 1-(1H-benzimidazol-2-yl) ethanone (bzen) was prepared by reported method^[17] and coordinating ligand Hbethtr was synthesised by refluxing molar proportion of amtrz and bzen in aqueous ethanol (50:50) containing 2-3 ml acetic acid. The recrystallized ligand has m.pt 258⁰c and nitrogen found 27.89%, required for $C_{13}H_{11}N_5OS$, nitrogen 28.01%.

B. Preparation of complexes: $[M(Hbethtr)Cl_2]$ ($M = Co^{II}$, Ni^{II} , Cu^{II} , Zn^{II} or Cd^{II})

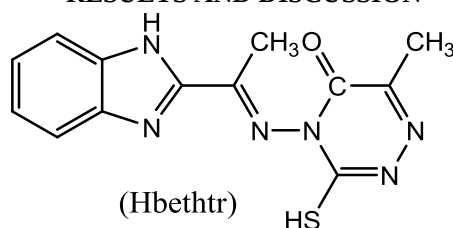
About one millimole of metal chloride was dissolved in 30-35 ml aqueous ethanol was added with stirring to one millimole of ligand dissolved in hot dry ethanol. The resulting solutions were concentrated with stirring when dichloro complexes of Cu (II) and Cd(II) separated while other separated on concentration and cooling overnight. The products were collected on a buckner funnel, washed with cold ethanol and dried over $CaCl_2$. Yield 60-80%.

C. Preparation of complexes: $[M(bethtr)_2]$ ($M = Mn^{II}$, Co^{II} , Ni^{II} , Cu^{II} , Zn^{II} and Cd^{II})

About one millimole of metal chloride or acetate was dissolved in 25 ml aqueous ethanol and refluxed with two millimole of ligand dissolved in ethanol. The p^H of solution was raised by adding sodium acetate. The bisligated complex separated when resulting solution was diluted with excess of water. The products were digested on a steam bath, cooled and collected on a buckner funnel, washed with water and dried over $CaCl_2$. Yield 92-98%. The dried products were analysed and results of elemental analyses are recorded in Table-I.

The metal chloride or acetate used were E.Merck, extra pure or B.D.H. Anal-R grade chemicals. The i.r spectra were recorded in KBr disc on Shimadzu FTIR spectrophotometer, ¹HNMR spectra were recorded on Bruker Model DRX-400MHz, NMR spectrophotometer, U-V spectra were recorded in the range 200-850 nm. The metal and chloride contents of complexes were estimated by standard method^[18].

III. RESULTS AND DISCUSSION



The ligand Hbethtr is a potent N, N and S donor benzimidazolyl triazine derivative. It forms complexes either as tridentate neutral or monoanionic coordinating molecule yielding complexes of composition $[M(Hbethtr)Cl_2]$, ($M = Cu^{II}$, Ni^{II} , Co^{II} , Zn^{II} or Cd^{II}) and bischelates $[M(bethtr)_2]$, ($M = Cu^{II}$, Mn^{II} , Co^{II} , Ni^{II} , Zn^{II} or Cd^{II}). The dichloro complexes are formed in dry ethanol while the neutral complexes are formed in aqueous ethanol in basic medium. The neutral bis complexes are almost insoluble in water but partially dissolved in ethanol but fairly soluble in DMF. The DMF solutions of both $[M(bethtr)_2]$ and $[M(Hbethtr)Cl_2]$ type of complexes show negligible electrical conductance value in DMF ($\Lambda_m = 6-12 \text{ ohm}^{-1} \text{ mol}^{-1} \text{ cm}^2$) indicating their non-ionic nature and chloride ions to be coordinated to metal atom^[19]. The complexes are stable to heat below 300^0-330^0C . Except $Zn(II)$ and $Cd(II)$, the complexes of $Cu(II)$, $Ni(II)$, $Co(II)$ and $Mn(II)$ are paramagnetic and moment value of $[M(bethtr)_2]$ occur in the range of high spin octahedral complexes^[20]. The magnetic moment value of $Cu(Hbethtr)Cl_2$, (1.89 B.M) indicated that complex is magnetically dilute^[20]. The magnetic moment values of $Ni(II)$ and $Co(II)$ complexes $[M(Hbethtr)Cl_2]$ are similar to high spin five coordinated trigonal bipyramidal complexes^[20,22].

The electronic absorption spectra of ligand shows three electronic transitions located at 212, 243 and 287 nm assignable to $\sigma \rightarrow \sigma^*$, $\sigma \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions. The copper (II) complexes $[Cu(Hbethtr)Cl_2]$ displays a strong absorption at 430 nm and a broad band at 630-665 nm attributable to ${}^2B_{1g} \rightarrow {}^2E_g$ and ${}^2B_{1g} \rightarrow {}^2B_{2g}, \rightarrow {}^2A_{1g}$ transitions in distorted octahedral field^[21,22]. The bisligated complex $[Cu(bethtr)_2]$ displays a broad band at 610-650 cm^{-1} attributed to ${}^2B_{1g} \rightarrow {}^2B_{2g}, \rightarrow {}^2A_{1g}$ transition^[21,22]. The cobalt(II) complex $[Co(bethtr)_2]$ display a shoulder at 450 nm and broad band at 520-530 nm assignable to ${}^4T_{1g} \rightarrow {}^4T_{1g} (P)$, ${}^4T_{1g} \rightarrow {}^4A_{2g}$ transitions in distorted octahedral field^[21]. The neutral complex $[Ni(bethtr)_2]$ displays two transitions located at 430 nm and 585 nm assigned as ${}^4A_{2g} \rightarrow {}^4T_{1g} (P)$ and ${}^4A_{2g} \rightarrow {}^4T_{1g} (F)$ transitions in approximately octahedral field^[21,22]. The expected third spin allowed transition ${}^3A_{2g} \rightarrow {}^3T_{2g}$ could not be located due to limitation of the range of instrument (200-850 nm). The electronic absorption spectra of $[Ni(Hbethtr)Cl_2]$ display a electronic absorptions at 415 and weak bands at 518, 620 nm similar to five coordinated $Ni(II)$ complexes^[22].

A. I.R spectra

The i.r spectrum of ligand display (NH), (C-H) phenyl ring, (CH_3) and aldimino (CH) stretches between $2840-3340 \text{ cm}^{-1}$. The $\nu(C=O)$ of triazine ring oxo group was observed at 1720 cm^{-1} and the band is retained in almost all complexes indicating the non involvement of CO oxygen in coordination^[23]. The $\nu(C=N)$ of azomethene group and imidazole ring were observed in the region $1622-1595 \text{ cm}^{-1}$ and these are shifted to lower frequency by $10-15 \text{ cm}^{-1}$ suggested their bonding with metal atoms. The ligand displays $\nu(C=S)$ band at 968 cm^{-1} which is shifted to lower frequency by $10-20 \text{ cm}^{-1}$ in dichloro complexes $[M(Hbethtr)Cl_2]$ and by $150-200 \text{ cm}^{-1}$ in bisligated complexes $[M(bethtr)_2]$ suggesting coordination of (C=S) sulphur as thione group in former and as deprotonated thiol sulphur in latter complexes $[M(bethtr)_2]$ ^[23,24]. The (N-H) bending band of imidazole ring located at 1502 cm^{-1} is not affected in complexes suggested that imidazole (N-H) nitrogen is not involved in bonding. The i.r spectrum of ligand displays a number of i.r bands between $1450-550 \text{ cm}^{-1}$ due to various mode of i.r vibrations of phenyl, imidazole ring, triazine ring skeletal and ring deformation vibrations^[23,24]. The i.r spectrum of complexes and ligand displays four to five i.r bands between $635-450 \text{ cm}^{-1}$ and therefore definite M-N or M-S stretches could not be predicted.

The ${}^1\text{H}$ NMR spectrum of Hbethtr shows two singlet at $\delta = 2.874$ and 4.158 ppm in DMSO attributed from azoacetyl $-CH_3$ and triazine ring linked CH_3 proton signals. The phenyl ring proton signals were located at $7.235-7.845 \text{ ppm}$ as multiplet. The (N-H) proton signal of imidazole and triazine ring (N-H) were located as broad signal near $5.465-5.785 \text{ ppm}$. Due to poor solubility of complexes in DMF and $CDCl_3$, the ${}^1\text{H}$ NMR of complexes could not be determined.

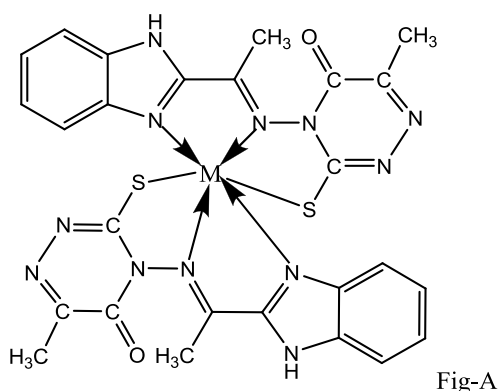
From analytical results, electrical conductance values, magnetic susceptibility values, and infrared spectral data, the probable structure of complexes are shown in figure A and B.

B. Antibacterial and antifungal activity

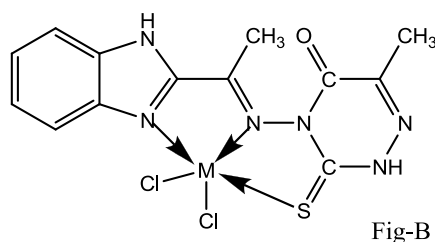
The antifungal activity of the ligand and its complexes have been evaluated by radial growth method^[25]. Czapek agar medium prepared by dissolving 20g starch, 20g agar agar and 20g glucose in one litre distilled water. The

resulting medium was added requisite amount of test compound to get 50 and 100 ppm of solutions. The medium was then poured into petri plates and the spores of fungi were placed on medium with the help of inoculum needle. These petri plates were wrapped in polythene bags containing two to three drops of ethanol and then placed in an incubator at $30 \pm 0.05^\circ\text{C}$. The linear growth of fungus was evaluated by measuring the fungal colony diameter after five days. The percentage inhibition was calculated from the relation $100(C-T)/C$ where C and T are the diameter of the fungus colony and control test plates respectively. The fungi used in present investigation included *A. niger* and *Candida albicans*. The control solution was Mycostatin. The result of activity is shown in Table-II. The results show that antifungal activities of complexes are larger than free ligand. The activity increases with increasing concentration of the substances from 50 to 100 ppm. The greater fungal activity may be due to large electron cloud delocalization which increases lipophilic nature of the central metal atom causing permeation through the lipid layer of cell membrane.

Structure of complexes $M(\text{bethtr})_2$, ($M = \text{Mn}^{\text{II}}, \text{Co}^{\text{II}}, \text{Ni}^{\text{II}}, \text{Cu}^{\text{II}}, \text{Cd}^{\text{II}}, \text{Zn}^{\text{II}}$)



Structure of $[M(\text{Hbethtr})\text{Cl}_2]$, ($M = \text{Cu}^{\text{II}}, \text{Co}^{\text{II}}, \text{Cd}^{\text{II}}, \text{Ni}^{\text{II}}, \text{Zn}^{\text{II}}$)



The bacterial activity against *E. coli*, *S. aureus* and *Salmonella typhi* were evaluated by inhibition zone technique^[26]. The nutrient agar medium was prepared taking 5g peptone, 5g beef extract 5g NaCl and agar 20g in one litre distilled water. The solution was pipetted into petri plate dried and added seeded agar with bacteria. The compound was dissolved in DMF having 250 and 500 ppm strength. The discs of Whatman no.1 filter paper soaked with these solutions to 5mm diameter discs were dried and placed on the medium previously seeded with organism in petri plate at suitable distance. The petri plates were stored in an incubator at $30 \pm 1^\circ\text{C}$ for twenty four hours. The zone of inhibition was formed around each disc. The zone of inhibition was measured accurately in millimetre. The results are shown in Table-II. It is found that metal complexes show higher activity than free ligand.

IV. Conclusion

The ligand Hbethtr coordinates as tridentate molecule forming bond through both imidazole tertiary nitrogen ($\text{C}=\text{N}$), azomethine ($\text{C}=\text{N}$) nitrogen and thione or deprotonated thiol sulphur in complexes. The copper (II) and Cadmium (II) complexes $[M(\text{bethtr})_2]$ show positive antibacterial and antifungal activity.

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Table-I: Analytical results and physical data of complexes

Compound	Elemental results found (Calc.)					Colour	Magnetic moment (B.M)
	M	N	C	H	Cl/S		
CoL ₂	9.10(8.95)	25.31(25.53)	47.18(47.43)	3.65(3.34)	9.62(9.72)	brown	5.18
NiL ₂	9.11(8.93)	25.31(25.54)	47.21(47.44)	3.92(3.35)	9.63(9.73)	Reddish brown	3.23
ZnL ₂	9.71(9.85)	25.41(25.32)	47.21(47.03)	3.44(3.31)	9.39(9.64)	Green yellow	Dia
CdL ₂	15.61(15.76)	23.41(23.64)	43.31(43.41)	3.29(3.09)	9.21(9.01)	Yellow	Dia
MnL ₂	8.40(8.42)	25.61(25.72)	47.61(47.78)	3.43(3.36)	9.69(9.80)	Dull yellow	5.92
CuL ₂	9.71(9.60)	25.11(25.39)	47.32(47.16)	3.51(3.32)	9.47(9.67)	brown	1.85
Co(LH)Cl ₂	13.61(13.70)	20.13(20.00)	36.11(36.29)	2.92(2.79)	16.63(16.51)	pink	4.71
Ni(LH)Cl ₂	13.73(13.60)	19.84(20.01)	36.10(36.30)	2.97(2.79)	16.41(16.52)	Greenish yellow	3.41
Cu(LH)Cl ₂	14.37(14.62)	19.52(19.79)	35.71(35.90)	9.39(2.76)	16.61(16.34)	Grey	1.89
Zn(LH)Cl ₂	14.71(14.98)	19.61(19.70)	35.45(35.71)	2.81(2.74)	16.10(16.26)	White	Dia
Cd(LH)Cl ₂	23.21(23.25)	17.46(17.79)	32.17(32.27)	2.53(2.48)	14.73(14.69)	Cream	Dia

Table-II: Antimicrobial activity of [M(Hbethtr)₂] and its complexes, antifungal inhibition after 120 hours and antibacterial inhibition after 24 hours

Fungi	Conc.	HL	NiL ₂	CuL ₂	ZnL ₂	CdL ₂	Cu(LH)Cl ₂	CoL ₂	Cd(LH)Cl ₂	Ref
A.niger	50	35	38	51	40	48	46	42	46	72
	100	43	46	65	50	57	57	48	61	93
C.albicans	50	43	45	50	42	48	47	41	48	70
	100	52	55	64	53	61	60	53	63	95

Antibacterial inhibition

E.coli	250 500	— +	+ ++	++ +++	+ ++	+ ++	++ +++	+ ++	+ ++	++++ ++
S. aureus	250 500	+ ++	+ ++	++ +++	+ ++	+ ++	++ +++	+ ++	+ ++	+++ ++++
S. typhi	250 500	+ ++	++ +++	++ +++	+ ++	+ ++	++ +++	+ ++	+ ++	+++ ++++

- Not active, + slightly active, ++ less active, +++ active, ++++ most active

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