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BIOASSAY STUDIES OF METAL(II) COMPLEXES OF 2,2'-(ETHANE-1,2-DIYLDIIMINO)DIACETIC ACID

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ABSTRACT. Ni(II), Cu(II) and Zn(II) coordination compounds with modified diammine 2,2'-(ethane-1,2-diyldiimino)diacetic acid (EDDA) were prepared and characterized. Coordination complexes of the EDDA were characterized by physical measurements including elemental analysis, IR, UV-Visible, magnetic susceptibilities and conductance measurements. The complexes were screened against four pathogenic bacteria like *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus* and their concentrations for maximum inhibition zones were obtained.

KEY WORDS: EDDA, Coordination complexes, Antibacterial studies

INTRODUCTION

Many studies stressed the role of metal ions in biological processes. Whereas the inorganic pharmacology started to be an important field, pursuing more than 25 inorganic compounds being used in various therapies as antibacterial, antiviral and anticancer drugs [1-9].

In many such cases the chelating agent is a complex polyfunctional moiety which can virtually enclave the metal in an organic sphere. The geometries of the corresponding metal complexes also offer potency in biological applications. It has also been shown that chelation to the metal center tend to make biologically inactive compounds active by reorienting and forefronting the active sites for interaction with the biological systems [10-15].

All these evidences highlighted the importance of metal coordination with chelating agents in drugs and therapeutics. In the present work EDDA was complexed with Ni(II), Cu(II) and Zn(II) metal ions keeping in view the importance of these metals in biological system [10-26].

Transition metals being the centre of many metal proteins like vitamin B_{12} [16], hydrogen transfer enzymes like glutamate mutase, methylmalonyl CoA isomerase, dioldehyrase, ethanolamine ammonia lyase, ribonucleotide reductase and many others [17-26], therefore we got interest in complexation of transition metals with modified diammine adding to our previous work carried out on diammines and their complexation [10, 11, 30]. These ligands by themselves are very potent offering poly chelating sites [29, 30].

In the context of our current interest in methodologies for constructing substituted diammines, we envisioned a new and versatile access to novel chiral diammine. This strategy involves the synthesis of the ligand by the removal of primary hydrogens from the corresponding amines with alkyl halide to bear diammines of the same class with different substituents. The prepared complexes were then screened against various pathogenic strains by using agar well diffusion method.

EXPERIMENTAL

All the chemicals and solvents used were of Analar grade. Metal(II) salts used were chlorides and carbonates (Riedel-de-Haen, Germany) and were used as such without further purification. Solvents were distilled at least twice before use. Elemental analysis were taken using Vario Elementar III (Germany). Melting points were recorded on Gallenkamp apparatus and reported

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as such. Biological activities were carried out by the stated procedure at Pakistan Medical Research Center, Khyber Medical College, Peshawar (PMRC).

Instrumentation

Molar conductances of the solution of the metal complexes were determined with a conductivity meter type HI 8333. Conductometric measurements were carried out at room temperature using freshly prepared solution.

Magnetic susceptibilities were measured by Gouy method at room temperature using $Hg[Co(SCN)_4]$ as standard, magnetic moments of complexes were calculated using method cited [34]. The cations were estimated by using analytical procedure [32].

Infrared spectra were taken in the range of 4000-600 cm^{-1} on Pye Unicam Infrared Spectrophotometer as KBr disc. The far IR spectra were examined as KBr discs in the region of 400- 200 cm^{-1} (T-IR SHIMADZU).

The absorption spectra of solution of complexes in the range of 400-800 nm using different solvents were obtained on Jasco DEC-1 Spectrophotometer with 1 cm matched quartz cells.

Bioactivities were investigated using agar-well diffusion method [27]. Two to eight hours old bacterial strains inoculums containing approximately 104–106 colony forming units (CFU)/mL were used in these assays. The wells were dug in the media with the help of a sterile metallic borer with centers at least 24 mm. Recommended concentration (100 µL) of the test sample 1 mg/mL in DMSO was introduced in the respective wells. Other wells supplemented with DMSO and reference antibacterial drug, maxipime served as negative and positive controls, respectively. The plates were incubated immediately at 37 ^oC for 20 hours. Activity was determined by measuring the diameter of zones showing complete inhibition (mm). Growth inhibition was compared with the standard drug maxipime for the selected bacterial strains. In order to clarify any participating role of DMSO in the biological screening, separate studies were carried out with the solutions alone of DMSO and they showed no activity against any bacterial strains. All these complexes were found to be potentially active against these bacterial strains.

Synthesis of 2,2'-(ethane-1,2-diyldiimino)diacetic acid (EDDA)

2 g (0.033 mol) of ethylenediamine in 10 mL of dry ethanol was taken and stirred at room temperature for about 10 min. 6.237 g (0.066 mol) of chloroacetic acid was added to this mixture and stirring continued for one hour. Solid KOH 1.84 g (0.033 mol) was added to this reaction mixture and refluxed for one hour. White precipitate of KBr was filtered off and the filtrate containing EDDA was evaporated through rotary evaporater to get solid product. Solid EDDA was purified by dissolving in dry methanol. Purity was checked by TLC technique. Yield for EDDA obtained was 38%. $C_6H_{12}N_2O_4$ calc. C (40.91%), H (6.87%), N (15.90%). Found: C (40.11%), H (6.70%), N (16.30%).

Synthesis of [M(EDDA)2H_2O]

0.3 g (0.0028 mol) of $M(II)CO_3$ [where M = Ni(II), Cu(II) and Zn(II)] was dissolved in minimum amount of distilled water. The reaction mixture was stirred 0.4 g (0.0025 mol) of ligand EDDA dissolved in minimum amount distilled water was added to this reaction mixture and the resulting solution was heated up to 60 ^oC in inert atmosphere until the CO_2 emission completed. Almost it took 10 min. The mixture was left overnight and then concentrated by rotary evaporator. On cooling different colored product was precipitated out. The product was

filtered through sintered glass crucible and washed by 5 mL mixture of ice-cold water, ethanol and acetone. The dried product appeared to be stable in air.

$C_6H_{14}N_2NiO_6$ yield = 68%, calc. C (26.80%), H (5.25%), N (10.42%), Ni (21.83%); found C (27.73%), H (5.85%), N (10.22%), Ni (20.01%). $C_6H_{14}CuN_2O_6$ yield = 40%, calc. C (26.33%), H (5.16%), Cu (23.21%), N (10.23%); found C (26.13%), H (5.76%), Cu (23.20%), N (10.67%). $C_6H_{14}N_2O_6Zn$ yield = 53%, calc. C (26.15%), H (5.12%), N (10.16%), Zn (23.73%); found C (26.10%), H (6.03%), N (10.02%), Zn (24.23%).

RESULTS AND DISCUSSION

The ligand and solid complexes were characterized by elemental analyses, the % composition of carbon, hydrogen and nitrogen are considerably within the permissible limit to those calculated values. ^{1}H and ^{13}C NMR spectrum were obtained for ligand, the ^{1}H NMR for EDDA showed triplet at 2.63 ppm for the methylene protons at 2 and 3 positions (Figure 1), triplet at about 3.05 ppm for methylene protons at position 1 and 2 whereas the carboxylic singlet appears at round about 7 ppm in the spectrum.

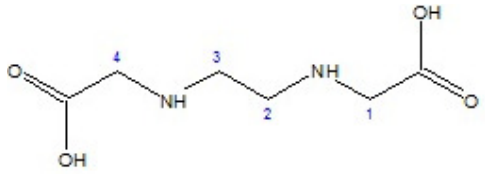

Figure 1. Structure of ligand EDDA.

Similarly the ^{13}C NMR spectrum of EDDA also showed three singlet peaks which are assigned to methylene carbon atoms at position 2 and 3, 1 and 4 and to the carboxylic carbon. The methylene occupying positions 2 and 3 appeared at around 50 ppm, the methylene at 1 and 2 appeared at 54 ppm and the 180 ppm is assigned to carboxylic carbon.

Elemental analytical data of the ligands and its complexes showed very closeness to the theoretical values and the percentage well fits to the calculated formula. The ligand EDDA behave as tetradentate ligand. In Table 1 are listed molar conductance, melting points and magnetic moment values. The molar conductance values indicate that the complexes are non-electrolyte.

Table 1. Conductance, melting points and magnetic moments data.

Complex	M.p. (ºC)	Solvent	Molar conductance (S/cm)	μ_{eff} (B.M.)
$C_6H_{12}N_2O_4$	281	---	---	---
$[Ni(C_6H_{12}N_2O_4)2H_2O]$	185	DMSO	10.2	3.2
$[Cu(C_6H_{12}N_2O_4)2H_2O]$	163	DMSO	60	1.7
$[Zn(C_6H_{12}N_2O_4)2H_2O]$	200	DMSO	73	Diamagnetic

The far IR spectra show M-N stretch around 500 cm^{-1}. The infrared spectrum of EDDA showed an alteration of N-H and carboxyl frequency, which suggest characterization of tetradentate ligand. There is alteration in the N-H frequency and a complete disappearance of carboxylic O-H frequency from the spectra of coordination complexes which depicts

coordination from these two sites. A broad peak at 3340 cm^{-1} the IR band observed is assigned to the metal to water coordination [33, 34]. The IR data are given in Table 2.

Table 2. Assignment of absorption bands in IR spectra for EDDA and its complexes (cm^{-1}).

Complex	N-H_{str}	C-N_{str}	Other significant bands	M-OH_2
$C_6H_{12}N_2O_4$	3330 sh	1220 m	2943 w, 3480 b, 1720 m	-----
$[Ni(C_6H_{12}N_2O_4)\ 2H_2O]$	3370 b	1215 m	2940 w, 1700 m	3460 b
$[Cu(C_6H_{12}N_2O_4)\ 2H_2O]$	3400 b	1222 m	2940 w, 1710 m	3400 b
$[Zn(C_6H_{12}N_2O_4)\ 2H_2O]$	3360 b	1220 m	2930 w, 1700 m	3400 b

sh = sharp, w = weak, m = medium, b = broad.

[Ni(EDDA) $2H_2O$] complex

The magnetic moment of nickel EDDA complex showed three unpaired electrons. The solution spectrum of nickel(II) EDDA complex showed bands at 19230, 15500 and 14800 cm^{-1} which are assigned to the transitions $^3A_{2g} \rightarrow {}^3T_{2g}$; $^3A_{2g} \rightarrow {}^3T_{1g}$ (F) and $^3A_{2g} \rightarrow {}^3T_{1g}$ (P), respectively. The intensities and bandwidths are also in accordance with O_h symmetry (Figure 2).

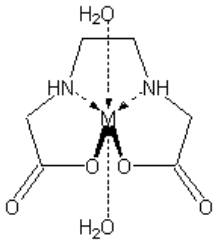

Figure 2. Proposed octahedral geometry for complexes.

[Cu(TEEDA)Cl_2] complex

The magnetic moment of copper complex is 1.7 B.M which indicates unpaired electron. The solution spectrum of copper(II) complex showed a set of three bands in range of 14000 to 15000 cm^{-1} which are assigned to $^2T_{2g} \rightarrow {}^2E_g$ transition. The intensities and bandwidths are in accordance with O_h symmetry as shown in Figure 2 [28, 29].

Zinc complexes

The conductance data of Zn(II) complexes indicate the non-ionic nature of the complex. The non-ionic nature and the elemental data suggest the proposed formula and octahedral geometry may be assigned for the complex [19].

Bio-assay investigations

The complexes of EDDA were investigated for their bioactivity. The study showed that the metal complexes become more biologically active as compared to neat organic moiety. The complexes of EDDA have been screened against *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus*. The results are reported in Table 3-6 which reveal increase in inhibition properties upon complexation.

Growth of inhibition zone was compared with standard available drug maxipime. The participating role of the solvent used was also clarified by running the biological screening against DMSO only.

Table 3 shows almost no inhibitory activities for *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, moderate activities were observed for *Klebsiella pneumoniae*. From the data presented it can be deduced that ligand alone cannot account for inhibition of pathogenic organisms.

Table 3. Antibacterial activity of EDDA against four pathogenic bacteria.

Concentration (µg/20µL)	Zones of inhibition (mm)			
	Escherichia coli	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*Staphylococcus aureus*
20	0	0	1.0	0
40	0	0	1.0	0
60	0	0	1.0	0
80	0	0	2.0	0
100	0	0	4.0	1.0
120	0.6	0.4	4.0	1.0

$[Ni(EDDA)(H_2O)_2]$ was observed to show biological activities against pathogens like *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* at high concentrations. But no activity was observed for *Staphylococcus aureus* as shown in Table. 4.

Table 4. Antibacterial activity of $[Ni(EDDA)(H_2O)_2]$ against four pathogenic bacteria.

Concentration (µg/20µL)	Zones of inhibition (mm)			
	Escherichia coli	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*Staphylococcus aureus*
20	0	0	2.0	0
40	0	0	2.0	0
60	0	2.0	4.0	0
80	4.0	2.0	4.0	0
100	4.0	5.0	4.0	0
120	4.0	6.0	5.0	0

Similarly $[Cu(EDDA)(H_2O)_2]$ showed moderate activity against *Staphylococcus aureus* and *Klebsiella pneumoniae* whereas for *Escherichia coli* and *Pseudomonas aeruginosa* significant activity was observed at high concentration as shown in Table. 5.

Table 5. Antibacterial activity of $[Cu(EDDA)(H_2O)_2]$ against four pathogenic bacteria.

Concentration (µg/20µL)	Zones of inhibition (mm)			
	Escherichia coli	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*Staphylococcus aureus*
20	0	0	1.0	1.0
40	0	0	1.0	2.0
60	0	0	1.0	2.0
80	3.0	1.0	2.0	2.0
100	4.0	2.0	2.0	2.0
120	6.0	4.0	2.0	2.0

$[Zn(EDDA)(H_2O)_2]$ also show the same trend in biological activities as shown by $[Cu(EDDA)(H_2O)_2]$ as can be depicted from Table 6.

Table 6. Antibacterial activity of $[Zn(EDDA)(H_2O)_2]$ against four pathogenic bacteria.

Concentration (µg/20µL)	Zones of inhibition (mm)			
	Escherichia coli	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*Staphylococcus aureus*
20	0	0	1.0	0
40	0	0	1.0	0
60	1.0	2.0	2.0	0
80	2.0	2.0	2.0	0
100	2.0	2.0	2.0	1.0
120	3.0	2.0	2.0	1.0

CONCLUSIONS

The synthesized coordination metal complexes of EDDA showed octahedral geometries. Magnetic moment studies proved the assigned geometries. The synthesized ligand showed antibacterial properties. In comparison, the metal(II) complexes of EDDA showed more activity against one or more bacterial strains, thus introducing a novel class of metal-based bactericidal agents.

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REFERENCES

1. Louie, A.Y.; Meade, T.J. *Chem. Rev.* **1999**, 99, 2711.
2. Scozzafava, A.; Menabuoni, L.; Mincione, F.; Mincione, G.; Supran, C.T. *Bioorg. Med. Chem. Lett.* **2001**, 11, 575.
3. Scozzafava, A.; Supran, C.T. *Bioorg. Med. Chem. Lett.* **2000**, 43, 3577.
4. Williams, R.J.P. *Bioinorganic Chemistry*, American Chemical Society: Washington; **1971**.
5. Brown, D.H.; Smith, W.E.; Teape, J.W.; Lewis, A.J. *J. Med. Chem.* **1980**, 23, 729.
6. Gillard, R.D. *Inorg. Chim. Acta Rev.* **1961**, 1, 60.
7. Walsh, C. *Science* **2001**, 409, 226.
8. Bertini, I.; Gray, H.B.; Lippard, S.J.; Valentine, J.S. *Bioinorganic Chemistry*, University Science Books: Mill Valley, California, USA; **1994**.
9. Kirchner, S.; Wei, Y.K.; Francis, D.; Bergman, D. *J. Med. Chem.* **1966**, 9, 369.
10. Hussain, M.S.; Rehman, S.U. *Z. Naturforsch.* **1978**, 33b, 67.
11. Hussain, M.S.; Rehman, S.U. *Inorg. Chim. Acta* **1982**, 60, 233.
12. Sorenson, J.R.L. *Inflammatory Diseases and Copper*, Human Press: New Jersey; **1982**.
13. Nagar, R.; Mohan, G. *J. Inorg. Biochem.* **1991**, 42, 9.

14. Oga, S.; Taniguchi, S.F.; Najjar, R.; Souza, A.R. J. *Inorg. Biochem.* **1991**, 41, 45.
15. Barboiu, M.; Cimpoisu, M.; Guran, C.; Supran, C.T.; *Metal-based Drugs* **1996**, 3, 227.
16. Hodgkin, D.C.; Pickworth, J.; Robertson, J.H.; Prosen, R.J.; White, J.G.; Bonnett, R.; Trueblood, K.N.; Cannon, J.R.; Johnson, A.W.; Sutherland, I.; Todd, A.; Smith, E.L. *Nature* **1955**, 176, 325.
17. Barker, H.A.; Rooze, V.; Suzuki, F.; Iodice, A.A. *J. Biol. Chem.* **1964**, 239, 3260.
18. Cannata, J.J.B.; Focisi, A.; Mazumder, R.; Warner, S.C.; Ochoa, S. *J. Biol. Chem.* **1965**, 240, 3249.
19. Flavin, M.; Ochoa, S. *J. Biol. Chem.* **1957**, 229, 965.
20. Kaziro, Y.; Ochoa, S. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1964**, 26, 283.
21. Abeles, R.H.; Lee, H.A. *J. Biol. Chem.* **1961**, 236, 2347.
22. Babior, B.M. *J. Biol. Chem.* **1969**, 244, 449.
23. Abrams, R.; Duraiswami, S. *Biochem. Biophys. Res. Co.* 1965, 18, 409.
24. Burke, G.T.; Mangum, J.H.; Brodie, J.D. *Biochemistry-US* **1970**, 9, 4297.
25. Wood, J.M.; Kennedy, F.S.; Wolfe R.S. *J. Biol. Chem.* **1968**, 7, 1707.
26. Ghambeer, R.K.; Wood, H.G.; Schulman, M.; Ljungdahl, L. *Arch. Biochem. Biophys.* **1971**, 143, 471.
27. Atta-ur-Rahman; Choudhary, M.I.; Thomsen, W.J. *Bioassay Techniques for Drug Development,* Harwood Academic: Amsterdam; **2001**.
28. Musker, W.K.; Hussain, M.S. *Inorg. Chem.* **1966**, 5, 1416.
29. Musker, W.K.; Hussain, M.S. *Inorg. Chem.* **1969**, 8, 528.
30. Rehman, S.U.; Noreen, S.; Rashida, I.M.; Ali, K.; Ahmad, I.; Arshad, M.; Bukhari, I.H. *The Nucleus* **2009**, 46, 501.
31. Nickolov, Z.; Georgieva, G.; Stoilovab, D.; Ivanova, I.; *J. Mol. Struct.* **1995**, 354, 119.
32. Jeffery, G.H.; Bassett, J.; Mendham, J.; Denney, R.C. *Vogel's Text Book of Quantitative Inorganic Analysis*, 4th ed., Longmann: London; **1978**.
33. Legg, J.I.; Averill, D.F.; Smith, D.L. *Inorg. Chem.* **1972**, 11, 2344.
34. Figgis, B.N.; Lewis, J.; Wilkins, R.G. (Eds.), *Modern Coordination Chemistry*, Interscience: New York; **1960**, p 412.