

SYNTHESIS, CHARACTERIZATION, ANTIMICROBIAL ACTIVITY AND CYTOTOXICITY STUDIES OF 2-((6-METHOXYBENZO[D] THIAZOL-2-YLIMINO) METHYL)-6-ETHOXYPHENOL AND ITS METAL COMPLEXES

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ABSTRACT

Objective: Metal complexes of imine bases have clinical, analytical and industrial applications and play important role in catalysis and organic synthesis.

Methods: Keeping in view the various applications of heterocyclic imine derivatives, an attempt was made for the design and synthesis of a new title imine compound.

Results: The title compound 2-((6-methoxybenzo[d]thiazol-2-ylimino) methyl)-6-ethoxyphenol was synthesized and characterized by elemental analysis, Mass, IR, ¹H, ¹³C -NMR and UV-Visible spectra. Metal complexes of title compound were prepared from chloride salts of Cu (II), Ni (II) and Co (II) in ethanol medium. The chemical structures of metal complexes were proposed by the data from IR, Mass and UV-Visible spectra, SEM-EDX and TGA.

Conclusion: All the imine metal complexes were evaluated for their antimicrobial activity against two gram positive, three gram-negative bacteria and aspergillus family. The study on HeLa cell line cytotoxicity affirmed more activity for 1:2 Cu (II) complex and moderate activity for Ni (II) and Co (II) complexes. The lower IC₅₀ values for metal complexes under study than in the free uncoordinated form of title compound obviously reveal enhanced therapeutic activity of metal complexes.

Keywords: 2-((6-methoxybenzo[d]thiazol-2-ylimino) methyl)-6-ethoxyphenol (2-MbtyeH), TGA-DTA, antibacterial, anti fungal activity, Cytotoxicity.

INTRODUCTION

The title compound has a functional group that contains a carbon-nitrogen double bond with the nitrogen atom connected to benzothiazole[1]. The donor atoms such as N, O and S in the compound have structural similarities with natural biological systems. Imines prepared from various heterocyclic derivatives were reported to have wide biological activities such as antitumor, antiameobic, antihistaminic, antihelminthic, cytotoxic, anticonvulsant, antiproliferative and antifungal activities[2]. The investigation of metal complexes with sulphur containing imine bases is a potential field and it has been shown that many of them possess anticancer activity.[3-4].

The use of transition metal complexes with imine nitrogen as one of the donor, in catalysis has been receiving significant attention during past two decades. A wide number of different imine base ligands have been used as cation carriers in potentiometric sensors as they have shown excellent selectivity, sensitivity, and stability for metal ions such as Co (II), Cu (II), Ni (II) etc. [5-7].

The imine bases derived from aldehydes containing ortho-substituted hydroxy group have ability to act as bidentate ligands for transitional metal ions[8-12]. Keeping in view all these properties, quite a good amount of research has been engaged in all over the world on the metal complexes of imine bases[13-22]. In the present investigation the structural aspects of 2-MbtyeH, and its Cu (II), Co (II) and Ni (II) complexes have been studied employing spectro-analytical techniques. Further antimicrobial activity and cytotoxicity studies of title imine base compound and its metal complexes were also carried out.

MATERIALS AND METHODS

Chemicals

All chemicals and solvents used were analytical grade. 2-Amino-6-methoxy benzothiazole and 3-Ethoxy 2-hydroxy benzaldehyde were Sigma Aldrich Chemicals. Cobalt Chloride, Nickel Chloride and Copper Chloride are SD's fine chemicals. All the chemicals and

solvents used were dried and purified by standard methods. The moisture was excluded from the glass apparatus using CaCl₂.

Instrumentation

FT-IR spectra of ligand and metal complexes were recorded on Perkin Elmer Infrared model 337 spectrophotometer in the range of (4000 - 400 cm⁻¹) (Table 1). The UV-Visible spectra were recorded on a Shimadzu UV spectrophotometer in the wavelength range 200-900 nm.

The ¹H NMR spectrum of 2-MbtyeH, was recorded on Varian Gemini Unity Spectrometer by employing TMS as internal standard. The mass spectra were recorded by ESI technique on VG AUTOSPEC mass spectrometer. Melting points of 2-MbtyeH and decomposition temperature of complexes were determined on Polmon instrument (model No.MP-96). The practical size and morphology of title compound and its Cu(II) complex were recorded on ZEISS SCANNING ELECTRON MICROSCOPE. INCA EDX instrument is used for elemental analyses. The thermal studies were carried out in a dynamic nitrogen atmosphere (20 ml min⁻¹) with a heating rate of (10°C min⁻¹) using a Shimadzu TGA-50H.

Synthesis of 2-((6-Methoxybenzo[d]thiazol-2-ylimino)methyl)-6-ethoxyphenol

The title compound 2-MbtyeH, was prepared by condensation of 3-Ethoxy, 2- hydroxyl benzaldehyde (0.01 mol) with 2-Amino-6-methoxy benzothiazole (0.01 mol) in ethanol medium and the mixture was refluxed for 5- 6 hrs at 50-60 °C. After completion of the reaction, as confirmed by TLC, the reaction mixture was treated with 5 % NaHCO₃ solution, followed by the addition of ethyl acetate. The organic layer was separated, dried over Na₂SO₄ and evaporated under vacuum, purified with column chromatography using pet ether: ethyl acetate as eluent to get pure product. The product collected is recrystallized from hot ethanol and dried in a vacuum desiccator. The yellow color solid product was separated out and dried under vacuum at room temperature. The product yield is 95 % and m.pt:198°C (Table 2).

Synthesis of metal complexes

A mixture of 2-MbtyeH (0.005 mol) and metal salt (MX_2 , where M= Ni (II), Co (II), or Cu (II); ($X=Cl/NO_3/acetates$)) (0.005 mol or 0.01 mol) in ethanol medium was refluxed on water bath for 4-5 hours at 70-80°C by adjusting pH in the range 4.0 – 6.5 to enable complex formation. The precipitated complex was filtered off, washed with anhydrous ethanol and dried in vacuum at room temperature. The product was dried in air and stored in a desiccator over anhydrous $CaCl_2$ under vacuum. All the metal complexes are colored and stable to air and moisture. The complexes yields and m.pt given in (Table 2)

Cytotoxicity assay

HeLa cells were purchased and maintained in National Cell Repository at NCCS, Pune, India. Cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10 % heat inactivated (56°C for 30 min) fetal bovine serum (FBS) at 37 °C in humidified chamber with 5 % CO_2 .

RESULTS AND DISCUSSION

Infrared and mass spectra

The IR spectra give valuable information regarding the type of donor sites attached to the metal atom in a complex. The main FT-IR bands and their assignments for title compound and its complexes (Fig. 1a-1f) are presented in (Table 1).

Table 1: FT-IR Spectral Data of 2-MbtyeH and its metal complexes

Compound	$\nu_{CH=N}$	ν_{M-N}	ν_{M-O}	ν_{OH/H_2O}
2-MbtyeH	1635.63	—	—	3732.25
Ni(II)-2-Mbtye	1604.41	543.92	424.33	3358.92
Co(II)-2-Mbtye	1602.84	540.14	432.05	3304.06
Cu(II)-(2-Mbtye) ₂	1608.63	520.78	426.26	3414.00
Ni(II)-(2-Mbtye) ₂	1614.41	541.99	428.19	3568.30
Cu(II)-2-Mbtye	1633.70	551.64	433.98	3421.65

The(C = N) stretching frequency of the imine is found in the free ligand at (1635 cm^{-1}). This band is shifted to lower wave numbers (1633–1602 cm^{-1}) in all complexes indicating the participation of the azomethine nitrogen in coordination (M -N). The shift towards lower wave number is ascribable to slight bonding character of nonbonding electrons of frontier orbitals at azomethine nitrogen donor site.[23]. The existence of the broad band in the region 3438–3452 cm^{-1} is due to coordinated water molecules. In addition to other bands[24].New bands, which are not present in the spectrum of ligand, appeared in the spectra of complexes in the range of (505 – 562 cm^{-1}) corresponding to ν_{M-N} [25-26], and (414-436 cm^{-1}) to ν_{M-O} vibrations. The other major bands observed at 2358–2852 cm^{-1} can be assigned to the stretching frequency of C-H bond from alkoxy chain [27].

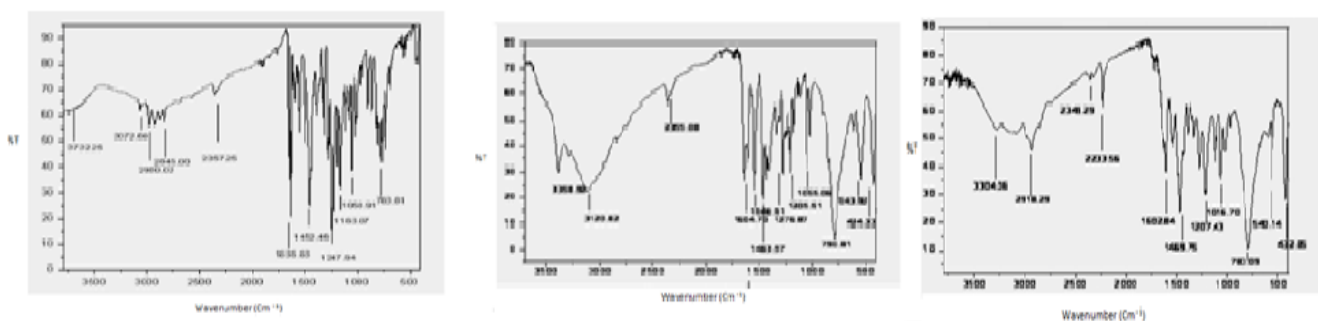


Fig. 1: IR Spectra of (a) 2-MbtyeH, (b) [(Ni(II)- (2-Mbtye)] and (c) [Co(II)- (2- Mbtye)]

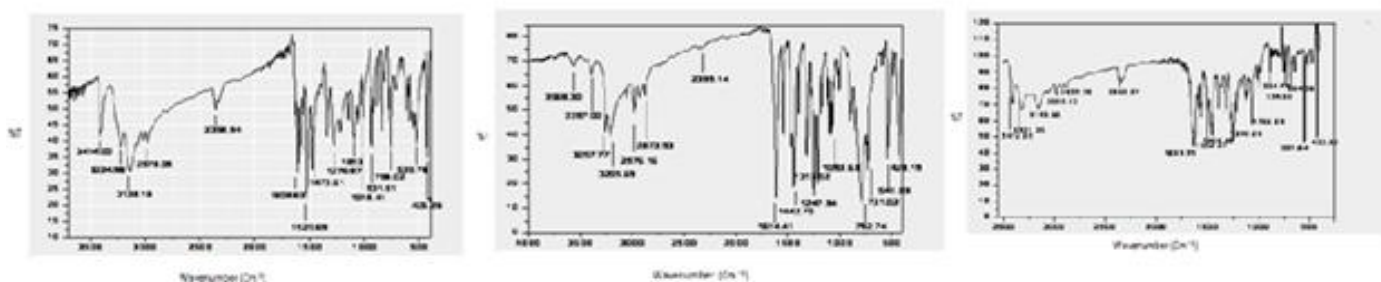


Fig. 1(d): IR Spectra of (d) [Cu(II)-(2-Mbtye)₂], (e)[Ni(II)- (2-Mbtye)₂] and (f) Cu(II)- (2- Mbtye)

The mass spectra of 2-MbtyeH and its metal complexes are presented in (Fig.2a-2b) and the analysed data is presented in (Table 2).

Table 2: Mass Spectral Data of 2-MbtyeH and its Metal complexes

S. No.	Compound	Calculated mass	Obtained Mass	Peak Assigned	M.P (°C)	Yield (%)	Color
1	$C_{17}H_{16}N_2O_3S$	328	329	M+1	198	95	Yellow
2	$Cu(C_{34}H_{30}N_4O_6S_2)$	718	719	M+1	>300	65	Green
3	$Cu(C_{17}H_{17}N_2O_4S_2)$	442	465	M+Na	>300	45	Green
4	$Ni(C_{34}H_{30}N_4O_6S_2)$	712	734	M+Na	>300	58	Brown
5	$Ni(C_{17}H_{19}N_2O_5S_2)$	457	483	M+Na+3H	>300	50	Brown
6	$Co(C_{17}H_{19}N_2O_5S_2)$	455	479	M+H+Na	>300	55	Brown

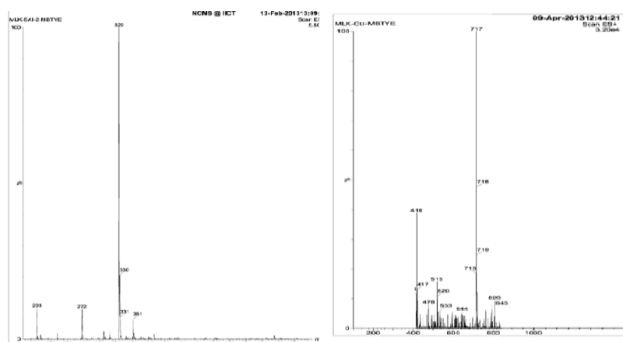


Fig. 2(a): Mass Spectrum of 2-MbtyeH, (b): Cu (II) -(2-Mbtye)₂ complex

¹H NMR and ¹³C-NMR

The ¹H NMR data of 2-MbtyeH is recorded in CDCl₃ (Fig. 3a-3b). The aromatic protons are observed in the region of 6.73-7.88 ppm (m,6H). Azomethine proton is observed at 9.26 ppm as a single and the protons of ethoxy group are observed at 1.66 ppm (t, CH₃) and 4.82 ppm (q, CH₂). A singlet at 3.83 ppm is attributable to methoxy protons. And the phenolic hydroxyl proton peak is observed at 12.54 ppm (s) (Table 3). The experimental spectral data is in good accordance with the structure of 2-MbtyeH, ¹³C spectrum displayed distinct chemical shifts corresponding to total of 17 carbon atoms (Table 4).

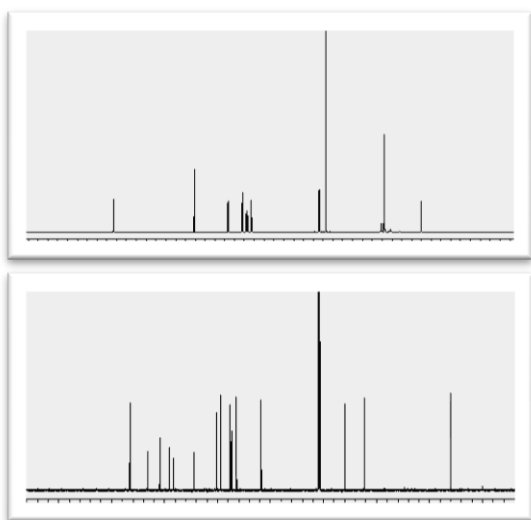


Fig. 3a: ¹H-NMR Spectrum of 2-MbtyeH Fig. 3b: ¹³C-NMR Spectrum of 2-MbtyeH

Table 3: ¹H-NMR spectral data of 2-MbtyeH

Chemical shift (ppm)	Multiplicity	Number of Proton	Type of Proton(s)
6.73-7.88	Multiplicity	6	Aromatic Protons
9.26	Singlet	1	-HC=N
4.13	Quartet	2	-OCH ₂
3.86	Singlet	3	-OCH ₃
12.55	Singlet	1	-OH

Table 4: ¹³C-NMR data of 2-MbtyeH

Chemical shift (ppm)	Number of Carbon	Carbon Group Assigned
110-155	12	Aromatic
158	1	Thiazole
55-65	2	O-CH ₂ , O-CH ₃
15	1	-CH ₃
167	1	N=CH

Electronic spectra

The UV-Vis spectra of 2-MbtyeH and its complexes of Co (II), Ni (II) and Cu (II) were recorded in dimethylsulfoxide (DMSO solvent) (Fig. 4a - 4d). The ligand spectrum displayed an absorption band in the region 30000 - 45000 cm⁻¹, which is due to $\pi - \pi^*$ and $n - \pi^*$ transitions in the ligand. The absorption peaks recorded at 24,154, 22,675, 16,447 cm⁻¹ in 1:1 Cu (II) complex and at 29,498, 25,188 and 19,960 cm⁻¹ in 1:2 Cu (II) complex are assigned to d-d transitions under square planar and tetragonal geometry in respective systems. The Ni (II) complex exhibited a broad electronic spectral band in the region 25,000 - 26,000 cm⁻¹ which may be due to overlap of two transitions. The third transition is off the scale of spectrum. The brown colour Co (II) complex exhibited a weak band in the visible region of the spectrum.

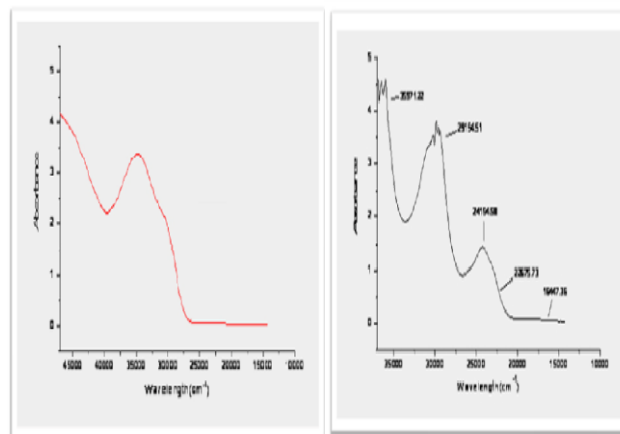


Fig. 4(a): UV-visible spectra of 2-MbtyeH, Fig. 4(b) UV-visible spectra of Cu-2-Mbtye

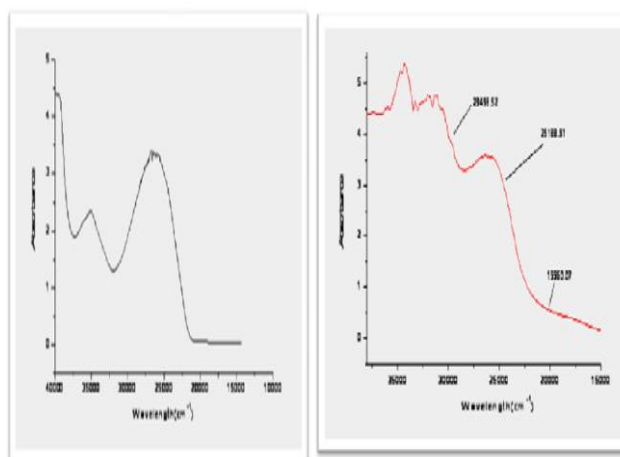


Fig. 4(c): UV-Visible spectrum of Ni (II)-2-Mbtye complex, Fig. 4(d): UV-Visible spectrum of Cu (II) - (2-Mbtye)₂ Metal complex

Thermal studies

Thermo gravimetric analysis (TGA) is commonly used to determine selected characteristics of materials that exhibit either mass loss or gain due to decomposition, oxidation, or loss of volatiles. Thermo gravimetric analysis of Cu (II) complex at the atmosphere of nitrogen in the range of temperature upto (1000°C) is carried out. The correlations between the decomposition steps of the complex with the corresponding weight loss are discussed in terms of the proposed formula of the complex. The TGA curve obtained for Cu (II) -2-Mbtye (1:1) complex showed decomposition in three steps (Fig. 5). The first step 7.16 % loss in weight up to (250 °C) is probably due to the loss of coordinated water. The second step decomposition (250-450 °C) corresponds to the loss of 75.99 % which may be due to the removal of the organic moiety. The residual mass left after 17.12 % loss in third step (451-992 °C) corresponds metal oxide.

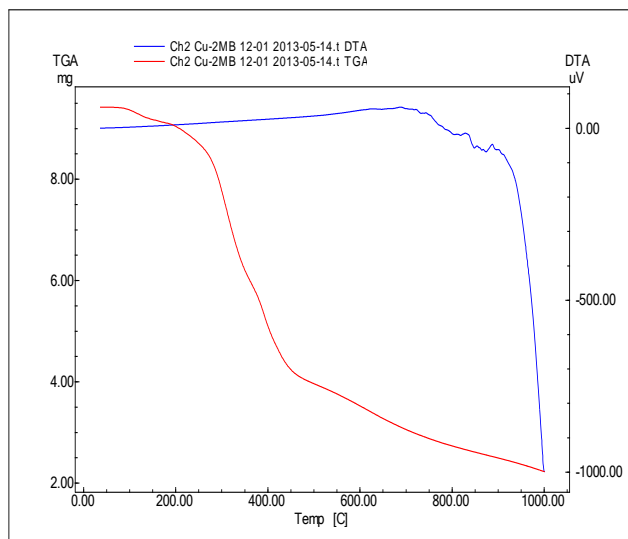


Fig. 5: TGA curve of Cu (II) 2-Mbtye complex

The above TGA data agree well with the proposed formula conformed by elemental analyses with EDX and IR and mass spectral analysis.

Scanning electron microscopy

The 2-MbtyeH and its Cu (II) complex exhibited different surface morphology (Fig. 5.a (a-b)). The image of 2-MbtyeH showed a rubber type layer on particles of size in the range of 10 μm . After complex formation irregularly shaped agglomerated particles are observed. EDX graphs are informative in elemental analyses of the compounds under investigation (Fig. 5.b (a-b)).

Molecular modeling studies

Molecular mechanics attempts to calculate energies, molecular geometries and other features by adjusting bond lengths and bond angles are dependent on the hybridization of an atom and its bonding. In the present study, the possible geometries of metal complexes were evaluated using the molecular modeling with Arguslab 4.0.1 version software.

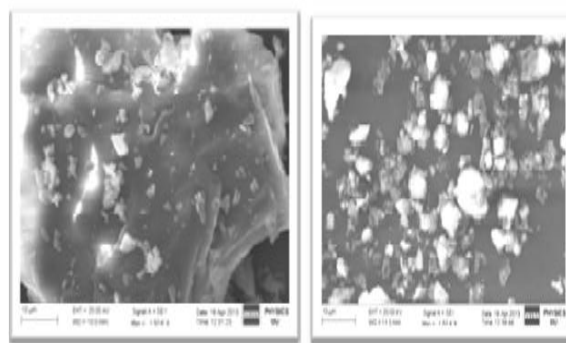


Fig:5(a) Morphology images of (a) 2-mbtyeH, (b) Cu(II)-2-Mbtye complexes.

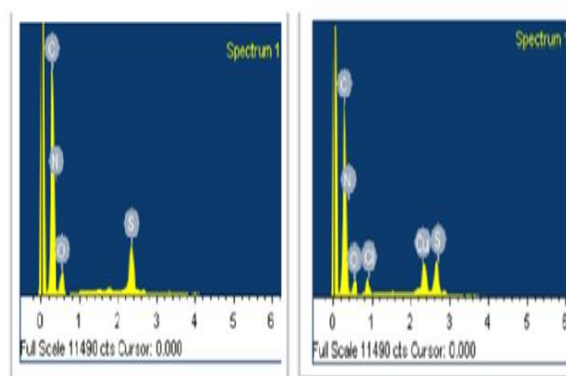


Fig: 5(b) EDX graph of (a) 2-MbtyeH, (b) Cu(II)-2-Mbtye complexes.

The metal complexes were built and geometry optimization was done using molecular mechanics uniform force field (UFF) method. Argus lab 4.0.1 version software was used to calculate the geometry optimization energy values of 2-MbtyeH and its metal complexes. Molecular modeling structures of the metal complexes are presented below. The energy values in all metal complexes are negative inferring their stability in chelated form

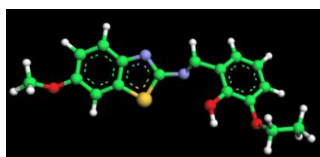


Figure 6(a):2-MbtyeH(– 43.69 kcal/mol),

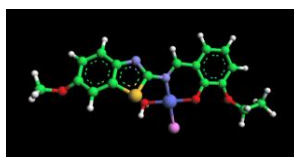


Figure 6(b): Cu (II)- 2-Mbtye, (– 108.75 kcal/mol),

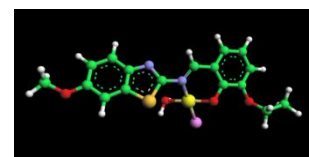


Figure 6(c): Ni (II)- 2-Mbtye (– 85.96 kcal/mol)

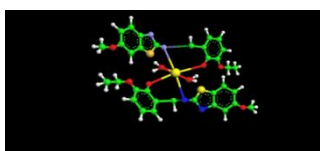


Figure 6(d): Co (II)-(2-Mbtye)₂ with coordinated water molecule (– 154.52 kcal/mol)

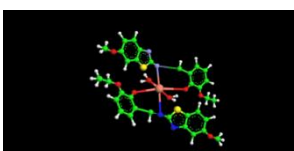


Figure 6(e): Cu (II) (2-Mbtye)₂ with coordinated water molecules (– 109.52 kcal/mol)

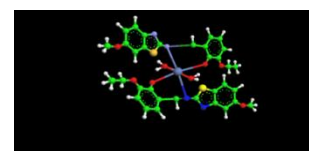


Figure 6(f): Ni (II) - (2-Mbtye)₂ with coordinated water molecules (– 171.52 kcal/mol)

Biological studies

Antimicrobial studies

In general imine bases of concentration of 1 $\mu\text{g}/1 \mu\text{l}$ were highly active against gram positive and negative bacteria. The vitro

biological screening effects of the imine base and its metal complexes under present study were tested against the two gram+ve bacteria, Bacillus subtilis; Staphylococcus aureus and three gram-ve bacteria; Escherichia coli, Klebsiella pneumonia and Pseudomonas putida. The stock solutions were prepared by

dissolving the compounds in DMSO. The minimum inhibition concentration (MIC) was determined. The nutrient agar medium was poured into Petri plates. A suspension of the tested microorganism (0.1 ml) was spread over the solid nutrient agar plates with the help of a spreader. Different dilutions of the stock solutions and ampicillin as standard were applied on the 6 mm diameter sterile discs.

The discs were placed on the inoculated plates. The Petri plates were placed at low temperature for two hours to allow the diffusion of the chemical and then incubated at a suitable optimum temperature 37°C for overnight. The diameter of the inhibition zones was measured in millimeters (mm) and compared with the diameter of standard. The results are presented in (Table 5).

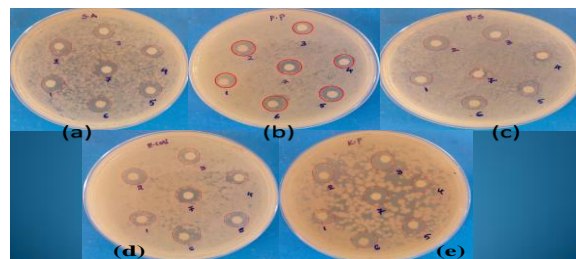


Fig. 7(a): Showing zone of inhabitation areas against (a)Staphylococcus aureus(b) Pseudomonas putida,(c) Bacillus subtilis,(d) Escherichia coli, and (e) Klebsiella pneumonia

Table 5: Anti bacterial activity of 2-MBTYE and its metal complexes

Name of organisms	2MBTY-EH(10 µl) (1)	Cu-2MBTYE (1:2)(10µl) (2)	Ni-2MBTYE (1:1)(10µl) (3)	Cu-2MBTYE (1:1)(10µl) (4)	Ni-2MBTYE (1:2)(10µl) (5)	Co-2MBTYE (1:1)(10µl) (6)	AMP (5µl) (STND)
Staphylococcus aureus	8	11	8	10	11	8	10
Pseudomonas putida	9	11	10	12	11	10	13
Bacillus Subtilis	11	13	10	11	11	9	13
Escherichia coli	9	13	8	13	11	9	11
Klebsiella Pneumonia	8	11	7	11	11	7	13

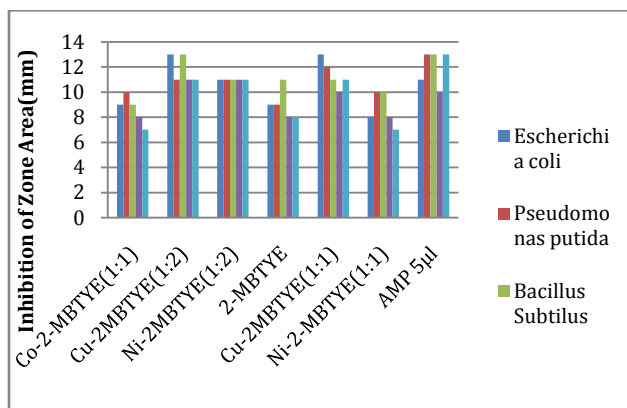


Fig. 7(b): Antimicrobial Activity of MbtYE and its Metal Complexes

Antifungal activity studies

The antifungal activity.[28-29], studies were carried out using fluconazole as standard. The inhibition activity of the newly prepared imine base MbtYE and its 1:1 and 1:2 Cu (II) complexes were measured.

Table 6: Antifungal Activity of 2-MbtYE and its Cu (II) complexes

S. No.	Name of Fungi	Zone of Inhibition in (mm)			Fluconazole
		2-MBTYE	Cu-2-MBTYE (1:1) (10 µl)	Cu-2-MBTYE (1:2) (10 µl)	
1	Aspergillus Niger	5	14	16.5	21
2	Aspergillus Flavus	5	17	19	22

The antifungal activity data indicate that the complexes show an appreciable activity against Aspergillus Niger and Aspergillus Flavus. When compared with the fluconazole[30],which is clinically

useful therapeutic agent, the metal complexes under present study exhibited almost similar activity on the fungi. The imine base however has shown lesser activity as compared to its 1:1 and 1:2 Cu(II) complexes. The experimental results of the compounds are expressed as inhibition zone diameter in (mm) (Table 6).

Cytotoxicity assay in in vitro studies

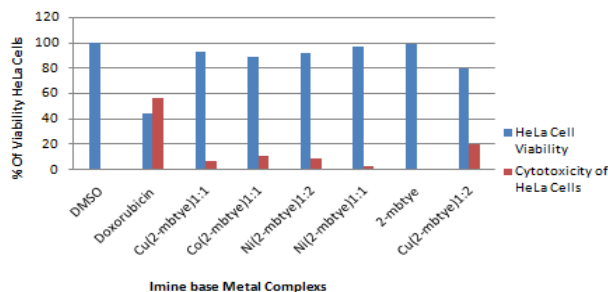
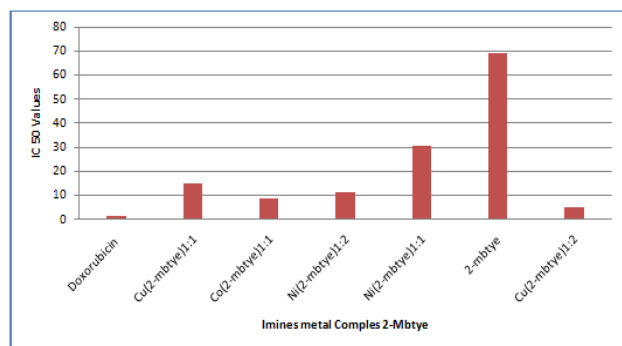
The cytotoxicity of Cu(II), Ni(II) and Co(II) complexes was tested against human cervical carcinoma (HELA) cell lines. Cell Viability was measured by MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrasodiumbromide] reduction assay[31].

The concentrated stock solutions were initially prepared by dissolving imine base 2-MBTYE and its metal complexes in DMSO. Doxorubicin was used as a positive control and the wells containing untreated cells were the negative control. Cells were seeded in each well containing 100 µl medium at a final density of 1×10⁴ cells/well, in 96 well microtitre plates at identical conditions. After overnight incubation, the cells were treated with imine base metal complexes (3 µg/ml) in a final volume of 200 µl with three replicates in each sample. After 48 h, 10 µl of MTT (5 mg/ml) was added to each well and the plate was incubated at 37 °C in the dark for 4 hrs. Reduction of MTT was quantified by absorbance at 570 nm in a spectrophotometer. The cell viability data were subjected to linear regression analysis (Fig. 8(a) – 8(b)). The IC₅₀ (inhibition of cell viability) concentrations were calculated by using the respective regression equation.

The investigation inferred cytotoxic effects of imine base metal complexes of present study on human HeLa cell lines. After 4 hrs of treatment, the numbers of live cells were measured by MTT assay and IC₅₀ values for each cell line were determined (Table 7). The HeLa cells were sensitive to test solution 1 i.e; Doxorubicin and metal complexes 2, 3 and 6, with an IC₅₀ value of 1.782, 14.856, 9.090, 11.455 and 5.0154 respectively. The 1:2 Cu(II) complex is more effective compared to all the systems studied [32]. The cytotoxicity of Cu(II) and Ni(II) complexes was found to be dependent on their metal to ligand ratio. The comparison of data revealed that 1:2 metal complexes of Cu(II) and Ni(II) complexes were more effective than corresponding 1:1 metal complexes. In 1:1 metal complexes, the cytotoxicity is more pronounced in 1:1 Co(II) complex. Overall study indicates that the therapeutic activity is enhanced in metal complexes than in free imine base.

Table 7: Effect of 2-MbtyeH and its metal complexes on the viability of HeLa- cells, following continuous incubation for 24 h, at different concentration

S. No.	Con.C ($\mu\text{g/ml}$) Imines base metal Complexes	Absorbance	% of viability (HeLa cells)	%of Cytotoxicity (HeLa Cells)	IC ₅₀ Vales with respect to HeLa Cell lines
Control	DMSO	0.36696	100	-	-
Test	Doxorubicin	0.16113	43.9094	56.0905	1.7821
1	Cu(2-mbtye)1:1	0.34226	93.2690	6.7309	14.856
2	Co(2-mbtye)1:1	0.32661	88.9998	11.0001	9.0908
3	Ni(2-mbtye)1:2	0.33493	91.2706	8.72930	11.4556
4	Ni(2-mbtye)1:1	0.35496	96.7298	3.27013	30.5798
5	2-mbtyeH	0.36166	98.5556	1.4443	69.237
6	Cu(2-mbtye)1:2	0.29384	80.0616	19.9383	5.0154

**Fig. 8(a): Effect of 2-MbtyeH and its metal complexes on the viability of HeLa-cells, following continuous incubation for 24 h, with increasing different concentration.****Fig. 8(b): Comparison of IC₅₀ values of 2-MbtyeH and its metal complexes with standard Doxorubicin.**

CONCLUSION

The title compound 2-MbtyeH and its complexes containing Cu (II), Co (II) and Ni(II) metallic core have been synthesized thoroughly characterized with a aim to design novel cancer chemotherapeutic agents. The metal complexes were characterized by UV-Vis, IR, Mass and TGA&DTA as well as Scanning Electron Microscopy. The structural optimization energy calculations were performed by Argus lab 4.0.1 version Online software. The antimicrobial activity results show that metal complexes are more effective than its title imine base MbtyeH. This implies that the process of chelation dominantly affects the overall biological behavior of the compounds. The IC₅₀ values obtained on HeLa cell line viability follow the order: 2-MbtyeH > 1:1 Ni(II)- 2-Mbtye > 1:1Cu(II) -2-Mbtye > 1:2Ni(II)- (2-Mbtye)2 > 1:1Co(II)-2-Mbtye > 1:2Cu(II)-(2-Mbtye)2 > Doxorubicin. As the lower IC₅₀ values correspond to higher therapeutic activity, from the above order it is inferred that 1:2 Cu(II) complex is less active than the employed standard Doxorubicin, but more active against Hela cells compared to other compounds in the series.

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