SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL SCREENING OF LIGAND 2- $\{[2-(5\text{-BENZOYL-1H-1,2,3-}$ **BENZOTRIAZOLE-1-YL)-2-OXOETHYL]AMINO}** PROPIONIC ACID

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ABSTRACT

2- amino propionic acid (Alanine), is extensively used as complex-forming reagents. Alanine was condensed with 1-(5-benzoyl-1H-1,2,3 benzotriazole-1-yl) 2-chloroethanone and yield 2- $\{[2-(5\text{-benzoyl-1H-1,2,3 benzotriazole-1-yl}) - 2\text{-oxoethyl}] \text{amino}\}$ propionic acid. The newly synthesized ligands and their complexes with first transitions series metal had been prepared, were analyzed and characterized by using different techniques, such as elemental analysis, infrared and electronics spectra. The IR spectra, NMR spectra and atomic absorption analysis show that the benzotriazole ligand forming chelates with 2:1 (Ligand: Metal) stoichiometry. Metal complexes were evaluator in-vitro antibacterial activity against Negative and Positive bacteria. The complexes showed significant antibacterial activity.

Keywords: 2-Amino propionic acid, 5- Benzoyl benzotriazole, Metal Complexes, Antifungal Activity, Antibacterial Activity.

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INTRODUCTION

Compounds containing triazole have attracted much interest because of their biological application.¹⁻⁴ Triazole-containing compounds show in several metabolic products of fungi and primitive marine animals. The coordination chemistry of triazole and benzotriazole derivatives was studied due to their importance in industry, agriculture, and their biological activity.⁵⁻⁸ Benzotriazole derivatives function as an agonist for several proteins. As vorozole and alizapride have valuable inhibitory properties in contrast to distinct proteins and benzotriazole esters have been started to perform as a mechanism-based fascinator for many respiratory syndromes 3CL proteases.⁹ Benzo condensed azoles are a class of heterocyclic molecules of that area attractive to the medicinal chemistry field owing to their properties and applications. Benzo-fused azole consists of three heteroatoms, which are benzoxazole, benzothiazole and benzotriazole^{10,11} and have been widely investigated for their thorough length biological activity. Benzotriazole and its derivatives are well-known as a multitask entity with a broad range of applications in various fields. Benzotriazoles are a class of organic composites that have been used as metal anticorrosive and UV stabilizer derivatives in a wide range of commercial and industrial applications. These composites have been in profit-making manufacturer and use since the 1950s with many compounds being produced at large volume over and above one million kilograms per annum. Some benzotriazole derivatives show signs of behaviors of importunate organic pollutants, and existing evidence indicates long-term preservation and persistence in dregs. Other applications of benzotriazoles associated with anticorrosion, its appear to be highly resistant to degradation and toxic to the marine organism. Benzotriazoles are complexing agents employed as antifreeze and corrosion-inhibiting agents¹², as copolymers in donor-acceptor polymers for solar cells. Their exceptional property and precise biological activity have attracted much attention from research chemists all over the world because they have found extensive applications in different branches of pharmaceutical chemistry¹³, antitubercular¹⁴, anticonvulsant¹⁵, CCR4 antagonist¹⁶, bio-imaging, organic-gels and valuable intermediates for the creation of organic compounds. The benzotriazoles thereof are known to be

important intermediates in the synthesis of organic compounds such as β -amido ketones, aldehydes¹⁷, β -keto-esters¹⁸, Ionic liquid and reagents for acylation and thioacylation reactions.¹⁹⁻²¹ 1H-Benzotriazoles are also known to exhibit a broad spectrum of pharmacological activities.²²⁻²⁴

Benzotriazole is continuing prompt growth in the synthesis of heterocycles since it can be used as a shape to construct novel biologically active molecules.^{25,26} Nitrogen atom-containing organic compounds are some of the leading complexing agents used in industries. Several types of the research reported electrochemical studies of amino-containing ligands such as Amino acid, EDTA and En on Cu.^{27,28} Many researchers synthesized several ligands on 5-benzoyl benzotriazole moiety and prepared complexes and observed their antimicrobial activity.²⁹⁻³³ Therefore, the present paper comprises the synthesise and characterize the solid colored complexes of the novel ligands containing the triazole moieties-2- $\{[2-(5-benzoyl-1H-1,2,3 \text{ benzotriazole-1-yl}) -2-oxoethyl]amino\}$ propionic acid with first transition series elements Zn^{2+} , Cu^{2+} , Ni^{2+} , Mn^{2+} , Co^{2+} and observed their antimicrobial and antifungal activity.

EXPERIMENTAL

Materials and Methods

5-benzoyl benzotriazole(**1**) was prepared by the method reported in literature.³⁴ The synthesis of N-(1-chloro acetyl)-5-benzoyl benzotriazole (**2**) done from the reaction of 5-benzoyl-1H-benzotriazole with chloro acetyl chloride respectively by the method reported in the literature (Fig.-1).³⁵ Rest of the chemicals were used analytical grade. The melting point of all complexes was determined by the open capillary method and was uncorrected.

Fig.-1: Structure of N-(1-chloro acetyl)-5-benzoyl benzotriazole (**2**)

Fig.-2: Structure of 2- $\{[2-(5-benzoyl-1H-1,2,3 \text{ benzotriazole-1-yl}) -2-oxoethyl]amino\}$ propionic acid

Synthesis of Ligand 2- $\{[2-(5-benzoyl-1H-1,2,3 \text{ benzotriazole-1-yl}) -2-oxoethyl]amino\}$ propionic Acid

N-(1-chloro acetyl)-5-benzoyl benzotriazole, (2.99 gm, 0.01 mole) and 2-amino propanoic acid (0.89 gm, 0.01 mole) in Ethanol with little HCl were suspended. To this suspension sodium bicarbonate (0.84 gm, 0.01 mole) was added and the mixture was warmed on the steam bath for about six hours. On cooling the solid crystals separated were filtered, washed with little alcohol, and dried. The yield of the ligand compound L-2 is 70% and having m.p- 201°C. Molecular Formula: $C_{18}H_{16}N_4O_4$. Molecular Weight: 352 gm/mole(Fig.-2).

General Synthesis of Metal Chelates with L-2

The Cu^{+2} , Co^{+2} , Ni^{+2} , Mn^{+2} and Zn^{+2} metal ion complexes of L-2 ligand have been prepared by the following common method. 0.01-mole corresponding ligands were dissolved in ethanol and 0.005 mole metal salt was also dissolved in a minimum quantity of alcohol. Both the solutions were mixed with constant stirring and raised pH (4-5 for Cu^{+2} , 6 for Ni^{+2} and Co^{+2} , 5.6 for Mn^{+2} and Zn^{+2}) by the addition of sodium acetate solution. The resulting mixture was refluxed for 4 hours. The solid complex thus obtained was filtered, washed and dried and re-crystallized. The yield of complexes was in the range of 64-68%. All the chelates were powdered well and dried at 70°C over 24 hours (Fig.-3).

Where M=Cu,Ni,Co,Mn,Zn

Fig.-3: Structure of MetalComplex with L-2

Measurements

The elemental analysis of C, H and N elements of metal chelates was carried out on elemental analyzer Thermo Finnigan 1101 Flash EA (Italy). The metal contents were estimated using standard method.³⁶ The halogen content was determined by the Carius method.³⁷ The infrared spectra (KBr) were examined in the range $4000\text{-}600\text{ cm}^{-1}$ with Nicolet 760 FTIR spectrophotometer. A diffused reflectance spectrum of solid metal complexes was recorded on the Backman DK2A spectrophotometer using MgO as reference. Magnetic susceptibility measurement of the entire metal complexes was carried out at room temperature (303K) by Gouy method³⁸ using mercury tetrathiocyanato cobaltate (II) as calibrant.³⁹ The values of effective magnetic moment μ_{eff} were calculated by using equation (1) and Diamagnetic corrections were made by using Pascal's constant⁴⁰.

$$\mu_{\text{eff}} = 2.84 \sqrt{X'_m \times T} \quad (1)$$

The ligand and their metal chelates were screens at 1000 ppm concentration in vitro for their antifungal activity against three fungi viz. *Penicillium expansum*, *Nigrospora* sp. and *Aspeginus niger*. The antifungal activity of the compound was measured by the cup-plate method⁴¹. Five days old cultures were suspended in potato dextrose agar medium and autoclaved at 120°C for 15 minutes at 15 atmospheric pressure. The percentage of inhibition of fungi was calculated after 5 days using equation (2).

$$\% \text{ of Inhibition} = \frac{100 (A-B)}{A} \quad (2)$$

Where 'A' is an area of the colony in the control plate and 'B' is an area of the colony in the test plate.

RESULTS AND DISCUSSION

A novel tetradentate ligand L-2 was synthesized by condensation 1-(5-benzoyl-1H-2,3-benzotriazole-1-yl) 2-chloroethanone with alanine. The synthesized ligand was further used for the synthesis of Cu(II) , Ni(II) , Co(II) , Zn(II) , Mn(II) complexes. The synthesized ligand and its metal complexes were characterized by FTIR, mass, ^1H NMR, elemental analysis was found to be stable at room temperature. The ligand was soluble in methanol, ethanol, DMF and DMSO, whereas metal complexes were soluble in DMF/DMSO. The physical properties of ligand and metal complexes are listed in Table-1.

Figures 4 and 5 are respectively for IR and NMR spectra and the data obtained from spectra for samples are shown in Table-2. The structures of ligand and complexes are confirmed from their IR and NMR

spectra. The anticipated IR spectral inflections around 1480 and 1450 cm^{-1} attributed to asymmetric and symmetrical stretching vibrations frequencies of CH_2 presence in COCH_2NH linkage. The bands around 1500 cm^{-1} , 1600 cm^{-1} and 3030 cm^{-1} in the region of double bonds appear, there might arise from aromatic breathing. The strong band around at $1690 \pm 10 \text{ cm}^{-1}$ largely responsible for $\text{C}=\text{O}$ of COOH group of aromatic acid and strong band at region 1700-1725 cm^{-1} due to $\text{C}=\text{O}$ of COOH group of aliphatic acid. The other bands in the fingerprint region appear at their respective positions. The bands around 1220 cm^{-1} and 1020 cm^{-1} are mainly due to C-N bending vibrations while the C-N stretching vibration features appear around 1690 cm^{-1} . The weak bands due to out-of-plane deformation of the 1,3,5-substituted benzene ring, and appear near 896 cm^{-1} . The band appears at 2341, 1488 and 1558 cm^{-1} due to the benzotriazole ring and the band at 723 cm^{-1} may arise from 1,2,3-triazole system.

Table-1: Characterization of Metal Chelates of Ligand L-2

Metal Chelates	Molecular Formula	M.Wt gm/mole	Yield %	% Metal Analysis		Elemental Analysis					
						%C		%H		%N	
				Cald	Found	Cald.	Found	Cald.	Found	Cald.	Found
L-2	$\text{C}_{18}\text{H}_{16}\text{N}_4\text{O}_4$	352	70	-	-	61.36	61.35	4.54	4.53	15.90	15.88
(L-2) ₂ Cu ⁺²	$\text{C}_{36}\text{H}_{30}\text{N}_8\text{O}_8 \cdot 2\text{H}_2\text{O}$	785.54	68	8.08	8.07	54.99	54.97	4.32	4.31	14.25	14.23
(L-2) ₂ Ni ⁺²	$\text{C}_{36}\text{H}_{30}\text{N}_8\text{O}_8 \cdot 2\text{H}_2\text{O}$	780.69	65	7.51	7.50	55.33	55.31	4.35	4.34	14.34	14.32
(L-2) ₂ Co ⁺²	$\text{C}_{36}\text{H}_{30}\text{N}_8\text{O}_8 \cdot 2\text{H}_2\text{O}$	780.93	67	7.54	7.52	55.31	55.30	4.35	4.32	14.34	14.33
(L-2) ₂ Mn ⁺²	$\text{C}_{36}\text{H}_{30}\text{N}_8\text{O}_8 \cdot 2\text{H}_2\text{O}$	776.93	65	7.07	7.06	55.60	55.58	4.37	4.35	14.41	14.40
(L-2) ₂ Zn ⁺²	$\text{C}_{36}\text{H}_{30}\text{N}_8\text{O}_8 \cdot 2\text{H}_2\text{O}$	787.38	64	8.30	8.28	54.86	54.85	4.31	4.30	14.22	14.20

All the IR spectra have identical bands at their respective positions. Most of the bands that appeared in the spectral of corresponding ligand are observed at a similar position in the IR spectra of their metal chelates. The band due to $\text{C}=\text{O}$ of COOH group appeared in the spectra of the ligand is almost vanished in the spectra of chelates. The new strong band around 1600 cm^{-1} appears and this might be responsible for COO^- anion. This is expected as the COOH group of the ligand is participating in metal chelate formation. A new band at 1077 cm^{-1} had appeared in the spectra of metal complexes. This may be assigned to $\nu_{\text{C-O}}$ of C-O Metal bond formation. The selected IR spectra of metal chelate are reflected in Fig.-4 (a and b).

Fig.-4(a): IR Spectrum of Ligand (L-2).

Fig.-4(b): IR Spectrum of Ligand (L-2)₂-Mn²⁺

Fig.-5: NMR Spectrum of Ligand L-2

Table-2: NMR Data of Ligand L-2.

NMR DMSO	Multiplet of Aromatic	δ ppm	7.0 to 7.7
	Singlet of CH ₂ linkage	δ ppm	3.66
	Singlet of NH	δ ppm	2.0
	Singlet of benzotriazole	δ ppm	8.0, 8.4
	Singlet of COOH	δ ppm	11.0
	Singlet of CH-CH ₃	δ ppm	1.23

¹H-NMR (Nuclear Magnetic Resonance) spectroscopy is one of the modern physical methods of investigating organic molecules. The scale of the spectrum is generally marked in parts per million (ppm) of the applied field or frequency units (Hz). ¹H-NMR spectra were recorded on Bruker WM400FTMHzNMR instrument using CDCl₃ or DMSO-d₆ as solvent and TMS as an internal reference. The data derived from Fig.-5 of ligand L-2 is summarized in Table-2.

Table-3: Magnetic Moment and Reflectance Spectral Data of Ligand L-2.

Complex	Absorption Band	Transitions	Magnetic Moment μ_{eff} (B.M.)
(L-2) ₂ Cu	23370, 15655	CT, B _{1g} → ² A _{1g}	1.95
(L-2) ₂ Ni	24478, 15607	³ A _{2g} → ³ T _{1g} (P), ³ A _{2g} → ³ T _{1g} (F)	3.85
(L-2) ₂ Co	8928, 18867, 24660	⁴ T _{1g} (F) → ⁴ T _{2g} (F), ⁴ T _{1g} (F) → ⁴ A _{2g} , ⁴ T _{1g} (F) → ⁴ T _{2g} (P)	3.87
(L-2) ₂ Mn	24045, 18657, 16222	⁶ A _{1g} → ⁴ A _{1g} (4Eg), ⁶ A _{1g} → ⁴ T _{2g} (4G), ⁶ A _{1g} → ⁴ T _{1g} (4G)	5.50
(L-2) ₂ Zn		Zn ²⁺ Diamagnetic in nature	

In this present work, the magnetic moment (μ_{eff}) of Cu^{+2} complex is found to be 1.95 B.M. for ligand L-2 indicating the distorted octahedral geometry for this metal complex. These findings are in agreement with data reported by several research workers.^{42,43} The two absorption bands are observed in the region 15655 and 23370 cm^{-1} for L-2. In electronic spectra of Cu^{+2} metal complexes of the ligand may be assigned to ${}^2\text{B}_{1g} \rightarrow {}^2\text{A}_{1g}$ and charge transfer transition respectively. These results reveal the distorted octahedral geometry for this complex.

The magnetic moments of Ni^{+2} complexes studied and found 3.85 B.M., which indicate the octahedral distorted structure of Ni^{+2} complex and further the high magnetic moments may be due to orbital contribution. Such observations are supported by many researchers.^{44,45} Further, the diffuse reflectance spectra of Ni^{+2} complex shows two bands around at 15607 and 22741 cm^{-1} , which can be assigned to the transition ${}^3\text{A}_{2g}(\text{F}) \rightarrow {}^3\text{T}_{1g}(\text{F})$ and ${}^3\text{A}_{2g}(\text{F}) \rightarrow {}^3\text{T}_{1g}(\text{P})$ respectively suggesting octahedral geometry for Ni^{+2} complex. The assignments are shown in Table-3.

It is observed that the magnitude of magnetic moments of ligand L-2 with Co^{+2} complex is found to be 3.87BM. This value indicates the possibility of an octahedral structure complex. Examination of the electronic spectral data reported in Table-3 indicates that transitions observed in the range 8000-9000 cm^{-1} are assigned to ${}^4\text{T}_{2g}(\text{F}) \rightarrow {}^4\text{T}_{1g}(\text{F})$ transition and another band in the region 18667 cm^{-1} and 24660 cm^{-1} may be attributed to ${}^4\text{T}_{2g}(\text{F}) \rightarrow {}^4\text{A}_{2g}$ and ${}^4\text{T}_{2g}(\text{F}) \rightarrow {}^4\text{T}_{2g}(\text{F})$ transitions respectively. Based on the magnetic moment and electronic spectral, the octahedral structure can be predicted for the Co^{+2} complex, and similar observations are also reported in the literature.^{46,47}

The magnetic moment of some Mn^{+2} complexes gives the value coordination to three unpaired electrons instead of five unpaired electrons and many have been reported and show low magnetic moments⁴⁸. This may be due to aerial oxidation of $\text{Mn}^{+2} \rightarrow \text{Mn}^{+3}$ in the solid-state^{49,50} and/or due to spin exchange in the solid-state. Electronic spectra of six coordinated Mn^{+2} complexes have been extensive. Mn^{+2} complex shows two bands in the region 18000-20000 cm^{-1} and a weak band around the region 23600-24350 cm^{-1} for octahedral geometry. These three bands can be assigned as ${}^6\text{A}_{1g} \rightarrow {}^4\text{T}_{1g}(\text{G})$, ${}^6\text{A}_{1g} \rightarrow {}^4\text{T}_{2g}(\text{G})$ and ${}^6\text{A}_{1g} \rightarrow {}^4\text{A}_{1g}(\text{4Eg})$ transitions. The magnetic moment of the Mn^{+2} complex with L-2 ligands is found to be 5.5 BM. The low magnetic moment values of the present compounds may be due to either aerial oxidation of $\text{Mn}^{+2} \rightarrow \text{Mn}^{+3}$ or due to spin exchange in the solid-state. The absorption bands of this compound are found to be 16222 cm^{-1} for L-2 attributed to ${}^6\text{A}_{1g} \rightarrow {}^4\text{T}_{1g}(\text{G})$ and second around 24045 cm^{-1} due to ${}^6\text{A}_{1g} \rightarrow {}^4\text{T}_{2g}(\text{G})$ transition⁴⁵. The study of the data reveals that the Mn^{+2} complex has an octahedral structure. As the spectrum of Zn^{+2} complexes compound is not well resolved it is not interpreted but the μ_{eff} value indicates that it is diamagnetic as expected.⁵¹

Table-4: Antibacterial and Antifungal Activity of the Ligand and their Metal Chelates

Sample	Zone of inhibition (in mm)						
	Gram + Ve		Gram -Ve		Antifungal		
	<i>B. Subtills</i>	<i>S. aureus</i>	<i>S. typhi</i>	<i>E-Coli</i>	Penicillium Expansum	A. Niger	Nigras Pora Sp.
L-2	15	17	13	12	15	14	17
(L-2) ₂ Cu^{+2}	35	38	32	29	28	29	27
(L-2) ₂ Mn^{+2}	29	21	22	20	19	24	21
(L-2) ₂ Co^{+2}	30	29	22	26	21	20	22
(L-2) ₂ Zn^{+2}	23	25	20	19	22	20	24
(L-2) ₂ Ni^{+2}	26	28	23	27	23	25	22

The antibacterial activities of all synthesized complex compounds were screened against two Gram-positive bacteria (*Bacillus subtilis* and *S. aureus*) and two Gram-negative bacteria (*Escherichia coli* and *Salmonella typhi*). Antifungal activity was screened against three fungal species (*Penicillium Expansum*, *Nigras Pora Sp.* and *A. Niger*). The minimal inhibitory concentration (MIC) of all complex compounds was determined by the microdilution methods according to National Committee for Clinical Laboratory Standards (NCCLS)⁵². The inoculum concentration of the test strain was adjusted to 108CFU (colony-forming units) per cm^3 by comparing the sample turbidity. Mueller-Hinton broth was used as a nutrient medium to grow and dilute the drug suspension for the test. DMSO was used as the diluent to get the

desired concentration of compounds to test upon the standard bacterial stains. The obtained results are presented in Table-4. Upon investigation of antimicrobial screening data, it has been observed that the majority of the complex compounds showed good activity against Gram-positive bacteria. Complex compounds with Copper metal salt showed the highest potency of the synthesized all complexes. Whereas in inhibiting Gram-negative bacteria, copper metal complex compound with L-2 observed excellent activity against E. coli. The antifungal activity of all the complexes measured for various plant pathogens. Inspection of the results is reflected in Table-4 and indicates that all complex compounds have good inhibition efficiency for fungi. Out of all the compounds, the copper chelate is more toxic than others. This compound almost inhibits the fungi by about 70%. Hence produced metal chelate can be employed as garden fungicides.

CONCLUSION

A library of benzotriazole derivatives ligands was developed with complex forming reagent 2-amino propionic acid. The synthesized all complex compounds were characterized by analytical and spectral data and are in full compliance with the suggested structure of ligand 2- $\{[2-(5\text{-benzoyl-1H-1,2,3 benzotriazole-1-yl})-2\text{-oxoethyl}] \text{amino}\}$ propionic acid (L-2) ligand enhance their antibacterial and antifungal activity, as is noticeable from the biological evaluation results. Most of the complex compounds were found to be potent against tested microorganisms with moderate to good activity.

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