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# SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF MIXED LIGAND COMPLEXES OF INNER TRANSITION METAL WITH L-ASCORBIC ACID, ITS DERIVATIVE AND ADENINE BASE

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## ABSTRACT

Complexes of La(III), Ce(III), Nd(III) and Gd(III) with mixed Ligand of 2-aminopurene and L-ascorbic acid derivative were prepared in aqueous ethanol. The two types of mixed ligand complexes $[M(AA)(ADE)(H_2O)_2]^+$and $[M(IPAA)(ADE)(H_2O)_2]^+$ have been synthesized. All complexes were characterized on the basis of elemental analysis, molar conductance, magnetic susceptibility measurements, IR, UV-VISSIBLE, NMR, ESR and Mass spectral studies. IR spectra of the complexes reveal that the complex formation occurred through both nitrogen and oxygen atoms. On the basis of electronic spectral data and magnetic susceptibility measurement octahedral geometry has been proposed for the complexes. The ESR spectral data of the M (III) complexes showed that the metal-ligand bonds have considerable covalent character. The electrochemical behaviour of mixed Ligand M(III) complexes was studied which showed that complexes of IPAA appear at more positive potential as compared to those for corresponding AA complexes. In addition, biological activity of the synthesised metal complexes against *Salmonella typhi, Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae* bacteria and *Aspergillus niger, Aspergillus flavus, Fusarium oxisporium* and *Trichoderma viride* fungi respectively were examined *in-vitro*. Some of the metal complex displayed pounced biological activity.

**Keywords:** 2-Aminopurene, L-ascorbic acid derivative, Bidentate, Pyrimidine, Organometallic, Ligand, Metal-complexes, Antimicrobial activity.

## 1. INTRODUCTION

According to World Health Organization (WHO) infectious diseases are spreading faster and emerging more quickly than ever before. Due to the increasing incidents related with new and re-emerging infectious diseases, discovery and development of new anti-microbial compounds with diverse structures and action mechanism is urgently needed. The antibacterial effects of lanthanides (Ln) have been studied since the 19th century and have been employed since then with more or less success in treatment of various diseases. This paper gathers together all the studies dealing with the antimicrobial activity of Ln(III) complexes published until now. Overall, it was discovered that the antimicrobial activity of the Ln complexes is greater than the activity of the free ligand (L), indicating that the complexation with the Ln enhances the activity of the L. In some cases, Ln complexes exhibit antimicrobial activity similar or even better than the activity of standard antimicrobial agents. These results are encouraging, Ln complexes representing possible alternative to antimicrobial agents used currently.

The complexation of metal ions with adenine has been well studied and its binding sites has also been clarified [1, 2]. Various coordination sites have been observed for adenine in copper complexes as indicated by X-ray studies. Among the four nitrogen's N(1), N(3), N(7) and N(9) of adenine, the N(9) is the most basic and hence bears a proton rendering it is the most preferred metal binding site. The understanding of the structure and function of RNA and DNA systems depends partly on the binding sites of metal ions to nucleobases [3, 4]. Adenine (Ad) is a nucleo-base (a purine derivative) with a variety of roles in biochemistry including cellular respiration, in the form of both the energy-rich adenosine triphosphate (ATP) and the cofactors nicotinamide adenine dinucleotide (NAD) and flavin adenine dinucleotide (FAD), and protein synthesis, as a chemical component

of DNA and RNA. Adenine (Ad) is one of four chemical bases in DNA as in fig. 1, with the other three being cytosine (C), guanine (G), and thymine (T). Within the DNA molecule, adenine bases located on one strand form chemical bonds with thymine bases on the opposite strand [5] A form of adenine called adenosine tri phosphate (ATP) serves as an energy storage molecule and is used to power many chemical reactions within the cell. Adenosine tri phosphate is used in cellular metabolism as one of the basic methods of transferring chemical energy between reactions. In DNA, adenine bonds only to thymine. It does so with two strong hydrogen bonds, so the bond is difficult to break and the code is difficult to damage. Adenine has the chemical structure as shown in fig. 1a. which is known as organic compound belong to the purine family, characterized by its nucleic acid, in this case called nucleotides, where the phosphate esters of adenosine (Fig. 1b) and de-oxy adenosine (Fig. 1c) are clear in the structure, The importance of Adenine to RNA is similar to that of DNA. Adenosine tri phosphate is the nitrogenous base adenine bonded to a five carbon sugar. Adenosine tri phosphate is used in cellular metabolism as one of the basic methods of transferring chemical energy between, chemical reactions [6].

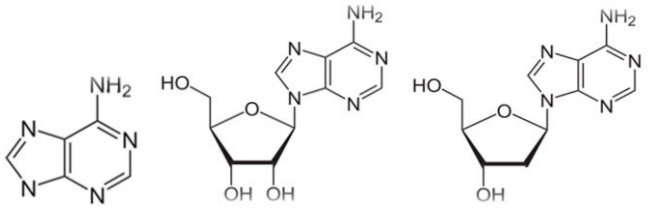


**Fig. 1: Typical chemical structure of Adenine (Fig. 1a), phosphate esters of adenosine (Fig. 1b) and de-oxy adenosine (Fig. 1c)**

Adenine is one of the two purine Nitrogenous bases used in forming nucleotides of the nucleic acids DNA and RNA. The derivatives of purine are called adenine (A) and guanine (G). The other three bases thymine (T), cytosine(C), and Uracil (U) are derivatives of pyrimidine, These four "code letters" allow cells to store their blueprint about how that life form is built. All cells of all living organisms, no matter how simple or complex, share this design [7]. In DNA, adenine binds to thymine via two hydrogen bonds to assist in stabilizing the nucleic acid structures. Adenine is one of the most interesting biological ligands which used for preparing and synthesizing different types of complexes [8].

L-Ascorbic acid (also called vitamin C), hereafter abbreviated as L-AA; is one of the most essential vitamins for both pharmaceutical and food processing industries. In view of its nutritional significance, varied uses in food and high daily doses necessary for optimum health, L-AA is a very significant vitamin for better public health [9, 10]. It has been reported that large doses of vitamin C increases greatly the rate of production of lymphocytes under antigenic stimulation and it is well established that such a high rate of lymphocyte blastomogenesis is associated with a favourable prognosis of cancer [11-13]. L-AA is known to kill HIV-positive cells and to be useful in HIV-positive patients as a consequence of the potentiating the immune system [14]. L-AA is a six-carbon keto-lactone, a strong reducing agent and serves as an antioxidant. The hydrogen donation from L-AA is considered to be primarily responsible for the antioxidant properties attributed to this molecule. It contains four OH groups (two enol OH groups on lactone ring carbons and two OH groups on the side chain C atoms). It can be very easily oxidized and changed to dehydroascorbic acid. Its four hydroxyl (OH) groups play important role in its antioxidant property.

## 2. EXPERIMENTAL

### 2.1. Analytical and physical measurements

The microanalyses of C, H, and N were carried out at SAIF, CDRI, Lucknow. The metal contents were determined by standard EDTA methods. Electronic spectra (DMF) were recorded on a Gary 14 spectro-photometer. The magnetic susceptibility measurements were carried out at SAIF, IIT Roorkee. The IR spectra were recorded on a Infrared spectrophotometer in the range 4000-200 $cm^{-1}$ using KBr at SAIF, Punjab University, Chandigarh. The NMR spectra were recorded on a Bruker NMR spectrometer (300 MHz). ESR spectra were recorded using an X-band (9.4GHz) EMX Bruker spectrometer equipped with an Oxford Helium continuous flux cryostat, allowing us to perform the experiments from 4K to room temperature (RT). The microwave power was varied from 2 mW to 350 mW in order to obtain the saturation curves. A field calibration was performed using an ESR standard, establishing that 0 dB (197 mW) corresponds to 0.11 mT. The ESR simulation spectra were made using the software EasySpin. The conductivity was measured on a digital conductivity meter (HPG System, G-3001).

Metal complexes were tested for in vitro antibacterial activity against some bacterial strains using spot on lawn on Muller Hinton Agar (MHA). Four test pathogenic

bacterial strains, viz., *Bacillus cereus* (MTCC 1272), where MTCC-Microbial type culture collections, *Salmonella typhi* (MTCC 733), *Escherichia coli* (MTCC 739), and *Staphylococcus aureus* (MTCC 1144), *Klebsiella pneumoniae* (MTCC 1377) bacteria and *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxisporium* and *Trichoderma viride* fungi were considered for determination of minimum inhibitory concentration (MIC) of selected complexes. The test pathogens were sub-cultured aerobically using Brain Heart Infusion Agar (HiMedia, Mumbai, India) at 37°C (24 h). Working cultures were stored at 4°C in Brain Heart Infusion (BHI) broth (HiMedia, Mumbai, India), while stock cultures were maintained at -70°C in BHI broth containing 15% (v/v) glycerol (Qualigens, Mumbai, India). The organism was grown overnight in 10 ml of BHI broth, centrifuged at 5.000 g for 10 min, and the pellet was suspended in 10 ml of phosphate buffer saline (PBS, pH 7.2). Optical density at 545 nm (OD_545) was adjusted to obtain 108 cfu/ml followed by plating serial dilution onto plate count agar (HiMedia, Mumbai, India).

### 2.2. Determination of MIC

MIC (minimum inhabitation concentration) is the lowest concentration of the antimicrobial agent that prevents the development of viable growth after overnight incubation. Antimicrobial activity of the compounds was evaluated using spot_on_lawn on MHA (HiMedia, Mumbai, India). Soft agar was prepared by adding 0.75% agar in Muller Hinton Broth (HiMedia, Mumbai, India). Soft agar was inoculated with 1% of 108 Cfu/ml of the test pathogen, and 10 ml were overlaid on MHA. From 1000X solution of compound (1 mg/ml of DMSO) 1, 2, 4, 8, 16, 32, 64, and 128X solutions were prepared. Dilutions of standard antibiotics (streptomycin and griseofulvin) were also prepared in the same manner: 5µl of the appropriate dilution was spotted on the soft agar and incubated at 37°C for 24h. Zones of inhibition of compounds were considered after subtraction of the inhibition zone of DMSO. Negative control (with no compound) was also observed.

### 2.3. Antimicrobial Studies

The antibacterial tests were prepared and characterized according to the standard method. All strains were isolated from laboratory of microbiology. The identity of all the strains was confirmed. A bacterial suspension was prepared and added to the Nutrient Agar, the fungi added to surrounded Agar, All this before medium solidification and under aseptic condition. Then different concentration of complexes were placed on the surface of the culture, The bacteria incubated at 37°C for 24 h, The fungi incubated at 28°C for 72 h.

### 2.4. Bacterial and Fungi Cultures

Plate cultures of nutrient agar medium were used for culture of bacteria. The medium was prepared by dissolving 14 g, and culture of fungi's 32.5 g of powder in 500 mL of sterile distilled water, the medium was then sterilized by autoclaving at 121°C for15 min.

### 2.5. Synthesis of Ligand IPAA

#### *2.5.1. Synthesis of 5, 6-O-Isopropylidine-L-Ascorbic Acid, Compound 1*

The Ligand (IPAA) was synthesised by refluxing a solution congaing 10 gm powdered L-ascorbic acid in 40 ml acetone while stirring about 30 minutes. After addition of benzyl chloride (1.5 ml), the reaction mixture was stirred for 2-3 hours at ambient temperature. The precipitate thus obtained, was washed several times with acetone- hexane mixture, cooled at 0°C.The final sediment has been dehydrated beneath miniature pressure to yield 10.1 g of needle-shaped crystals as a white solid, m. p. (206-208°C) Anal. Cald for $C_9H_{12}O_6$ : C 50.00 %,H 5.50 %, found C 48.96 %,H 5.47; IR: 3242(–OH), 2910 (-C-H), 1753 (-C=O), 1662 (-C=C-) 1436 (-C-H ), 1141, 898 (C-O; $^1$HNMR: δ 1.8 ppm (s, for the isopropylidene methyl proton) δ 2.4-2.9 ppm (m, $H_4$, $H_5$, $H_6$), δ 3.1 ppm (s, for the-OH); $^{13}$CNMR: δ 171.11 (-C=O), δ 152.26, 108.89 (=C-OH), δ 74.12, 64.73 (-CH-CH-$CH_2$), δ 25.70, 25.30 (-$CH_3$) ppm; Mass (FAB) :216 ($M^+$).

### 2.6. Synthesis of Binary Complex

#### *2.6.1. The general procedure for the synthesis of M (III) Ligand complexes*

La, Ce, Nd, Gd binary metal complexes with adenine. The binary metal complexes were synthesized by mixing 10 ml solution of metal salts (0.01mol) with 10 ml of Adenine (0.01 mol) in hot ethanol by keeping the metal-Ligand ratio (1:1 v/v). The mixture was refluxed for about 4 to 6 hours on a water bath with continuous stirring. The pH of the solution was adjusted about 5 to 6 by adding an acidic buffer solution in ethanol. The volume of the solution was reduced to half. The solid coloured products thus obtained were filtered, washed with distilled water and cold ethanol and then dried in vacuum over anhydrous calcium chloride a dedicator.

Metal Complexes **2(a-d)** were synthesized in the similar manner using compound 1 and various selected Metal Chlorides. Characterization data is presented in table 1.

### 2.7. Synthesis of Mixed Ligand Complexes

Mixed Ligand complexes of type $[M(AA)(ADE)(H_2O)_2$ and $[M(IPAA)(ADE)(H_2O)_2]^+$ were synthesized by adding 10 ml of binary complexes solution (0.01 mol) and 10 ml of AA/IPAA in hot ethanol by keeping the metal complexes- Ligand (1:1 v/v), as shown in Scheme 1. The reaction mixture was refluxed for 6 to 8 hours on a steam bath with continuous stirring and the volume of the solution was reduced to half of its original volume. The solid coloured compounds obtained were filtered off, washed with water, cold ethanol diethyl ether and dried in vacuum over anhydrous calcium chloride in desiccators.

Metal Complexes 3(a-d) and 4(a-d) were synthesized in the similar manner using compound 2(a-d) and selected Ligand L- Ascorbic acid (L-AA) and 5, 6-O-Isopropylidine-L-Ascorbic Acid (IPAA) respectively. Characterization data are presented in table 2 and 3.

**Table 1: Physical parameters of $[M(ADE)(H_2O)_4]^{3+}$**

| Complex | Colour | M.P. (°C) | Elemental Analysis Calculated (Found) | | |
|---|---|---|---|---|---|
| | | | C% | H% | N% |
| $[La(ADE)(H_2O)_4]^{3+}$ | White | 378-380 | 17.36 (17.21) | 3.75 (3.72) | 20.23(20.18) |
| $[Ce(ADE)(H_2O)_4]^{3+}$ | Red | 386-388 | 17.28 (17.19) | 3.74 (3.70) | 20.16(20.12) |
| $[Nd(ADE)(H_2O)_4]^{3+}$ | Brown | 390-392 | 17.08 (17.07) | 3.70 (3.68) | 18.21(18.17) |
| $[Gd(ADE)(H_2O)_4]^{3+}$ | Pink | 389-391 | 16.47 (16.41) | 3.56 (3.49) | 19.21(19.16) |

**Table 2: Physical parameters of $[M(ADE)(AA)(H_2O)_2]^+$**

| Complex | Colour | M.P. in °C | Elemental Analysis Calculated (Found) | | |
|---|---|---|---|---|---|
| | | | C% | H% | N% |
| $[La(ADE)(AA)(H_2O)_2]^+$ | White | 394-396 | 27.27 (27.11) | 3.09 (2.98) | 14.46(14.41) |
| $[Ce(ADE)(AA)(H_2O)_2]^+$ | Red | 398-400 | 27.20 (27.13) | 3.09 (2.96) | 14.42(14.39) |
| $[Nd(ADE)(AA)(H_2O)_2]^+$ | Brown | 382-384 | 26.97 (26.93) | 3.06 (2.98) | 14.30(14.27) |
| $[Gd(ADE)(AA)(H_2O)_2]^+$ | Pink | 389-391 | 26.28 (26.24) | 2.98 (2.96) | 13.93(13.91) |

**Table 3: Physical parameters of $[M(ADE)(IPAA)(H_2O)_2]^+$**

| Complex | Colour | M.P.in °C | Elemental Analysis Calculated (Found) | | |
|---|---|---|---|---|---|
| | | | C% | H% | N% |
| $[La(ADE)(IPAA)(H_2O)_2]^+$ | White | 375-377 | 32.06 (31.96) | 3.62 (3.61) | 13.36 (13.34) |
| $[Ce(ADE)(IPAA)(H_2O)_2]^+$ | Red | 380-382 | 31.99 (31.87) | 3.61 (3.59) | 13.33 (13.31) |
| $[Nd(ADE)(IPAA)(H_2O)_2]^+$ | Brown | 384-386 | 31.74 (31.71) | 3.58 (3.56) | 13.22 (13.19) |
| $[Gd(ADE)(IPAA)(H_2O)_2]^+$ | Pink | 376-378 | 30.98 (30.94) | 3.50 (3.47) | 12.91 (12.89) |

### 2.8. General Properties

The colour of ligand was changed from white colour of the free ligand to several different colours according to the type of metal ions, this change was mainly due to the effect the linkage between the ligand and the different electrons in 5f orbital's, where the attracting electrons between the ligand and the metal in f orbital's where the high and the less in energy, the magnetic frequency beam is proportion to the different in energy between the two states energy in atom. Some electrons rise into energy high level. The conductivity of the complexes depended mainly on the free electrons which non conjugation in the last orbital's, where the conductivity become less when conjugating occur between the metal and the ligand, this mean these electrons are bounded.

## 3. RESULTS AND DISCUSSION

Complexes of La(III), Ce(III), Nd(III) and Gd(III) with mixed Ligand of 2-aminopurene and L-ascorbic acid derivative were prepared in aqueous ethanol. The two types of mixed ligand complexes $[M(AA)(ADE)(H_2O)_2]^+$ and $[M(IPAA)(ADE)(H_2O)_2]^+$ have been synthesized. All complexes were characterized on the basis of elemental analysis, molar conductance, magnetic susceptibility measurements, IR, UV-Visible, NMR, ESR and Mass spectral studies. IR spectra of these complexes reveal that the complex formation occurred through both nitrogen and oxygen atoms. On the basis of electronic spectral data and magnetic susceptibility measurement, octahedral geometry has been proposed for the complexes. The ESR spectral data of the M (III) complexes showed that the metal-

ligand bonds have considerable covalent character. The electrochemical behaviour of mixed Ligand M(III) complexes was studied which showed that complexes of IPAA appear at more positive potential as compared to those for corresponding AA complexes. In addition, biological activity of the synthesised metal complexes against *Salmonella typhi*, *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae* bacteria and *Aspergillus niger, Aspergillus flavus, Fusarium oxisporium* and *Trichoderma viride* fungi respectively were examined *in-vitro*. Some of the metal complex displayed pounced biological activity.

### 3.1. Effect of Solvents on the Electronic Spectra of the Adenine

The solvent effects on the electronic absorption spectra are used to study the chemical properties of the excited state and to identify the electronic transitions in a molecule. The solvent polarity tends to move the absorption maximum towards lower energy, due to the stabilization of the excited state by the induced dipole interaction between the transition moment and the solvent molecule. Also, the frequency shift of the spectra bands from the vapour state to solution could be related to salvation stabilization energy of the excited and ground states depending on the various types of intermolecular interaction. In polar or hydrogen bonding solvents with permanent dipole moment for polar solute, blue shift occurs of λ max with increasing solvent polarity with the presence of "frank Condon" phenomena. If the excited state-dipole moment is less than that of the ground state, blue shift of λmax occurs with increasing solvent polarity. This explains the shift of n-π* transition on hydrogen bonding, relative to hydrocarbon solvents. In general, the functional groups with high bond moments are involved in H-bond formation. The non-polar solute in non-polar solvents leading approximate equal salvation energies of both ground and excited state is due to dispersion depending mainly the solvent refractive index, similar situation was found for the non-polar solute in polar solvents but with more H-bonding molecule with the increase of solvent cage molecules, the behaviour of the polar solute in the non polar or polar solvent was found to depend on the dipole moment of the solute (decreased or increased during excitation). The process of the reaction in the first case leads to a blue shift of the maximum absorption and in the second case a red shift occurs. It is expected that in the presence of polar solvents, accumulation of H-bonding forces occurs depending on the magnitude of the charge in dipole moment during the electronic transitions, the solvent dipole moment value, and the size of solvent and solute. Many empirical single-solvent polarity parameters have been introduced and have had varying degrees of success correlating solvent dependent data. Electronic spectra of Adenine ligand in presence of Ethanol solvent as an example, is illustrated in Fig. 2. Adenine in Ethanol, and DMF gave bands at 205 nm this bands is due to n-π* electronic transition, this band is blue shifted in presence of water to be at 202 nm, and appear strongly red shifted to be at 256, 218 nm in presence DMSO, and Methanol respectively.

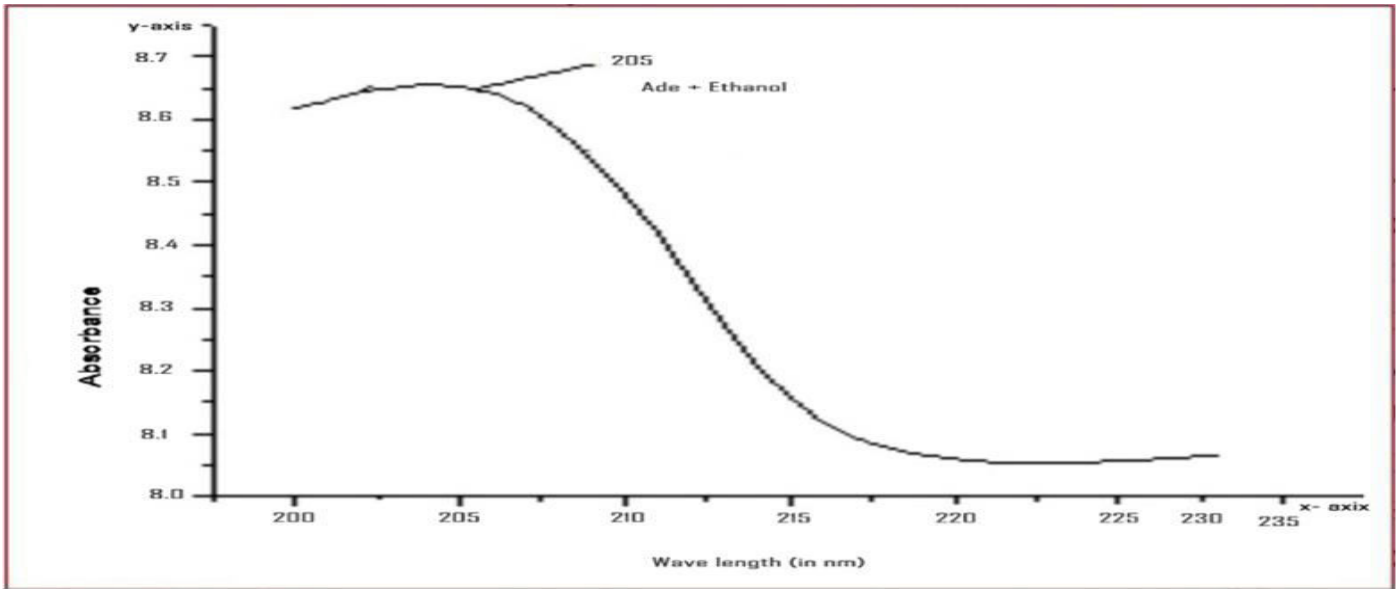


**Fig. 2: Typical electronic spectra of Adenine ligand in presence of Ethanol solvents**

### 3.2. Molar Absorbance

A series of metal-ligand aqueous solutions were prepared with different [L]/[M] ratios. The absorptions of these solutions were measured using UV spectrophotometer at λ max of the expected complex MLx. It was observed that the absorption increases linearly as the ligand concentration increase, because of the formation of the complex, until the solution reaches the actual molar ratio of the investigated complex. At this point, all of the added materials were completely reacted, and the absorbance observed is the absorption of the investigated complex alone. After this point, the excess amount of the added ligand causes an inflection in the straight line, that is because the ligand has an absorbance value differ from that of the complex at λ max of the complex. [L]/[M] ratio corresponding to the inflection point in (ABS-[L]/[M] curve) indicates to the actual [L]/[M] ratio of the investigated complex, referring to the Fig. 3 and Fig. 4, The data showed that of the studied complexes in this investigation are able to be stable in the form ML, and $ML_2$ were geometric isomerism.

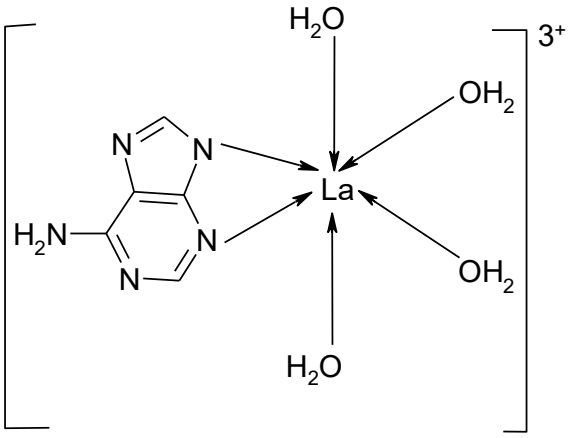


**Fig. 3: Typical (ABS-[L]/[M] curve) indicates to the actual [L]/[M] ratio of metal complex**

### 3.3. Characterization of compounds

#### *3.3.1. $[La(ADE)(H_2O)_4]^{3+}$ (2a)*

UV-Visible ( λ Maximum in nm) 272 (in DMSO); IR (In $cm^{-1}$): 3413-3391, 840-865 ( O-H in coordinated water), 3315-3216 (N-Hstr. in -$NH_2$), 2980, 1667, 869 (NH), 2910, 1504, 1447(-C=C), 457-426 ( M-N Bond) ; $^1$HNMR: δ 7.86 ppm (s, N-H), δ 7.08 ppm (s,-$NH_2$), δ 4.60 ppm (s, for $H_2O$), δ 7.56, 8.86 ppm (s, for-C-H); $^{13}$CNMR: δ 156.36, 153.42, 151.71, 140.30, 119.07 ($C_1$-$C_5$, Ade ); Mass (FAB) :346 ($M^+$), 310, 274, 210, 166, 136 (bp), 108, 81,80, 65, 54, 28 (fp).

#### *3.3.2. $[Ce(ADE)(H_2O)_4]^{3+}$ (2b)*

UV-Visible ( λ Maximum in nm) 269 (in DMSO), IR (In $cm^{-1}$): 3415-3393, 841-864 (O-H in coordinated water), 3317-3219 (N-Hstr. in –$NH_2$), 2982, 1669, 868 (NH), 2908, 1505, 1448(-C=C), 459-425 (M-N Bond ); $^1$HNMR: δ 7.87 ppm (s, N-H), δ 7.09 ppm (s,-$NH_2$), δ 4.62 ppm (s, for $H_2O$), δ 7.58, 8.88 ppm (s, for-C-H); $^{13}$CNMR: δ 156.41, 153.43, 151.73, 140.28, 119.09 ($C_1$-$C_5$, Ade ); Mass (FAB) :347 ($M^+$), 311, 275, 211, 167, 136 (bp), 108, 81,80, 65, 54, 28 (fp).

#### *3.3.3. $[Nd(ADE)(H_2O)_4]^{3+}$ (2c)*

UV-Visible ( λ Maximum in nm) 256 (in DMSO), IR (In $cm^{-1}$): 3418-3395, 842-865 ( O-H in coordinated water), 3318-3220, 1668, 918 (N-Hvib. in -$NH_2$), 2982, 1669, 868 (-NH), 2909, 1507, 1449(-C=C), 461-428 ( M-N Bond ); $^1$HNMR: δ 7.89 ppm (s, N-H), δ 7.07 ppm (s,-$NH_2$), δ 4.64 ppm (s, for $H_2O$), δ 7.59, 8.89 ppm (s, for -C-H) ; $^{13}$CNMR: δ 156.39, 153.38, 151.67, 140.28, 119.11 ($C_1$-$C_5$, Ade ); Mass (FAB) :351 ($M^+$), 315, 281, 215, 171,140 (bp), 108, 81,80, 65, 54, 28 (fp).

#### *3.3.4. $[Gd(ADE)(H_2O)_4]^{3+}$ (2d)*

UV-Visible ( λ Maximum in nm) 271 (in DMSO), IR (In $cm^{-1}$): 3419-3396, 844-866 (O-H in coordinated water), 3319-3222, 1679, 919 (N-Hvib. in -$NH_2$), 2983, 1670, 869 (-NH), 2911, 1508, 1450 (-C=C), 463-429 ( M-N Bond ); $^1$HNMR: δ 7.90 ppm (s, N-H), δ 7.09 ppm (s,-$NH_2$), δ 4.67 ppm (s, for $H_2O$), δ 7.60, 8.90 ppm (s, for –C-H) ; $^{13}$CNMR: δ 156.35, 153.43, 151.75, 140.35, 119.11 ($C_1$-$C_5$, Ade ); Mass (FAB) :364 ($M^+$), 328, 292, 184, 153 (bp), 108, 81,80, 65, 54, 28 (fp).

#### *3.3.5. $[La(ADE)(AA)(H_2O)_2]^{3+}$ (3a)*

**UV-**Visible ( λ maximum in nm) 262 (in DMSO); IR (In $cm^{-1}$): 3412, 3317,3392, 3220(-OHstr.), 3010, 2913 (-C-H), 1732 (-C=O), 1666, 1506, 1448 (-C=C-) , 1395 (C-O), 1254, 916 (C=N), 3339, 3220 (–$NH_2$), 2986 (-N-H), 457-426 ( M-N Bond ), 422-408 (M-O); $^1$HNMR: 4.86 (s, -OH), δ 3.45, 3.72, 4.72 ppm (m, $H_4$, $H_5$, $H_6$), δ 7.87 (s, N-H), δ 7.10 ppm (s,-$NH_2$), δ 4.6 ppm (s, for $H_2O$), δ 7.56, 8.86 ppm (s, for -C-H); δ $^{13}$CNMR: δ 156.33, 153.46, 151.68, 140.36, 119.10, ($C_1$ to $C_5$ Ade), 174.04, 118.68, 156.22, 77.03, 69.76, 62.93 ($C_1$ to $C_6$ AA); Mass (FAB): 484($M^+$), 448, 417, 386, 278, 232, 217, 161(bp), 116, 101, 85, 81, 80, 71, 65, 54, 28.

### 3.3.6. $[Ce(ADE)(AA)(H_2O)_2]^{3+}$ (3b)

**UV-**Visible ( λ maximum in nm) 268 (in DMSO); IR (In $cm^{-1}$): 3415, 3316, 3394, 3222 (–OHstr.), 3013, 2916 (-C-H), 1733 (-C=O), 1673, 1506, 1448 (-C=C-), 1397 (C-O), 1257,919 (C=N), 3339, 3220 (-$NH_2$), 2986 (-N-H), 459-428 ( M-N Bond ), 425-410 (M-O); $^1$HNMR: 4.88 (s, -OH), δ 3.47, 3.74, 4.73 ppm (m, $H_4$, $H_5$, $H_6$), δ 7.89 ppm (s, N-H), δ 7.12 ppm (s,-$NH_2$), δ 4.60 ppm (s, for $H_2O$), δ 7.58, 8.88 ppm (s, for -C-H); δ $^{13}$CNMR: δ 156.36, 153.42, 151.74, 140.30, 119.08, ($C_1$ to $C_5$ Ade), 174.03, 118.67, 156.26, 77.08, 69.75, 62.91 ($C_1$ to $C_6$, AA); Mass (FAB): 485($M^+$), 449, 418, 417, 387, 279, 233, 218, 137 (bp), 116, 101, 85, 81, 80, 71, 65, 54, 28.

### 3.3.7. $[Nd(ADE)(AA)(H_2O)_2]^{3+}$ (3c)

**UV-**Visible ( λ maximum in nm) 255 (in DMSO); IR (In $cm^{-1}$): 3416, 3321,3396, 3224(-OHstr.), 3014, 2917, (-C-H), 1736 (-C=O), 1674, 1509, 1453 (-C=C-), 1399 (C-O), 1258, 919 (C=N), 3343, 924 (-$NH_2$), 2982, 793 (-N-H), 461-430 ( M-N Bond ), 426-412 (M-O); $^1$HNMR: 4.89 (s, -OH), δ 3.45, 3.72, 4.78 ppm (m, $H_4$, $H_5$, $H_6$), δ 7.91 (s, N-H), δ 7.14 ppm (s,-$NH_2$), δ 4.62 ppm (s, for $H_2O$), δ 7.60, 8.90 ppm (s, for –C-H); δ $^{13}$CNMR: δ 156.31, 153.46, 151.73, 140.27, 119.15, ($C_1$ to $C_5$ Ade), 174.06, 118.62, 156.26, 77.05, 69.74, 62.96 ($C_1$ to $C_6$, AA); Mass (FAB): 489($M^+$), 453, 422, 371, 282, 237, 222, 166, 136(bp), 116, 101, 85, 81, 80, 71, 65, 54, 28.

### 3.3.8. $[Ce(ADE)(AA)(H_2O)_2]^{3+}$ (3d)

**UV-**Visible ( λ maximum in nm) 272 (in DMSO); IR (In $cm^{-1}$): 3418, 3323,3398, 3225(–OHstr.), 3017, 2919, (-C-H), 1738 (-C=O), 1676, 1510, 1456 (-C=C-), 1401 (C-O), 1259, 920 (C=N), 3346, 925(-$NH_2$), 2984, 794 (-N-H), 464-432 ( M-N Bond ), 428-413 (M-O); $^1$HNMR: 4.91 (s, -OH), δ 3.47, 3.79, 4.79 ppm (m, $H_4$, $H_5$, $H_6$), δ 7.93 (s, N-H), δ 7.16 ppm (s,-$NH_2$), δ 4.64 ppm (s, for $H_2O$), δ 7.61, 8.92 ppm (s, for-C-H); $^{13}$CNMR: δ 156.35, 153.46, 151.74, 140.35, 119.08, ($C_1$ to $C_5$ Ade), 174.11, 118.69, 156.25, 77.05, 69.73, 62.92 ($C_1$ to $C_6$, AA); Mass (FAB): 502($M^+$), 466, 435, 404, 342, 296, 281, 199, 180, 165, 136 (bp), 116, 101, 85, 81, 80, 71, 65, 54, 28.

### 3.3.9. $[La(ADE)(IPAA)(H_2O)_2]^{+}$ (4a)

UV-Visible ( λ Maximum in nm) 272 (in DMSO) ; IR ( in $cm^{-1}$): 3423-3398, 842-869 ( O-$H_{str}$. in coordinated water), 3242 (–OH), 2910 (-C-H), 1769 (-C=O), 1669, 1506, 1448 (-C=C-) 1436 (-C-H ), 1398 (C-O), 3334, 929 (–$NH_2$), 2996, 878 (-NH), 3027, 3178,1420 (-HC-$CH_2$), 1669, 1506, 1448 (-C=C-), 1254, 1160, 916 (C=N), 452-421 ( M-N Bond ); $^1$HNMR: δ 1.73 ppm (s, 6H,-$CH_3$ ), δ 3.42, 3.77, 4.66 ppm (m, $H_4$, $H_5$, $H_6$), δ 3.1 ppm (s, for the -OH) δ 12.71 ppm (s, N-H), δ 7.02 ppm (s,-$NH_2$), δ 4.62 ppm (s, $H_2O$), δ 7.52, 8.81 ppm (s, for -CH); $^{13}$CNMR: δ 170.11, 150.26, 118.06, 108.88, 74.12, 73.31, 64.73, 25.70, 25.30 ($C_1$ to $C_9$ IPAA), 157.11, 154.42, 151.74, 146.11, 119.16 ($C_1$ to $C_5$, Ade); Mass (FAB): 524($M^+$), 488, 460, 402, 344, 320, 264, 185 (bp), 113, 102, 87, 82, 72, 65, 54, 28.

### 3.3.10. $[Ce(ADE)(IPAA)(H_2O)_2]^{+}$ (4b)

UV-Visible ( λ Maximum in nm) 274 (in DMSO) ; IR ( in $cm^{-1}$): 3425-3399, 844-870 (O-$H_{str}$. in coordinated water), 3242, 866 (–OH), 2910 (-C-H), 1761 (-C=O), 1669, 1506, 1448 (-C=C-) 1436 (-C-H ), 1398 (C-O), 3334, 929 (–$NH_2$), 2998, 879 (-NH), 3027, 3180, 1423 (-HC-$CH_2$), 1672, 1507, 1449 (-C=C-), 1255, 1162, 917 (C=N), 462-428 ( M-N Bond ), 424-409 (M-O); $^1$HNMR: δ 1.78 ppm (s, 6H,-$CH_3$ ), δ 3.42, 3.77, 4.66 ppm (m, $H_4$, $H_5$, $H_6$), δ 7.89 ppm (s, N-H), δ 7.15ppm (s,-$NH_2$), δ 4.64 ppm (s, $H_2O$), δ 7.55, 8.84 ppm (s, for –CH); $^{13}$CNMR: δ 170.16, 150.24, 118.03, 108.87, 74.14, 73.32, 64.76, 25.73, 25.31 ($C_1$ to $C_9$ IPAA), 157.12, 154.46, 151.74, 146.12, 119.18 ($C_1$ to $C_5$, Ade); Mass (FAB): 525($M^+$), 489, 461, 403, 345, 321, 265, 185(bp), 113, 102, 87, 82, 72, 65, 54, 28.

### 3.3.11. $[Nd(ADE)(IPAA)(H_2O)_2]^{+}$(4c)

UV-Visible ( λ Maximum in nm) 274 (in DMSO) ; IR ( in $cm^{-1}$): 3425, 3399, 844-870 (O-$H_{str}$. in coordinated water), 3180, 1423 (-C-H), 1771 (-C=O), 1672, 1507, 1449 (-C=C-), 1401 (C-O), 3337, 931 (–$NH_2$), 2998, 879 (-NH), 3027, 3178, 1420 (-HC-$CH_2$), 1672, 1507, 1449 (-C=C-), 1255, 1162, 917 (C=N), 462-428 ( M-N Bond ), 424-409 (M-O) ; $^1$HNMR: δ 1.79 ppm (s, 6H,-$CH_3$ ), δ 3.44, 3.74, 4.68, ppm (m, $H_4$, $H_5$, $H_6$), δ 7.90 ppm (s, N-H), δ 7.18 ppm (s,-$NH_2$), δ 4.64 ppm (s, $H_2O$), δ 7057, 8.86 ppm (s, for -CH); $^{13}$CNMR: δ 170.11, 150.26, 118.06, 108.88, 74.12, 73.31, 64.73, 25.70, 25.30 ($C_1$ to $C_9$ IPAA), 157.11, 154.42, 151.74, 146.11, 119.16 ($C_1$ to $C_5$, Ade); Mass (FAB): 529($M^+$), 493, 465, 407, 349, 323, 269, 189 (bp), 117, 106, 91, 86, 78, 65, 54, 28.

### *3.3.12. $[Gd(ADE)(IPAA)(H_2O)_2]^+$ (4d)*

UV-Visible ( λ Maximum in nm) 277 (in DMSO) ; IR ( in $cm^{-1}$): 3428-3403, 844-873 ( O-$H_{str}$. in coordinated water), 1775 (-C=O), 1670, 1508, 1452 (-C=C-) 3186, 1425 (-C-H ), 1405 (C-O), 3340, 935 (–$NH_2$), 3002, 882 (-NH), 3178, 1425 (-HC-$CH_2$), 1670, 1508, 1452 (-C=C-), 1259, 1164, 923 (C=N), 465-429 ( M-N Bond ), 428-410 (M-O) ; $^1$HNMR: δ 1.81 ppm (s, 6H,-$CH_3$ ), δ 3.46, 3.76, .67 ppm (m, $H_4$, $H_5$, $H_6$), δ 7.92 ppm (s, N-H), δ 7.20 ppm (s,-$NH_2$), δ 4.67 ppm (s, $H_2O$), δ 7.55, 8.84 ppm (s, for -CH); $^{13}$CNMR: δ 170.12, 150.26, 118.06, 108.88, 74.12, 73.31, 64.73, 25.70, 25.30 ($C_1$ to $C_9$ IPAA), 157.15, 154.42, 151.74, 146.11, 119.16 ($C_1$ to $C_5$, Ade); Mass (FAB): 542 ($M^+$), 506, 478, 442, 384, 360, 304, 264, 184 (bp), 112, 101, 84, 81, 71, 65, 54, 28.

### 3.4. Antimicrobial Activity

The antimicrobial activities of the all prepared complexes were tested selected strain of bacteria and fungi as in **table 5**. The antibacterial tests were prepared and characterized according to the standard method. A bacterial suspension was prepared and added to the Nutrient Agar, the fungi added to surrounded Agar, All this before medium solidification and under aseptic condition.

**Table 5**: **Anti-bacterial activity of synthesised compounds**

| Compound code | *Salmonella typhi* | | *Bucillus Subsniss* | | *Staphylococcus aureus* | | *Klebsiella pneumoniae* | |
|---|---|---|---|---|---|---|---|---|
| | 50 ppm | 100 ppm | 50 ppm | 100 ppm | 50 ppm | 100 ppm | 50 ppm | 100 ppm |
| 2a | ++ | +++ | ++ | +++ | ++ | +++ | ++ | +++ |
| 2b | ++ | +++ | ++ | ++ | ++ | ++ | ++ | +++ |
| 2c | + | ++ | + | ++ | ++ | ++ | ++ | ++ |
| 2d | ++ | + | ++ | ++ | + | ++ | + | ++ |
| 3a | ++ | +++ | ++ | +++ | ++ | +++ | ++ | +++ |
| 3b | ++ | +++ | ++ | ++ | ++ | ++ | ++ | +++ |
| 3c | + | ++ | + | ++ | ++ | ++ | ++ | ++ |
| 3d | + | + | ++ | ++ | + | ++ | + | ++ |
| 4a | ++ | +++ | ++ | +++ | ++ | +++ | ++ | +++ |
| 4b | ++ | +++ | ++ | ++ | ++ | ++ | ++ | +++ |
| 4c | + | ++ | + | ++ | ++ | ++ | ++ | ++ |
| 4d | + | ++ | + | ++ | + | + | + | + |
| SM | +++ | ++++ | +++ | ++++ | +++ | ++++ | +++ | ++++ |

*[SM = streptomycin inhibition diameter in mm ,Highly active = +++ (inhibition zoes > 15), moderatively active= ++ (inhibition zone 10-15), slightly active = + (inhibition 10), inactive inhibition zone -6) for bacteria]*

**Table 6**: **Antifungal activity of the synthesized compounds**

| Compound code | *Aspergillus niger* | | *Aspergillus flavus* | | *Fusarium oxisporium* | | *Trichoderma viride* | |
|---|---|---|---|---|---|---|---|---|
| | 50 ppm | 100 ppm | 50 ppm | 100 ppm | 50 ppm | 100 ppm | 50 ppm | 100 ppm |
| 2a | ++ | ++ | ++ | +++ | ++ | +++ | ++ | +++ |
| 2b | + | ++ | + | ++ | + | ++ | ++ | +++ |
| 2c | ++ | +++ | + | ++ | + | ++ | ++ | +++ |
| 2d | ++ | ++ | + | ++ | + | ++ | + | +++ |
| 3a | + | ++ | + | ++ | + | ++ | + | +++ |
| 3b | + | ++ | + | ++ | + | ++ | + | +++ |
| 3c | ++ | +++ | ++ | +++ | ++ | +++ | ++ | +++ |
| 3d | ++ | +++ | ++ | ++ | ++ | ++ | ++ | +++ |
| 4a | + | ++ | + | ++ | ++ | ++ | ++ | ++ |
| 4b | + | ++ | + | + | + | ++ | + | ++ |
| 4c | ++ | +++ | ++ | +++ | ++ | +++ | ++ | +++ |
| 4d | ++ | ++ | + | ++ | ++ | +++ | ++ | ++ |
| GF | +++ | ++++ | +++ | +++ | +++ | ++++ | +++ | ++++ |

*[Std- Griseofulvin inhibition diameter in mm, Highly active = ++++ (inhibition zone > 20-25), More Active =+++ (inhibition zone > 12-20), Moderatively active= ++ (inhibition zone 6-12), slightly active = + (inhibition Zone less than 6), Inactive inhibition zone - for Fungi]*

Then different concentration of complexes were placed on the surface of the culture, The bacteria incubated at 37°C for 24 hours, The fungi incubated at 28°C for 72 h. Plate cultures of nutrient agar medium were used for culture of bacteria. The medium was prepared by dissolving 14 g, and culture of fungi's 32.5 g of powder in 500 mL of sterile distilled water, Then the medium was sterilized by autoclaving at 121°C for15 min. After incubation, the inhibition zones were recorded in mm. Diameter less than 10 mm indicates no effect.

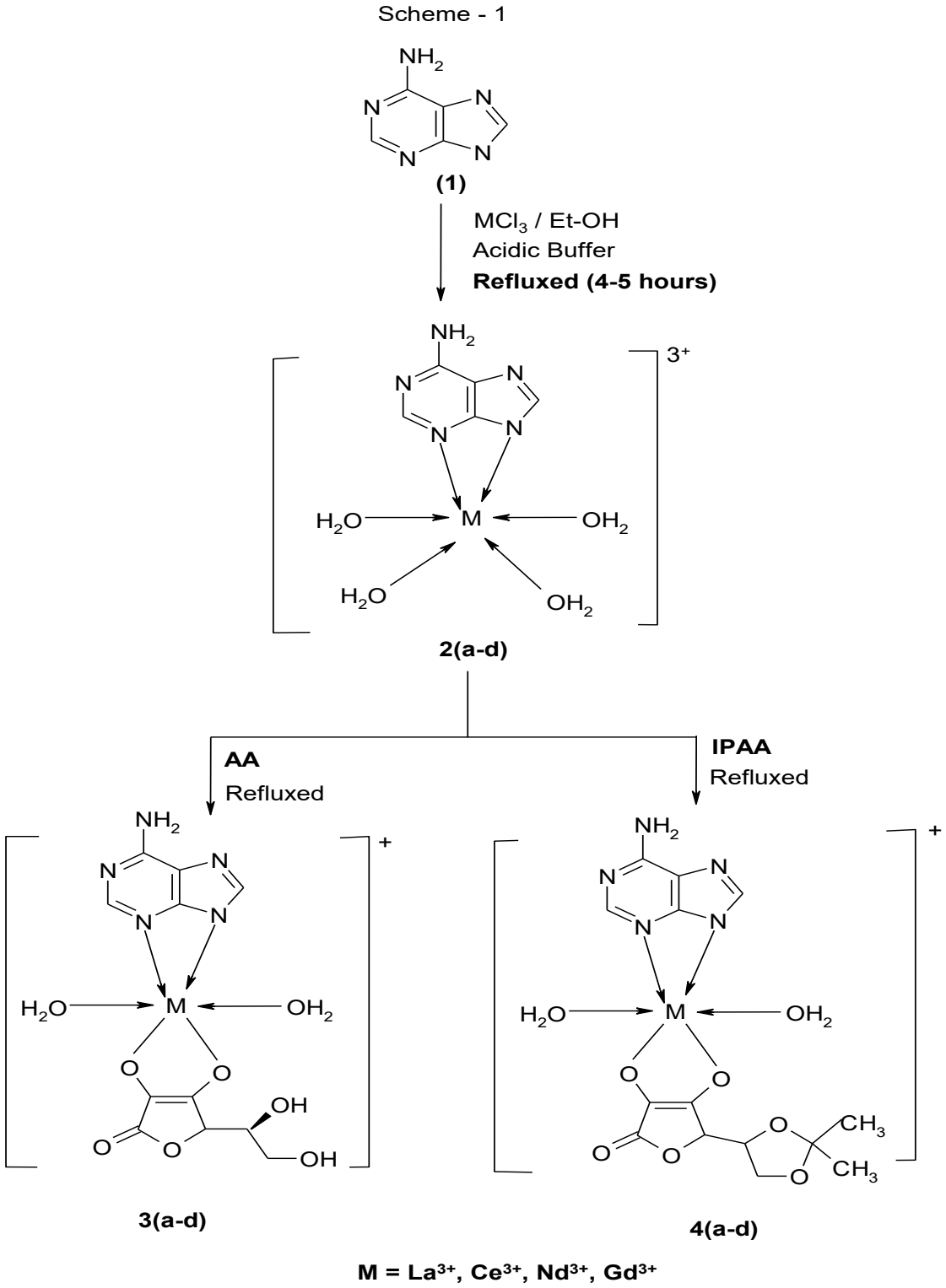


**Fig. 4: The Fig. showed that of the studied complexes ML, and $ML_2$ geometric isomerism.**

## 4. CONCLUSION

From the analysis of UV Visible, IR, NMR and mass studies and other results obtained in this work it was suggested that the coordination pattern of the ligands to the metal centre occurs in the second coordination sphere of complex. Based on these facts the following general proposed structure is assigned to metal complexes. The synthesised compounds will be assayed for their biological activities viz antimicrobial, anti-inflammatory, analgesic, anticonvulsant, Cardiovascular etc. using standard methods as given in the literature. As we know that many heterocycles and their derivatives show remarkable biological activities used in pharmaceutical and agro-based industries. Hence I fell that the proposed work after successful completion may have a contribution for the nation especially in drug chemistry and agriculture field.

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### *Conflict of interest*

The authors declare no Conflict of interest

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