

Biocoordination and computational modeling of novel ligands with Bi (V)

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Abstract : Novel ligands 12-((1R)-2, 2-dichloro-1-(1, 3-dihydroxy-1-(4-nitrophenyl)propan-2-ylamino)-1-hydroxyethoxy)-3, 6, 10, 12a-tetrahydroxy-4-isopropyl-6-methyl-1,11-dioxo-1, 2, 5a, 6, 11, 11a, 12, 12a-octahydrotetracene-2-carboxamide and 1-(4-(2,2-dichloro-1-(1,3-dihydroxy-1-(4-nitrophenyl)propan-2-ylamino)-1-hydroxyethoxy)-2-(4-(3,5-dihydroxy-6-(hydroxymethyl)-4(methylamino) - tetrahydro-2H-pyran-2-yloxy)-5-formyl-5-hydroxymethyltetrahydrofuran-3-yloxy)-3,-dihydroxy-5-(methylamino)cyclohexyl)guanidine have been synthesized by the reaction of chloramphenicol, tetracycline and streptomycin in an equimolar ratio in aqueous methanolic solution. Ligands and their complexes have been characterized by elemental analysis, spectral UV, IR ¹HNMR and XRPD studies. The optimized structure of the both complexes has trigonalbipyramidal. the complexes having orthorhombic crystal system.

Keywords: Antibiotics, tetracycline, chloramphenicol, XRPD, streptomycin, molecular modeling

Introduction

Streptomycin, tetracycline and chloramphenicol are member of antibiotics. They have potential donor atoms to coordinate metal ions. One of the Streptomycin having chemical name 1-(4-(4-(3,5-dihydroxy-6-(hydroxymethyl)-4-(methylamino)tetrahydro-2H-pyran-2-yloxy)-5-formyl-5-hydroxy-3-methyltetrahydrofuran-2-yloxy)-2,5,6-trihydroxycyclohexane-1,3-diyl)diguanidine is an aminoglycosidic antibiotic with three components: streptidine, streptose and N-methyl-L-glucosamine (Fig1). It is used to treat infections caused by Gram-negative bacteria and in the therapy of tuberculosis [1-4]. Serious toxicity is a major limitation of its usefulness, most notable is its ototoxicity, causing deafness in severe cases. It has been proposed that the mechanism of action of this antibiotic could be related to an enhancement of the biological availability of magnesium and to a reduction of calcium [5-6]. Some preliminary studies about the interaction in solution of some metal ions with streptomycin have been reported [7-9], but detailed characterization of the complexes are unavailable except for a neutral Cu(II) complex where Cu-O bonds with streptomycin are suggested [10-11]. Much of the structural effort has focused on aminoglycosides bound either to modular constructs of the ribosomal RNA decoding site in solution [12]. Furthermore, literature on streptomycin as a ligand reveals that there is clear disagreement about the metal – ligand binding sites in complexes of this ligand [13]. Therefore, in order to ascertain metal binding sites in streptomycin metal chelates, the synthesis and

spectroscopic characterization of complexes of streptomycin with these transition/non-transition metal ions is attempted in this investigation. The structure of streptomycin is given in Figure.1. Aminoglycosides kill bacteria primarily by inhibiting the translation step in microbial protein synthesis [14]. A major problem in therapy with aminoglycosides is their relatively high toxicity to the kidney and the inner ear. Nevertheless, streptomycin is currently the first choice antibiotic in developing countries and is still widely used in industrialized countries for the treatment of serious bacterial infections as tuberculosis. The adverse effects of aminoglycosides may result from complex formation with transition metal ions and the oxidative reactions the complexes subsequently promote. [15] co-administration of transition metal chelators and free radical scavengers, as well as over-expression of superoxide dismutase in model animals, suppress aminoglycoside-induced ototoxicity [16]. Systematic *in vitro* studies of iron interactions with streptomycin have led to a postulated mechanism of toxicity involving free radical formation by Fe(II)/Fe(III)-streptomycin complexes. Another body of evidence [17] has suggested that both pharmacological activity and toxicity of aminoglycoside antibiotics could be related to copper(II)-aminoglycosides complexes. Jezowska-Bojczuk et al. extensively investigated chelation of copper(II) ions by streptomycin-related aminoglycoside antibiotics using potentiometry and a variety of spectroscopic techniques. [18] Kanamycin B, tobramycin, gentamicin, and amikacin strongly bind

Cu(II) ions, forming monomeric complexes over a wide pH range. In naturally occurring aminoglycosides, the amino nitrogens and deprotonated alcoholic oxygens of the terminal aminosugar rings are involved in the coordination, forming five- and six-membered chelate rings about central ions[19]. Amikacin, a semisynthetic derivative of kanamycin A, having the 1-amino group on the 2-deoxystreptamine moiety modified by acylation with 4-amino-2-hydroxybutyric acid, exhibits different binding modes by involving the amidated nitrogen in coordination[20]. Further, Cu(II)-amikacin complexes catalyze hydrogen peroxide disproportionate at pH 7.4 mediated by hydroxyl radicals and involving Cu(I)/Cu(II) and Cu(II)/Cu(III) redox pairs.[21] These complexes mediate oxidation of 2'-deoxyguanosine to 7,8-dihydro-8-oxo-2'-deoxyguanosine, double-stranded DNA cleavage, and both hydrolytic and oxidative t-RNA^{Phe} strand scission at a specific site in the anticodon loop.[22] Under these circumstances, copper(II) ions are proposed to be involved in aminoglycoside toxicity. Therefore, in order to ascertain metal binding sites in streptomycin metal chelates, the synthesis and spectroscopic characterization antitumor activities of complexes of streptomycin (Figure 1) with this transition/main group metal ions provided in this investigation.

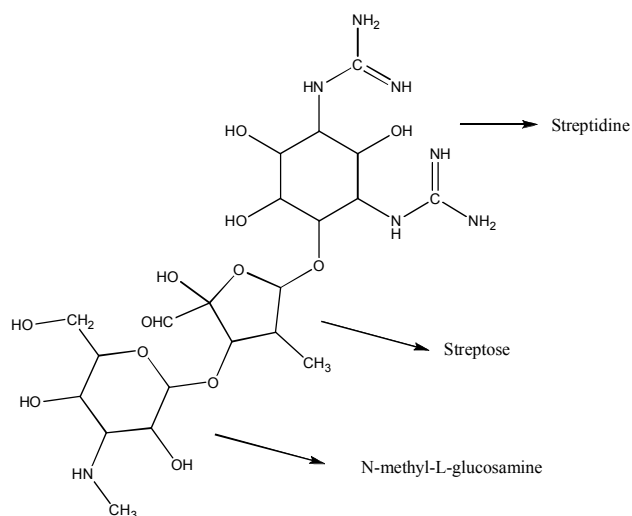


Figure1 (a)

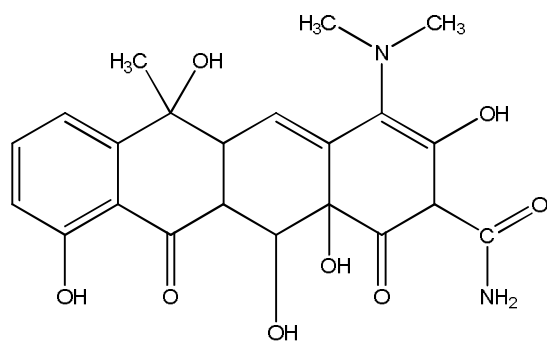


Figure1 (b).Tetracycline

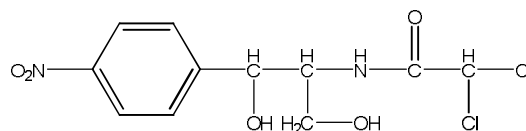


Figure 1(c). Chloramphenicol

Meanwhile tetracycline are broad- spectrum agents with activity against both Gram-positive and Gram – negative bacteria, chlamydia, mycoplasmas, rickettsia and protozoan parasites. Tetracycline having chemical name(4S,6S,12aS)-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydro-tetracene-2-carboxamidewhich in fact, allied to the low toxicity of tetracycline, their relatively low cost, and their oral administration led to their indiscriminate use and consequently to the appearance of bacterial resistance to these agents [23]. Besides the pharmacological importance of tetracycline (TC), this molecule possesses a very interesting structure with many potential metal – binding sites: oxygens at the C₁₀ –C₁₂ phenolic β- diketone system, the oxygen at C3 and the nitrogen at C4 and at the carboxamide group in ring A (figure.1b). Pharmacokinetics and bioavailability of TC is affected by metal coordination. In blood plasma, the drug is transported as calcium complexes [24]. In the intracellular medium, magnesium complexes seem to be more important as they probably play a role in binding to ribosome [25]. The presence of several metal coordination sites associated to different conformational states makes the assignment of bonding sites a difficult task. The main mechanism of TC action is the inhibition of protein synthesis. The strong binding of tetracycline to the bacterial 30S ribosomal subunit leads to the inhibition of protein synthesis by causing the rupture of codon – anticodon interactions between t-RNA and m-RNA and consequently the interruption of the bond between the aminoacyl-t-RNA and the ribosomal acceptor site [26]. Magnesium seems to play an important role in the binding to ribosome, because binding is significantly reduced by magnesium depletion. The actual pharmaceutical mechanism of TC and its derivatives has not been definitely established. However, it appears to be linked to the ability of the molecule to form complexes with a large variety of metal ions [27]. The question of which specific group of TC uses to bind the metal has not been completely affirmed, and therefore the behaviour of metal ion coordination by TC and its analogs has received appreciable attention [28]. Recently, it has been found that tetracycline give new hopes for ailing heart attack [29] and ulcer[30] patients. A novel single tetracycline-regulative adenoviral vector was investigated for tumor- specific bax gene expression and cell killing in vitro & vivo, which may become a potential therapeutic agent for the treatment of cancer. The TC-inducible gene expression system has become a commonly used approach to experimenter – controlled expression of genes for functional evaluation in mammalian cells[31]. Tetracycline is used to switch gene activity on and off. Through such precise regulation, researchers can

learn more about what specific substances do during different stages of an animal life [32]. It has been investigated TC –dependent gene regulations, and reported that combination of Trans – regulators yield a variety of expressions windows [33]. As same Chloramphenicol (chloromycetin, D-(7)-threo-dicholoacetamide-1-p-nitrophenyl propane-1-1,3-diol, CAP) is an antibiotic that finds applications in combating a wide range of infections caused by Gram-negative and some Gram-positive organisms. It is currently the first choice antibiotic in developing countries and is still widely used in industrialized countries for the treatment of serious bacterial infections [34]. The adverse effect to the eye of chloramphenicol appears to result from the interaction of the drug with metals ions. Chloramphenicol is still widely used in topical preparations (ointment and eye drops) for the treatment of bacterial conjunctivitis. Isolated cases report of aplastic anaemia following chloramphenicol eye drop application, but the risk is estimated to be low [35]. Chloramphenicol is bacteriostatic. It functions by inhibiting ribosomal activity and protein synthesis through prevention of the binding of aminoacyl-tRNA to the A site on the 50S subunits [36]. Chloramphenicol interacts with 50S ribosomal subunits directly and interferes with substrate binding [37]. The adverse affects of chloramphenicol may result from interaction with divalent metals by forming metal complexes.

In view of the rapid development and also challenging demands, it has become necessary to synthesize newer compounds, which may act potential antimicrobial activity. Furthermore, literature on synthesis of mixed ligand streptomycin, tetracycline and chloramphenicol as a ligand reveals that there is clear disagreement about the metal – ligand binding sites in complexes of this ligand. In naturally occurring novel ligands the amino nitrogen and deprotonated hydroxyl oxygen may be involved in the coordination with metal ions. The structure and mode of bonding in complexes of ligands with bismuth ions will be a great help in understanding the antibiotic – metal-ion interactions. Spectral characterization serves as an important tool for the interpretation of structures of the molecules 38. Powder diffraction data are especially useful to deduce accurate cell parameters as well as particle size in the absence of single crystal X-ray data [39] This paper describes the synthesis of novel ligands and complexation ability of the ligands with bismuth metal ions.

2. Experimental

2.1. Material and methodology

Streptomycin, tetracycline hydrochloride and chloramphenicol were purchased from Merck. All the chemicals used in this study were of analytical grade and used as procured. Solvents used were of analytical grade and were purified by standard procedures. The stoichiometric analyses (C, H and N) of the complexes were performed using Elementar vario EL III (Germany)

model. Metal contents were estimated on an AA-640-13 Shimadzu flame atomic absorption spectrophotometer in solution prepared by decomposing the respective complex in hot concentrated HNO₃. Their IR spectra were recorded on Perkins–Elmer FTIR spectrophotometer in KBr and polyethylene pellets. The electronic spectra were recorded in water on Beckman DU-64 spectrophotometer with quartz cells of 1 cm path length. ¹H NMR spectra were recorded in CDCl₃ solvent on a Bruker Advance 400 instrument. Rigaku model 8150 thermoanalyser (Thermafex) was used for simultaneous recording of TG-DTA curves at a heating rate of 10^omin⁻¹. For TG, the instrument was calibrated using calcium oxalate while for DTA, calibration was done using indium metal, both of which were supplied along with the instrument. A flat bed type aluminium crucible was used with α- alumina (99% pure) as the reference material for DTA. The activation energy and Arrhenius constant of the degradation process was obtained by Coats and Redfern method. The XRD powder pattern were recorded on a vertical type Philips 1130/00 x- ray diffractometer, operated at 40kV and 50Ma generator using the CuKα line at 1.54056 Å as the radiation sources. Sample was scanned between 5^o to 70^o(2θ) at 25°C. The crystallographic data was analyzed by using the CRYSFIRE –2000 powder indexing software package and the space group was found by the GSAS program. Debye – Scherer relation with the help of 100% peak width, determined the particle size. The density was determined by Archimedes method.

2.2. 3D - Molecular modeling

3D molecular modeling of the proposed structure of the complexes was performed using CsChem3D program package. The correct stereochemistry was assured through the manipulation and modification of the molecular coordinates to obtain reasonable low energy molecular geometries. The potential energy of the molecule was the sum of the following terms: $E = E_{str} + E_{ang} + E_{tor} + E_{vdw} + E_{oop} + E_{ele}$. Where all E's represent the energy values corresponding to the given types of interaction (kcal/mol). The subscripts str, ang, tor, vdw, oop and ele denote bond stretching, angle bonding, torsion deformation, van der waals interactions, out of plain bending and electronic interaction, respectively.

2.3. Preparation of Bi(V) solution

The solution of Bi(V) was prepared by usual method [16]

2.4 Synthesis of legands

Synthesis of novel ligand, 12-((1R)-2,2-dichloro-1-(1,3-dihydroxy-1-(4-nitrophenyl)propan-2-ylamino)-1-hydroxyethoxy)-3,6,10,12a-tetrahydroxy-4-isopropyl-6-methyl-1,11-dioxo-1,2,5a,6,11,11a,12,12a-ctahydrotetracene-2-carboxamide :The ligands were synthesized by mixing of chloramphenicol(0.5mmol) and tetracycline(0.5 mmol) in a aqueous solution of methanol and again mix chloramphenicol(0.5 mmol) and

streptomycin(0.5 mmol) in a aqueous solution methanol in separate round bottom flask. The reaction mixture was refluxed with stirring for 5h under reduced pressure followed by cooling to room temperature. The product obtained was washed with a small amount of methanol and air dried .The above product was redissolved in excess warm methanol and clear solutoion was left undisturbed for weeks to give beautiful crystals of the ligands separately. The other ligand 1,-(4-((R)-2,2-dichloro-1-((1R,2S)-1,3-dihydroxy-1-(4-nitrophenyl)propan-2-ylamino)-1-hydroxyethoxy)-6-(3-

(3,4-dihydroxy-6-(hydroxymethyl)-5-methylamino)tetrahydro-2H-pyran-2-yloxy)-5-formyl-5-hydroxy-4-methyltetrahydrofuran-2-yloxy)-2,5-dihydroxycyclohexane-1,3- was synthesized by mixing of 0.05(mmol) of chlramphenicol and streptomycin 0.05(mmol) in a round bottom flask adopting a similar method. The legands characterized by different physical techniques. Pertinent analytical and physico-chemical data for these ligands and their complexes are listed in Table 1.

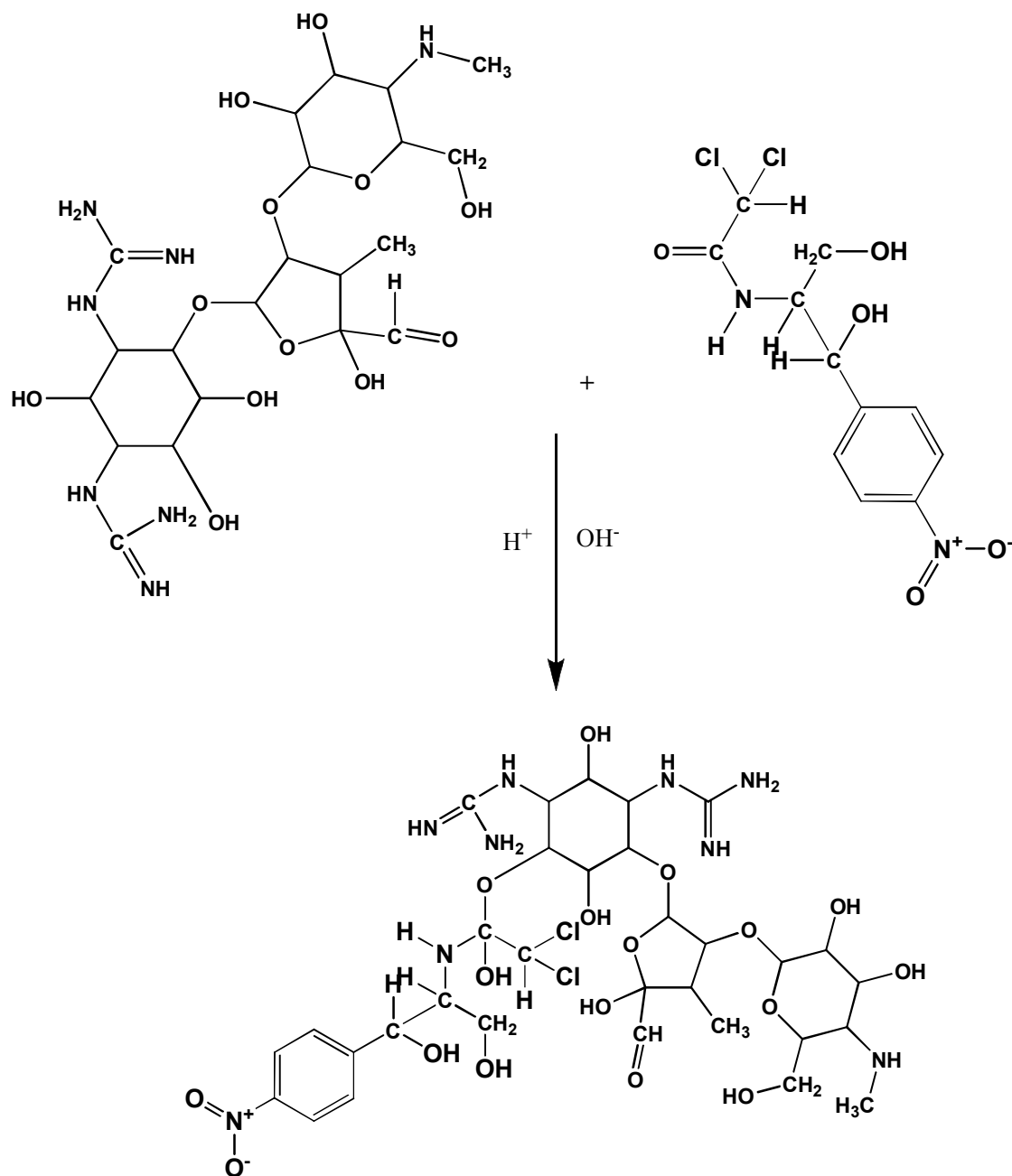


Figure2a.1,-(4-((R)-2,2-dichloro-1-((1R,2S)-1,3-dihydroxy-1-(4-nitrophenyl)propan-2-ylamino)-1-hydroxyethoxy)-6-(3-(3,4-dihydroxy-6-(hydroxymethyl)-5-(methylamino)tetrahydro-2H-pyran-2-yloxy)-5-formyl-5-hydroxy-4-methyltetrahydrofuran-2-yloxy)-2,5-dihydroxycyclohexane-1,3-diyl)diguanidine(L1)

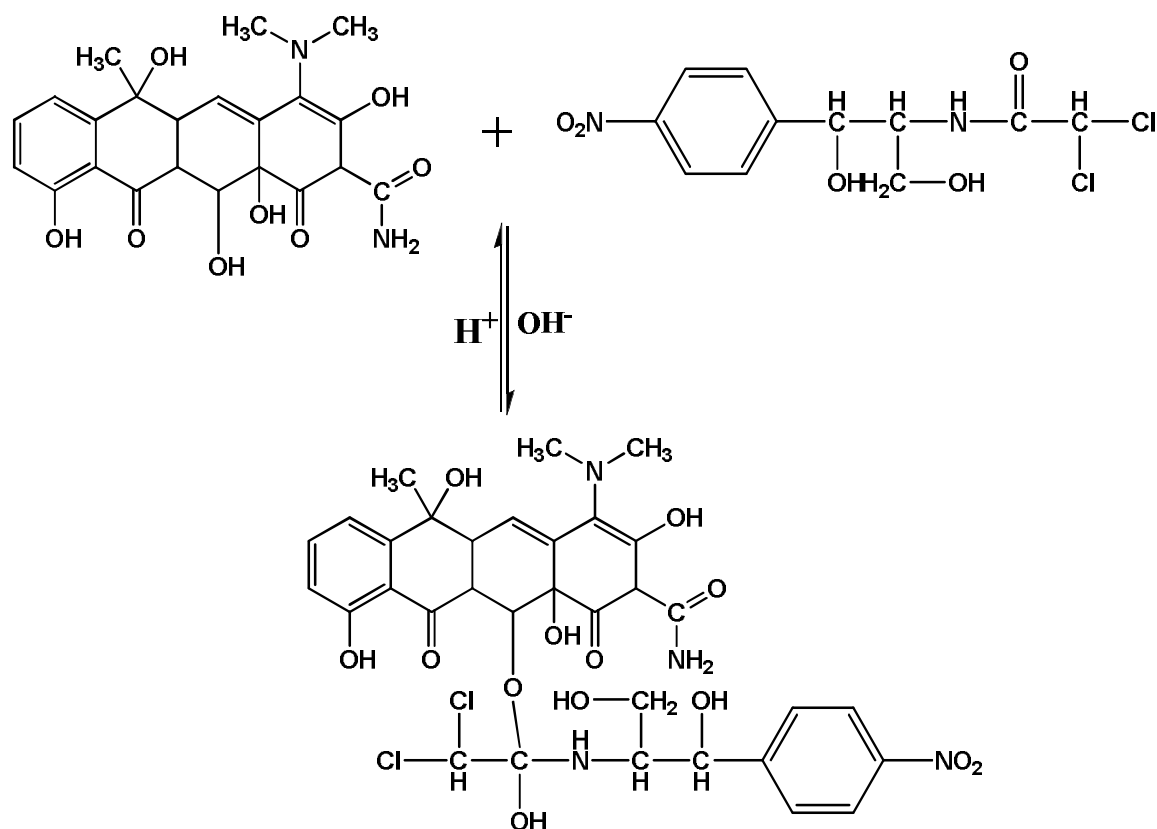


Figure 2b. 12-((1R)-2,2-dichloro-1-(1,3-dihydroxy-1-(4-nitrophenyl)propan-2-ylamino)-1-hydroxyethoxy)-4-(dimethylamino)-3,6,10,12a-tetrahydroxy-6-methyl-1,11-dioxo-1,2,5a,6,11,11a,12,12a-octahydro-tetracene-2-carboxamide(L2)

2.5. Synthesis of bismuth(V) complexes with 12-((1R)-2,2-dichloro-1-(1,3-dihydroxy-1-(4-nitrophenyl)propan-2-ylamino)-1-hydroxyethoxy)-3,6,10,12a tetrahydroxy-4-isopropyl-6-methyl-1,11-dioxo-1,2,5a,6,11,11a,12,12a-ctahydro-tetracene-2-carboxamide and 1,-(4-((R)-2,2-dichloro-1-((1R,2S)-1,3-dihydroxy-1-(4-nitrophenyl)propan-2-ylamino)-1-hydroxyethoxy)-6-(3-(3,4-dihydroxy-6-(hydroxymethyl)-5-(methylamino)tetrahydro-2H-pyran-2-yloxy)-5-formyl-5-hydroxy-4-methyltetrahydrofuran-2-yloxy)-2,5-dihydroxycyclohexane-1,3-diyl)diguandin. To a methanol solution of ligands in separate flask the solution of Bi(V) (0.01mmol) added drop wise and stirred 6h then left for a week to give crystals of the complexes of mixed ligands separately.

2.6. Antibacterial sensitivity assay

Antibacterial sensitivity assay was performed using Ligand1, ligand2 and their complex with bismuth(V) on *Agrobacterium sp* BN-2A. Various concentrations (0, 25,

50, 100, 150 and 200 $\mu\text{g/mL}$) were made and filter sterilized with 45mm sterile filter paper. Antibiotic discs were prepared with Watt's man filter paper to cut by paper punch machine. All paper discs were autoclaved and soaked with filter sterilized antibiotic solutions of different concentrations separately in sterile condition then excess water of solution was dried in oven. Now, antibiotic discs of different concentrations were ready to use. 100 μL aliquot of overnight nutrient broth grown culture (*Agrobacterium sp* BN-2A) was spread over Nutrient agar (NA) solid Petri plate and antibiotic discs were kept gently on the surface. The Petri plates were incubated in BOD incubator at 37°C for growth. Inhibition zone was visualized around antibiotics disc after overnight growth. The diameter of the zone of inhibition and antibiotic discs were recorded. Bacterial inhibition index (BII) was calculated using formula written below:

$$\text{Bacterial Inhibition Index (BII)} = \frac{\text{Diameter of inhibition zone} - \text{Diameter of antibiotic disc}}{\text{Diameter of antibiotic disc}}$$

The experiment was performed in triplicate and repeated three times. Average of all readings and standard deviations were calculated. Statistical calculations (t-test) were done and P-value was recorded. P value ($p \leq 0.005$) showed the significant data.

3. Results and Discussions

Table 1. Color, reaction yield and elemental analysis of complexes

Complex	Empirical formula	Color	Yield (%)	Analysis: found (calculated)(%)				
				C	H	N	M	M.P. ^o C
Ligand 1	C ₃₄ H ₃₇ Cl ₂ N ₃ O ₁₃	Pale	80	53.27 (53.32)	4.86 (4.87)	9.25 (4.62)	--	35
Complex 1	C ₃₄ H ₃₅ BiCl ₅ N ₃ O ₂₅	white	70	32.13 (32.11)	2.61 (2.77)	3.31 (3.30)	16.43 (9.61)	65
Ligand 2	C ₃₂ H ₅₁ C ₁₂ N ₉ O ₁₇	White	80	32.01 (32.11)	2.75 (2.77)	3.36 (3.30)	---	31
Complex 2	C ₃₂ H ₄₉ BiCl ₅ N ₇ O ₂₉	white	75	32.01 (30.65)	2.75 (2.77)	3.36 (3.30)	16.45 (16.43)	56.5

3.1 Kinetics of thermal decomposition

Recently, there has been increasing interest in determining the rate- dependent parameters of solid-state non- isothermal decomposition reactions by analysis of TG curves [25, 26]. Thermogravimetric (TG) and differential thermogravimetric (DTA) analyses were carried out for different metal-tetracycline complexes in ambient conditions. The thermogravimetric analysis revealed that the complexes of cobalt & nickel loses mass between 65°C and 140°C, corresponding to nearly 13 % of the total mass, followed by considerable decomposition up to 600°C, which corresponds to the decomposition of the ligand molecule leaving metal oxide (Bi₂O₅) in both complexes as residue. On the basis of thermal decomposition, the kinetic analysis parameters such as activation energy (E*), enthalpy of activation (ΔH^*), entropy of activation (ΔS^*), free energy change of decomposition (ΔG^*) were evaluated graphically by employing the Coats – Redfern relation [27]

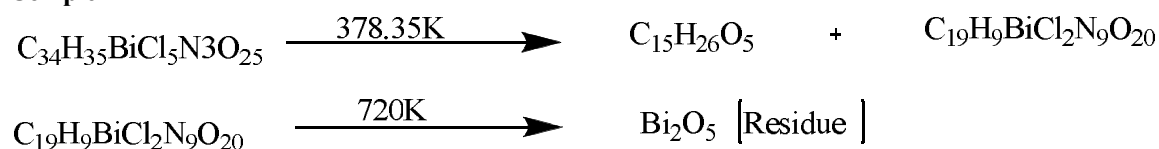
$$\text{Log} [-\text{Log} (1 - \alpha) / T^2] =$$

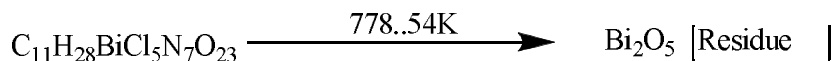
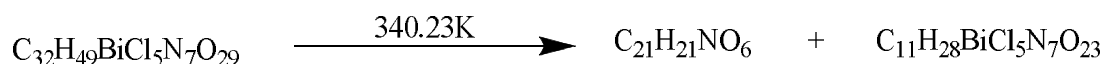
$$\text{log} [AR / \theta E^*(1-2RT/E^*)] - E^*/2.303RT \dots\dots(1)$$

Satisfactory results of elemental analysis (Table 1) and spectral studies revealed that the complexes were of good purity. Various attempts to obtain the single crystals have so far been unsuccessful. X-ray diffraction studies indicate crystalline nature of the metal complexes. The complexes were soluble in polar solvents water.

Where α is the mass loss up to the temperature T, R is the gas constant, E* is the activation energy in J mole⁻¹, θ is the linear heating rate and the term $(1-2RT/E^*) \cong 1$. A straight line plot of left hand side of the equation (1) against 1/T gives the value of E* while its intercept corresponds to A (Arrhenius constant). The Coats and Redfern linearization plots, confirms the first order kinetics for the decomposition process. The calculated values of thermodynamic activation parameters for the decomposition steps of the metal complexes are reported in table 3. According to the kinetic data obtained from the TG curves, the activation energy relates the thermal stability of the metal complexes. Among metal complexes, activation energy increases as complex III ~ complex II < complex IV < complex I, same trends happens with thermal stability of metal complexes. All the complexes have negative entropy, which indicates that the complexes are formed spontaneously. The negative value of entropy also indicates a more ordered activated state that may be possible through the chemisorptions of oxygen and other decomposition products. The negative values of the entropies of activation are compensated by the values of the enthalpies of activation, leading to almost the same values for the free energy of activation [28]. The plausible mechanism as follows for complex 1

Complex 1



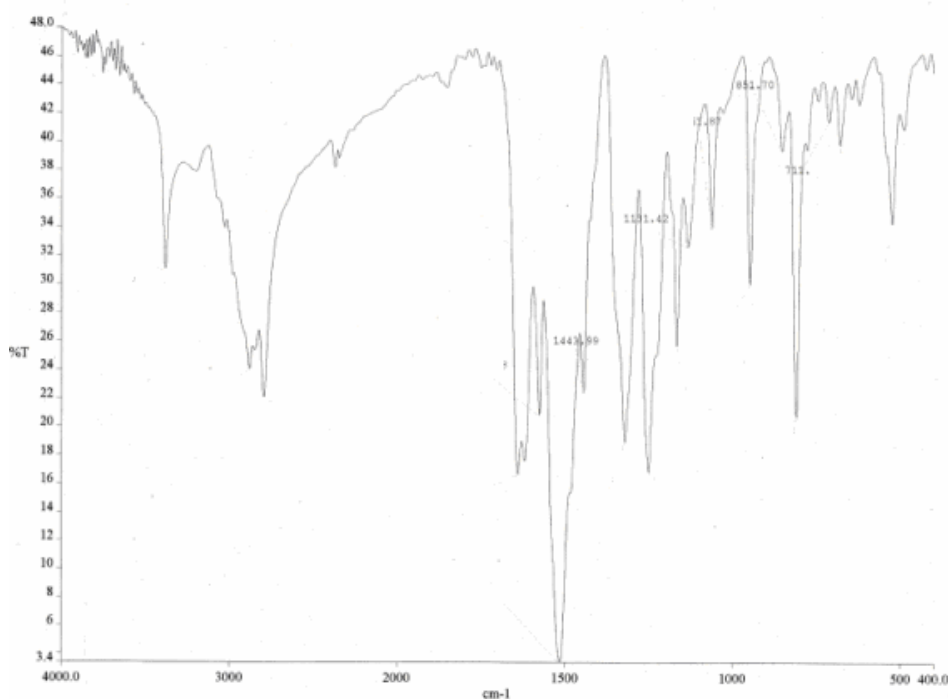
Complex2**Table 2. Thermodynamic activation parameters of the metal complexes**

Complex	Order/ n	Steps	E*/Jmol ⁻¹	A/sec ⁻¹	ΔS*/JK ⁻¹ mol ⁻¹	ΔH*/Jmol ⁻¹	ΔG*/kJmol ⁻¹	k×10 ⁴ m ⁻¹
C ₃₄ H ₃₅ BiCl ₅ N ₃ O ₂₅	1	I	28.83	4.24×10 ⁵	-12.312	55.13	42.16	9.953
		II	70.07	2.21×10 ⁵	-31.8..53	107.20	23.10	1.171
C ₃₂ H ₄₉ BiCl ₅ N ₇ O ₂₉	1	I	38.41	8.18×10 ⁵	-17..91	20.95	602.187	4.424
		II	93.49	5.16×10 ⁵	-34.69	27.89	27.566	1.626

3.2. IR spectra and mode of bonding

The IR spectra of free ligands and their complexes (shown in figure 3a, 3b, 3c and 3d) have been assigned in table 2. In both complexes ClO₄ ion appeared at 721, 1064, 962 cm⁻¹ which coordinate with Bi(V) ion

and in complex 2 922, 1035 and 724 cm⁻¹ and band at 1521 and 1624 cm⁻¹ appeared respectively suggesting that ligands occur through the oxygen atoms from hydroxyl groups coordinated. The M-O bonding appeared at 419 and 1340 cm⁻¹ and in complex 2 M—O at 919 cm⁻¹

**Figure 3a. IR spectra of ligand 1**

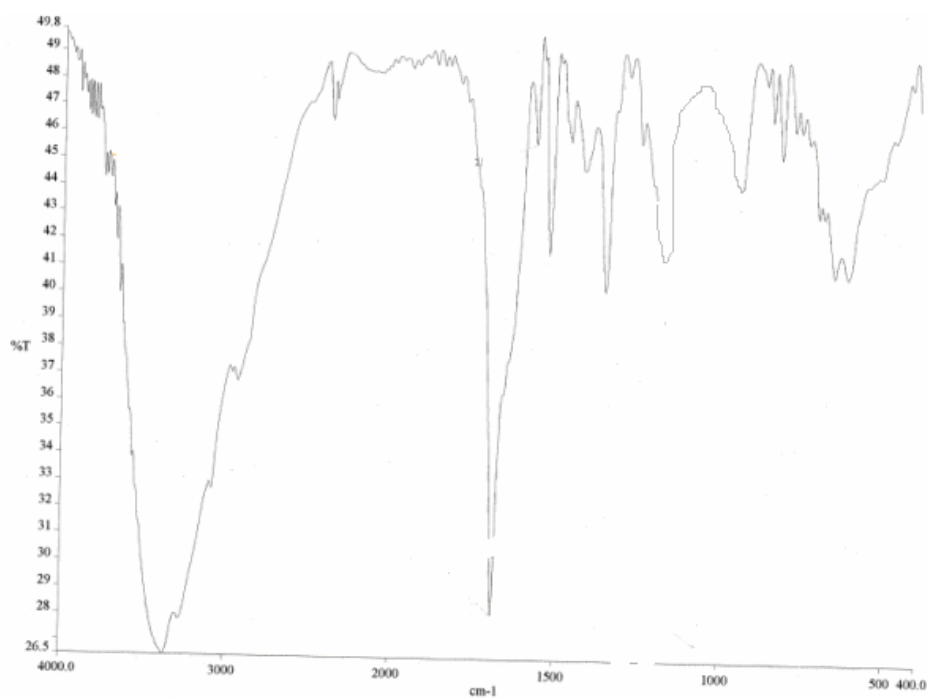


Figure3b. IR spectra o fligand2

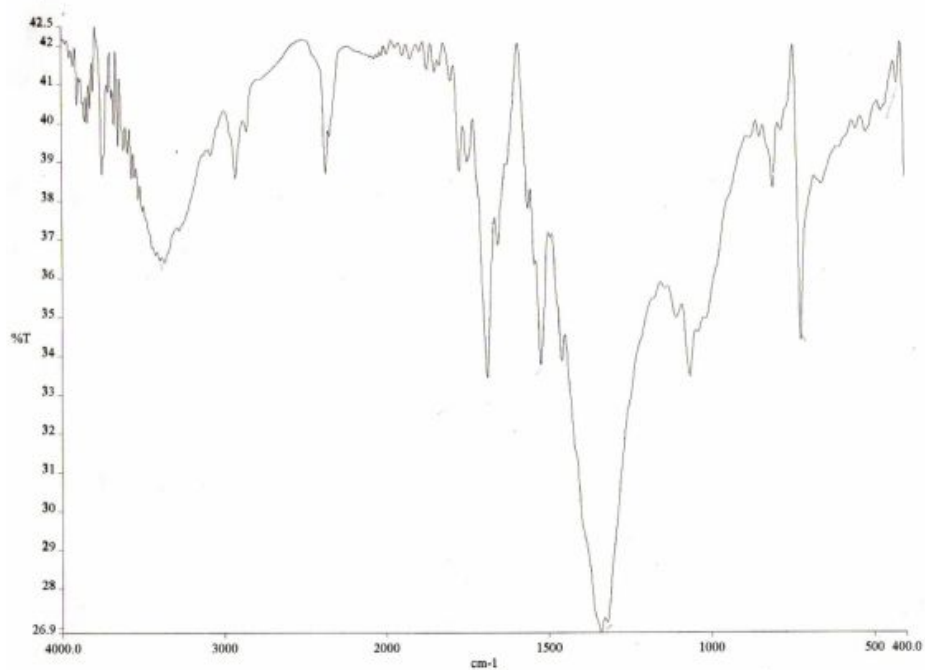


Figure3c. IR-spectra of complex1

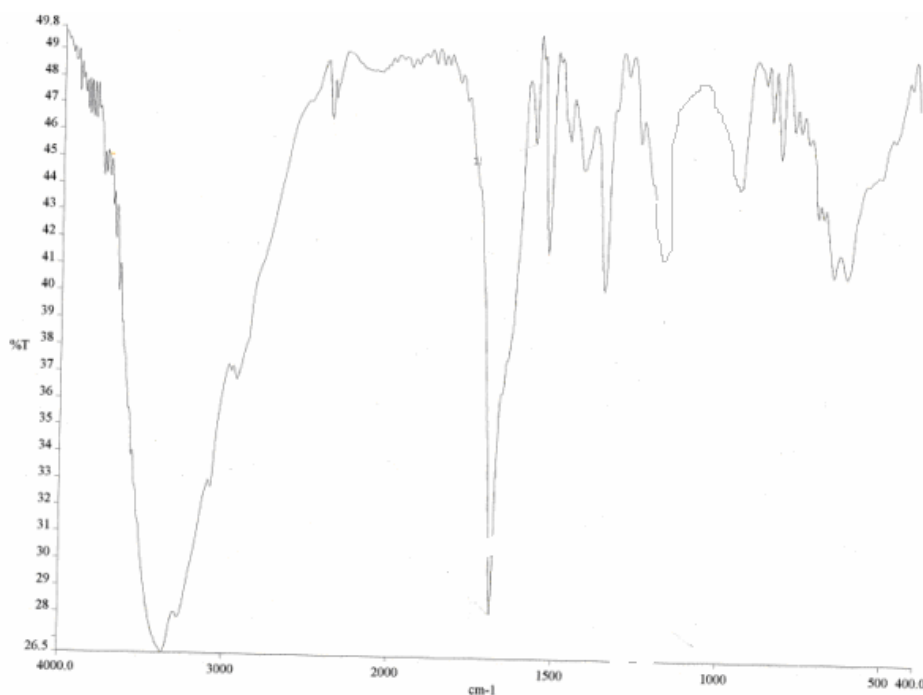


Figure 3d.I R spectra of complex 2

Table 2. IR spectral data (cm⁻¹) of the metal complexes

Frequency	$\nu_{\text{N-H}}$	OH	OH	NH ₂	NH ₂	M - O	M-ClO ₄
C ₃₄ H ₃₇ C ₁₂ N ₃ O ₁₃ Ligand 1	3381(s,b)	1639(m)	1514(s)	1222(m)	691(s)		
C ₃₄ H ₃₅ BiCl ₅ N ₃ O ₂₅ Complex 1	3329(s,b)	1635(m)	1521 (s)	1340(s)	695(m)	419(s),1 340	721,1064.962
C ₃₂ H ₅₁ C ₁₂ N ₉ O ₁₇ Ligand 2	3365(s,b)	1686(s)	1529(m)	1317(w)	613(m)	472(m)	
C ₃₂ H ₄₉ BiC ₁₅ N ₇ O ₂₉ Complex 2	3395(s,b)	1624(m)	1475(s)	1216(w)	627(s)	419(s)	922,1035,724

3.3. ¹H NMR spectra

The ¹H-NMR spectra of all ligands under study in D₂O which have been shown in figure 4a for ligand 1 and its complex in figure 4b. Table 3 summarises ¹H NMR data of the ligands and complexes. The peak positions as well as the integrated intensities of these compounds are consistent with the proposed structures for them. The protons of OH of ligand 1 at 5.35 and at 3.65 ppm

disappeared in the complex 1 which mean these proton are coordinated with Bi(V) in the ligands 2 the protons of OH appeared at 3.36ppm, 3.58 and 3.65 disappeared in complex 2 coordinated with Bi(V). the absence of the signal assigned to the OH protons of the both ligands confirms deprotonation and suggested the formation of a M-O bonds.

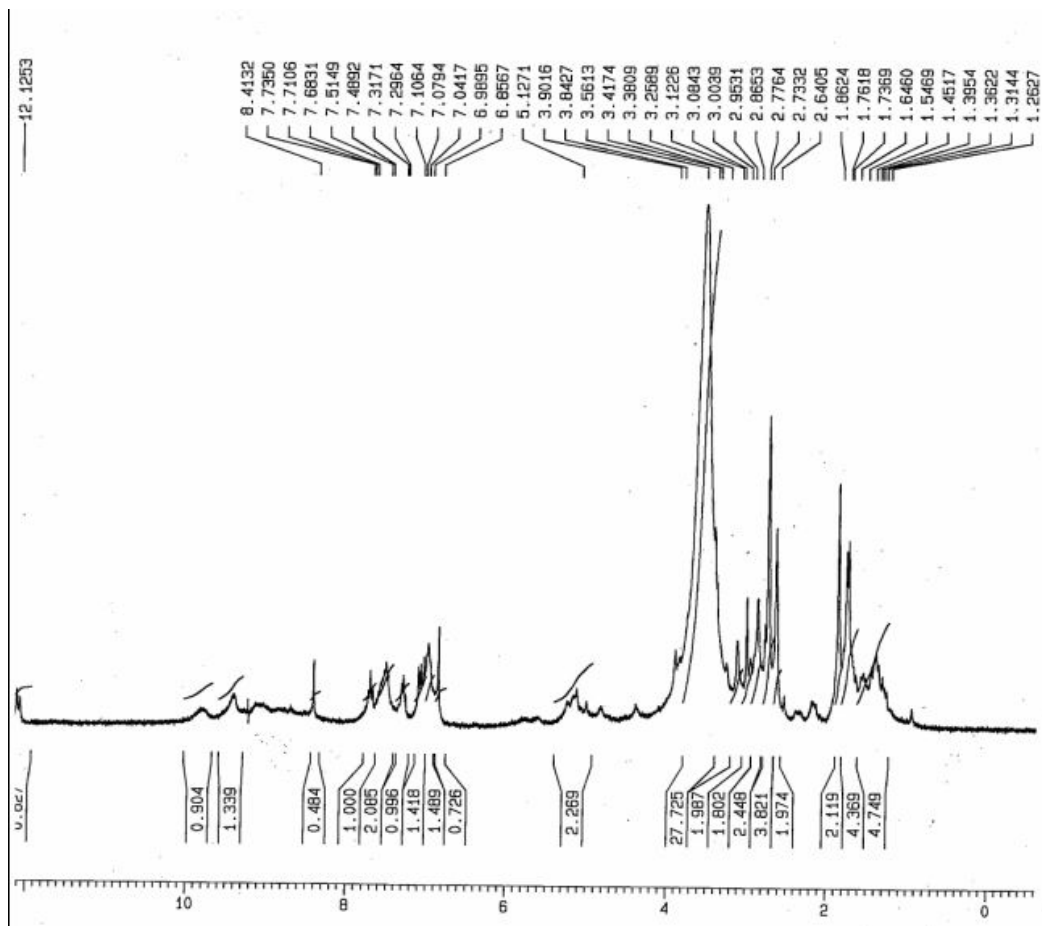


Figure 4a. ¹H N M R spectra of ligand1

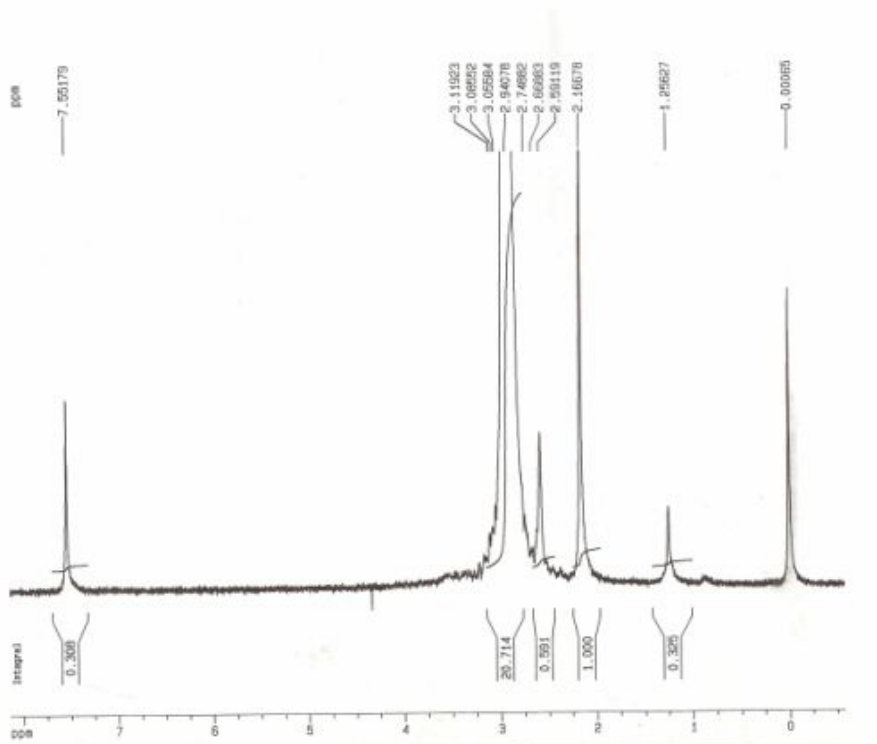


Figure.4b. ¹H N M R spectra of complex1

Table 3. H N M R data of free ligands and their complexes

Compounds	δ (ppm)
C ₃₄ H ₃₇ Cl ₂ N ₃ O ₁₃ Ligand 1	[3.35(s)1H,OH],16.77(s)1H,OH,16.77(s),1H,OH,3.65(m),5H,OH,2.0(s)1H,NH,7.16(s)1H,NH ₂ ,3.83(m)1H,CH,8.19(d)1H,CH(Ar),7.00(s)1H,CH(Ar),7.62(m)1H,CH(Ar),6.78(s)1H,CH(Ar),2.52---3.98(m)4H,(m)CH,3.98—4.73(d),2H,CH,1.35(s)3H,CH,1.06(s)6H,CH ₃].
C ₃₂ H ₅₁ Cl ₂ N ₉ O ₁₇ Ligand 2	[3.65(m),5H,OH],[3.58(s),4H,OH],[2.0(s)4H,NH],8.56(s)1H,NH ₂ ,]3.51—4.24(m)4H,CH],[5.03(d)1H,CH],3.76—3.81(m),6H CH],8.19(s)1H,CH]8.89(m)1H CH(Ar)],[7.62(s)1H,CH],[9.72(s)1H,CHO],4.73(m)1H CH,3.792H,CH ₂ ,3.17(m)6H,CH ₃ ,1.18(m)3H,CH ₃].

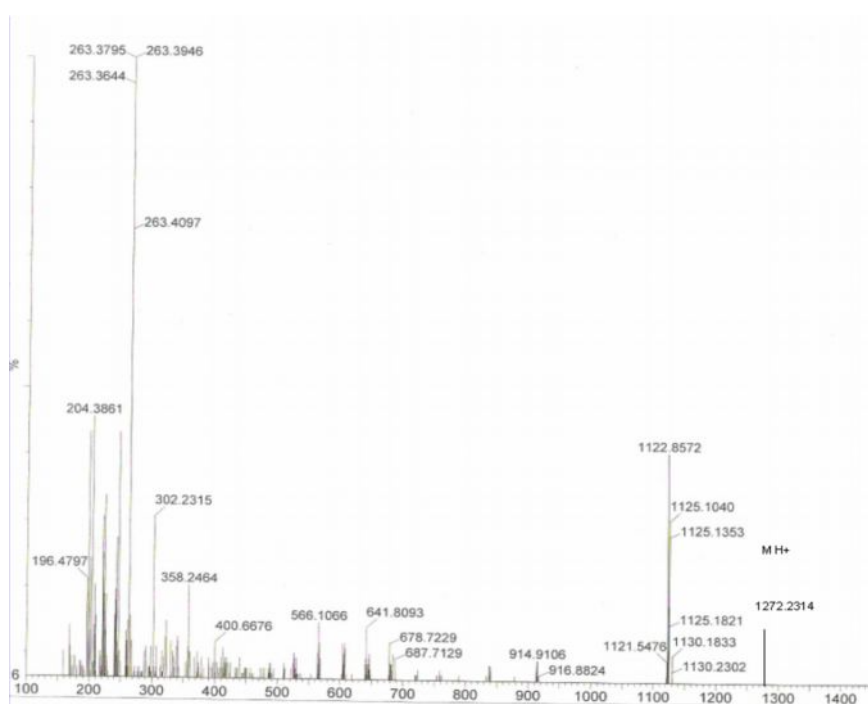
3.4. Electronic spectra measurements

The electronic absorption spectra of the free ligands in water solvent three confirming coordination of the ligands to metal, in addition to appearance of new bands maxima at 276nm,217nm and 208 nm in legand 1 which have their origin in the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transition within the organic ligand .The Bi(V) complex 1 show 345 nm and 295 nm in UV range due to charge transfer while in complex 2 367 nm,348 nm 277nm also strong absorption because of charge transfer and the high – energy band at 277nm is assigned to a charge-transfer, metal \rightarrow ligand or vice versa.

3.4. TOF–MS spectra

Mass spectrometry has been successfully used to investigate molecular species $[MH]^+$ in solution [17-25]. The molecular ion peaks of the ligands and complexes have been used to confirm the proposed formula (Table

4). The pattern of the mass spectrum gives an impression of the successive degradation of the target compound with the series of peaks corresponding to the various fragments. Their intensity gives an idea of stability of fragments. The ligand starts degradation and finally forms $[C_{23}H_{25}NO_7]^+$ e, 427/428(100 % m/z values. In the TOF–mass spectra of metal complexes initial fragmentation pattern is again similar (loss of two water molecules), a mononuclear nature for these complexes $[M(L)]^+$ can be deduced. The last two fragments appears in nearly all the complexes at positions (m/z values) 649(100% complex 1, 100 % complex 2, 100 % complex1 and 57 % complex (2) and 649/650 (10 % complex I, 50% complex 2 and 100 % complex 2 corresponds to $[C_{23}H_{42}Cl_2N_6O_{11}]^+$ and $[C_9H_{11}NO_4]^+$ respectively, which could be the result of degradation & demetallation of the complexes spectrum of complexes 2 with specific fragments..

Figure 5a. TOF-Mass spectra of complex 1

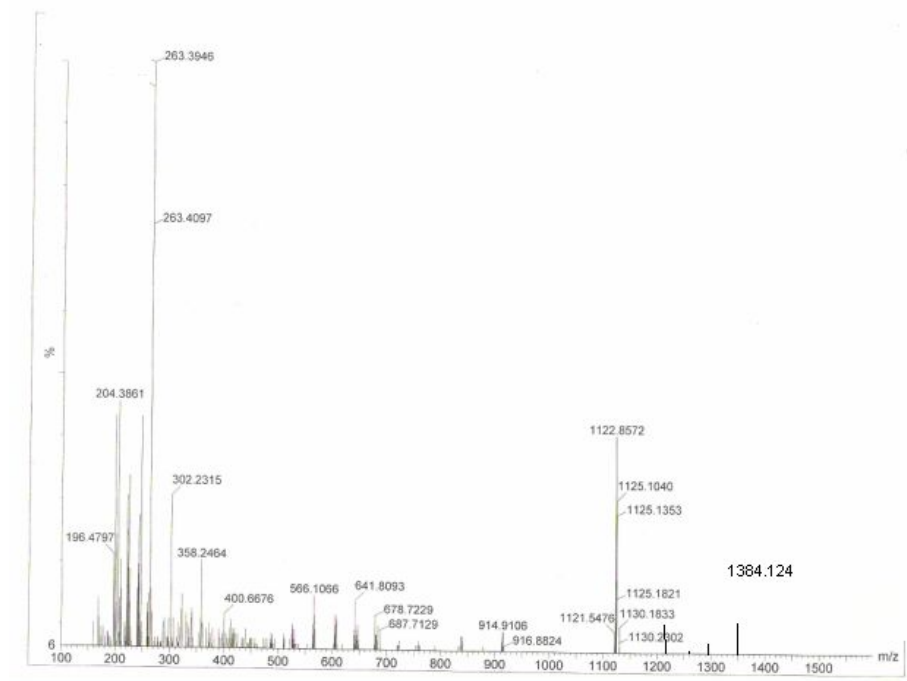


Figure 5b.TOF-Mass spectra complex 2

3.6. X-ray powder diffraction study

X-ray powder diffraction patterns of all ligands and their complexes were recorded between 9 and $80(2\theta)$. The value of (2θ) , interplanar spacing d (\AA) and the relative

intensities (I/I_0) of the compounds under study were recorded in table 4

Table 4. Crystallographic data for complexes

Compounds	Complex 1	Complex 2
Formula	$C_{34}H_{35}BiCl_5N_3O_{25}$	$C_{32}H_{49}BiCl_5N_7O_{29}$
FW	1271.89	1382.01
Temp (K)	293	293
Wavelength	1.54056	1.54056
Crystal System	Orthorhombic	Orthorhombic
Space group	P N M A	I M M A
Unit cell dimension		
a(\AA)	13.554540	22.094890
b(\AA)	9.496137	8.983012
c(\AA)	4.471746	8.483077
α	90.000	90.0000
β	90.000	90.000
γ	90.000	90.000
Volume (\AA^3)	575.58	1683.71
θ range ($^\circ$)	19.0-70.0	7.0-30.0
Limiting indices	$0 \leq h \leq 8$	$0 \leq h \leq 7$

	$0 \leq k \leq 3$	$0 \leq k \leq 2$
	$0 \leq l \leq 1$	$0 \leq l \leq 2$
Particle size(nm)	49.123	50.341
Intensity (%)	7.2–100	5.9–100
R indices	0.0000106	0.000131
Density	1.405	1.343
Z	1	2

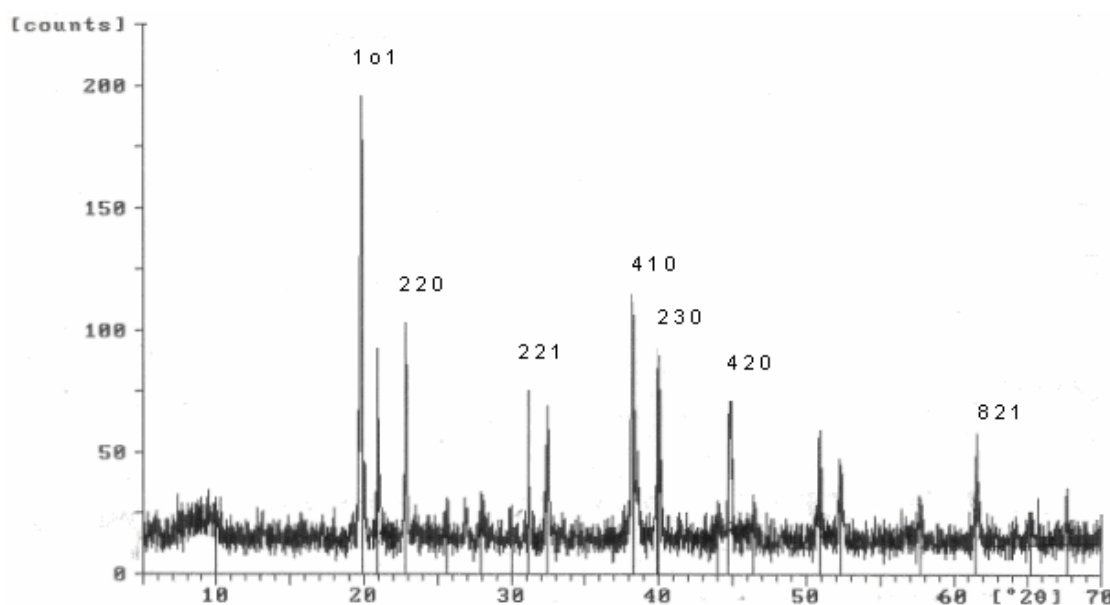


Figure 6.XRPD spectra of complex 1

3.7 Molecular modeling of complexes

To examine the structural properties, various traditional research techniques were used, but in this article, we were trying to assess observed data at molecular level with the help of molecular modeling. This modeling program was commonly known as computer assisted molecular design (CAMD). Molecular modeling had been successfully used to detect three dimensional arrangements of atoms in complexes Their utilization in the demonstration of molecular structure of the studied complex was presented in the article. Molecular

mechanics was a mathematical formalism, which attempted to reproduce molecular geometries, bond energies and other related features. Bond lengths, bond angles and atomic coordinates and their values were dependent on the hybridization of an atom and its bonding scheme [25-32]. So molecular models for Bi(V) complex was obtained by using the commercial available molecular modeling software Chem Office Ultra-11. These reported statistics have a good correlation with figures 2 – 4 confirmed trigonabipyramidal structure of both complexes.

Table 5. Data for selected bond lengths and bond angles of complex 1 and complex 2

Bond length A°		Bond angle°	
Complex1		Complex(1)	
O(60)-Bi(54)	2.1000	O(60)-Bi(54)	2.1000
Bi(54)-O(55)	2.1000	Bi(54)-O(55)	2.1000
Bi(54)-O(65)	2.1000	Bi(54)-O(65)	2.1000
O(28)-Bi(54)	2.1000	O(28)-Bi(54)	2.1000
O(46)-Bi(54)	2.0979	O(46)-Bi(54)	2.0979
		Bi(54)-O(65)-Cl(66)	120.0000
		Bi(54)-O(60)-Cl(61)	120.0000
		Bi(54)-O(55)-Cl(56)	120.0000
		O(60)-Bi(54)-O(55)	109.1483
		O(60)-Bi(54)-O(65)	115.5429
		O(60)-Bi(54)-O(28)	31.0659
		O(60)-Bi(54)-O(46)	111.0579
		O(55)-Bi(54)-O(65)	45.9776
		O(55)-Bi(54)-O(28)	95.1614
		O(55)-Bi(54)-O(46)	135.3548
		O(65)-Bi(54)-O(28)	125.2133
		O(65)-Bi(54)-O(46)	125.2133
		O(28)-Bi(54)-O(46)	109.5734
		Bi(54)-O(46)-C(44)	109.502
		Complex(2)	
		Bi(59)-O(60)-Cl(61)	120.0000
		O(65)-Bi(59)-O(70)	5.2237
		O(65)-Bi(59)-O(60)	122.4479
		O(65)-Bi(59)-O(13)	92.7153
		O(65)-Bi(59)-O(43)	48.3416
		O(70)-Bi(59)-O(60)	119.0320
		O(70)-Bi(59)-O(13)	92.2568
		O(70)-Bi(59)-O(43)	53.2914
		O(60)-Bi(59)-O(13)	125.1194
		O(60)-Bi(59)-O(43)	125.1194
		O(13)-Bi(59)-O(43)	109.7612
Complex2			
Bi(59)-O(65)	2.1000		
Bi(59)-O(70)	2.1000		
Bi(59)-O(60)	2.1000		
O(13)-Bi(59)	2.1017		
O(43)-Bi(59)	2.1000		

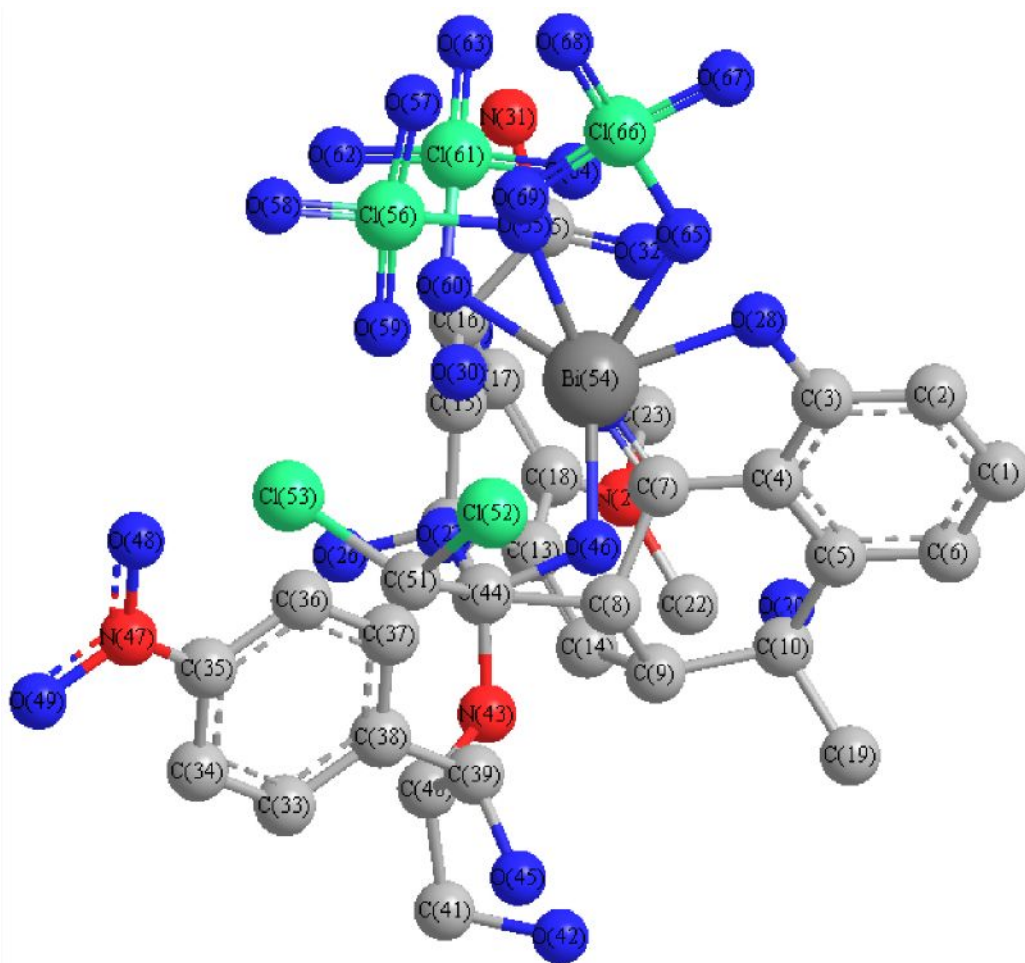


Figure7a. Optimised structure of complex 1.

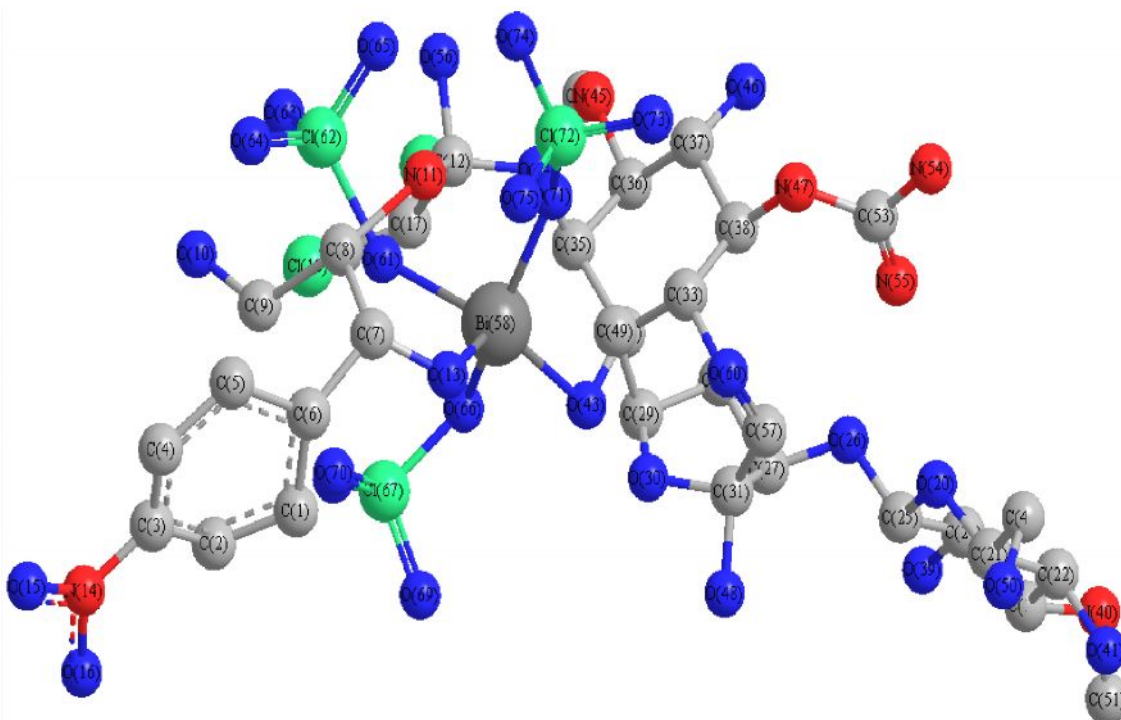


Figure7b. Optimised structure of complex 2

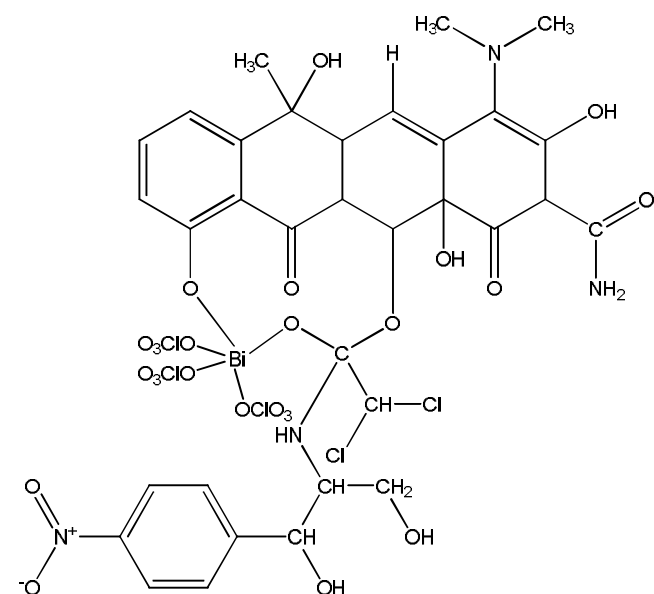


Figure8a. Graphical structure of complex 1.

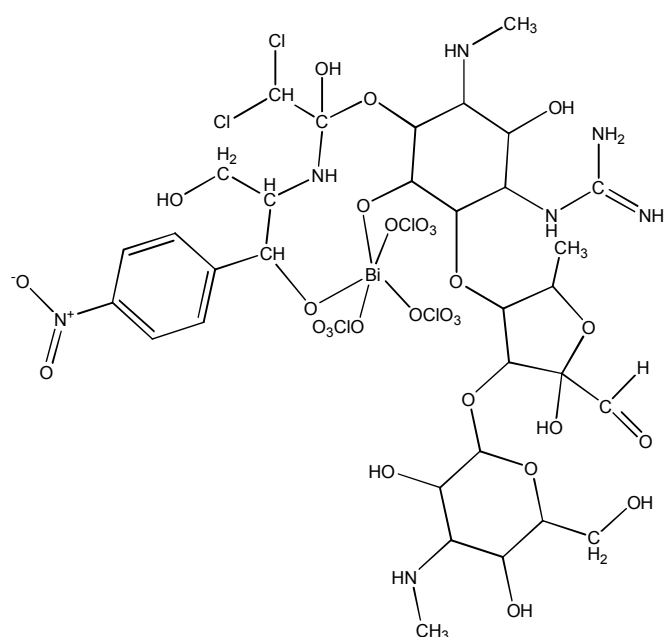


Figure8b. Graphical structure of complex 2

3.7. Antibacterial sensitivity

The antibacterial sensitivity assay shows that there is increase of inhibitory potentials of antibiotic ligand1 and ligand2 by the formation of complex with metal. The bacterial strain sensitive to ligand1 and ligand 2 easily

grow in the presence of 200 $\mu\text{g/mL}$ of complex1and complex2. the antibacterial activity increase in the complexes with bismuth. The results have been shown in (Table-6, figure9).

Table6. Antibacterial sensitivity assay of ligand1, ligand2 and its metal complexes on *Agrobacterium sp* BN-2A

Concentration ($\mu\text{g/mL}$)	BII of antibacterial Streptomycin-metal complexes [#]			
	Ligand1	Ligand2	Complex1	Complex2
Control (-)	0 \pm 0 (0)	0 \pm 0 (0)	0 \pm 0 (0)	0 \pm 0 (0)
25	0.8 \pm 0.047 (0.2)	0.5 \pm 0.05 (0.2)	0.9 \pm 0.01 (0.2)	0.842 \pm 0.04 (0.2)
50	1.2 \pm 0.082 (0.002)	1.3 \pm 0.01 (0.032)	1.543 \pm 0.023 (0.001)	2.43 \pm 0.021 (0.032)
100	1.6 \pm 0.163 (0.003)	1.87 \pm 0.0654 (0)	2.32 \pm 0.01 (0.021)	3.541 \pm 0.02 (0.21)
150	2.2 \pm 0.245 (0.004)	3.6 \pm 0.032 (0.332)	3.2 \pm 0.067 (0.01)	2.35 \pm 0.021 (0.021)
200	4.2 \pm 0.245 (0.001)	1.0 \pm 0.082 (0.169)	2.121 \pm 0.0654 (0.001)	3.321 \pm 0.02 (0.0012)

Values shown is the average \pm standard deviation of three readings performed three times.

[#]value in the parenthesis indicates p-value of the data based on t-test ($p \leq 0.005$).

The antibacterial sensitivity assay shows that there is reduction of inhibitory potentials of antibiotic ligand1 and ligand2 by the formation of complex with metal. The bacterial strain sensitive to ligand1 and ligand 2 easily grow in the presence of 200 $\mu\text{g/mL}$ of complex1and complex2. the antibacterial activity increase in the complexes with bismuth. The results have been shown in (Table-6, figure9).

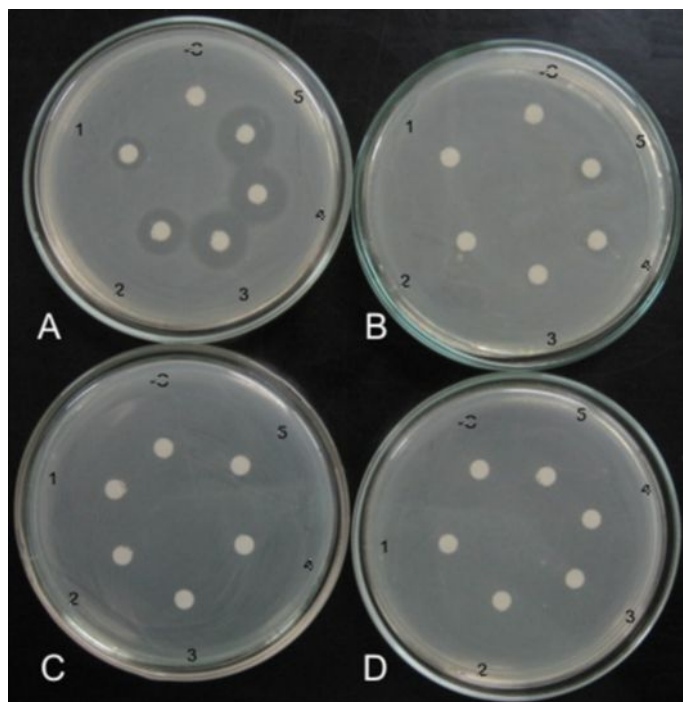


Figure9. Antibacterial sensitivity assay of metal added antibiotics ligand 1 and ligand 2 on *Agrobacterium* sp BN-2A. A. ligand 1 B. ligand2 C. complex 1, D. complex2 (-C, 0.0 µg/mL; 1, 25 µg/mL; 2, 50 µg/mL; 3, 100 µg/mL; 4, 150 µg/mL; 5, 200 µg/mL concentration in ligand1, ligand2 and metal complexes of ligands).

4. Conclusion

This asymmetric ligand has several potential N/O atoms with different reactivity and electron density. Our main interest was to understand the O/N bonding with

Bi(V) in the ligand system. The ligand moiety has different electronegativities, which influences the binding abilities of the ligand. Several spectroscopic techniques were used to understand the stabilities factors and coordination properties of mix ligand. By using of

spectroscopic techniques, an understanding of the stability and coordination ability of Bi(V) was determined. The coordination behaviors of the anions are variable due to different coordinating sites of the ligand. It is quite understandable that the metal ions are five coordinate with different atoms as well as anions. All the details about the studied complexes are given in figures 2, 4 and 5. Observed data suggested five coordinate configurations around the metal. The antibacterial sensitivity increase with the complexation.

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