



Synthesis, Characterization, Antimicrobial/Antitumor Activity of Binary and Ternary Neodymium (III) Complex with 2,2'-((1E,1'E)-(ethane-1,2-diylbis(azaneylylidene)) bis(methaneylylidene)) diphenol and Imidazole



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Abstract

Two new Neodymium(III) complexes with ligand, 2,2'-((1E,1'E)-(ethane-1,2-diylbis(azaneylylidene)) bis(methaneylylidene)) diphenol (**H₂L**) [**Nd(L)₂Cl₂Cl.H₂O** (**C₁**), and **H₂L** mixed with imidazole (**I**) [**Nd(L)₂(I)₂Cl₃.H₂O** (**C₂**) were prepared and characterized with physicochemical methods were used to clarify the structural makeup of the new compounds. [**Nd(L)₂Cl₂Cl.H₂O**] and [**Nd(L)₂(I)₂Cl₃.H₂O**] were effective antimicrobials, with larger inhibition zones than the reference antibiotic. The antimicrobial efficacy follows the order: gentamicin > [**Nd(L)₂(I)₂Cl₃.H₂O**] > [**Nd(L)₂Cl₂Cl.H₂O**] > **H₂L**. The cytotoxicity of ligand, binary and ternary complexes against human liver (Hep-G2) and breast (MDA-MB231) carcinoma cell lines was determined by using cisplatin as reference, the complex of [**Nd(L)₂(I)₂Cl₃.H₂O**] exhibited the highest activity against two cell lines.

Keywords: Complex, Imidazole, Neodymium, Antimicrobial, Cytotoxicity

Introduction

The complexes of lanthanide (III) ions is expanding quickly, due to the importance of these compounds in fundamental and practical research in numerous fields of science, including chemistry, material science, and life science [1-3]. Due to their distinctive structures and prospective uses in advanced materials including Lndoped semiconductors [4], magnetic [5], catalytic [6], fluorescent [7], and nonlinear optical materials [8], lanthanide coordination compounds are the focus of significant research, furthermore, due to their straight forward preparation process and diverse structural makeup of schiff base complexes their play crucial roles in the main group and transition-metal coordination chemistry [9, 10]. Also numerous imidazoles, including azomycine, clotrimazole, miconazole, ergothioneine, clonidine, and oxendine, have been synthesised as pharmacological agents. The imidazole nucleus is a significant synthetic motif in drug development, which are used in numerous antifungal medicines, have grown to be significant, antibacterial [11-14], medicines that are anti-inflammatory [15], analgesic [16], antitubercular, antidepressant [17],

antiprotozoal, and antihypertensive. Different kinds of metal complexes have been created recently in an effort to learn more about their potential to harm cancer cells as well as to prevent the growth of germs and fungi. Mixed ligand complexes are particularly desirable candidates for these projects because of the combination of pharmacological effects they provide, including DNA cleavage and antibacterial effects. Numerous studies of mixed ligand complexes with nitrogen-containing heterocyclic amines and Schiff base ligands have recently been conducted. The structure of nitrogen donors like imidazole contains heteroaromatic groups, which provide these ligands additional characteristics and pharmacological and therapeutic activities [18, 19]. In keeping with our ongoing fascination with Schiff base ligands and the characteristics/uses of their transition metal complexes [20, 21]. The purpose of this paper is to prepare and characterization of neodymium (III) complexes with 2,2'-((1E,1'E)-(ethane-1,2-diylbis(azaneylylidene)) bis(methaneylylidene)) diphenol (**H₂L**), In addition to studying the effect of imidazole on the interaction to form ternary complex. Also study the activation of binary and ternary complex against the different strain

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of microbial and the Hep-G2 liver carcinoma and MDA-MB231 breast cancer carcinoma cell lines.

Experimental

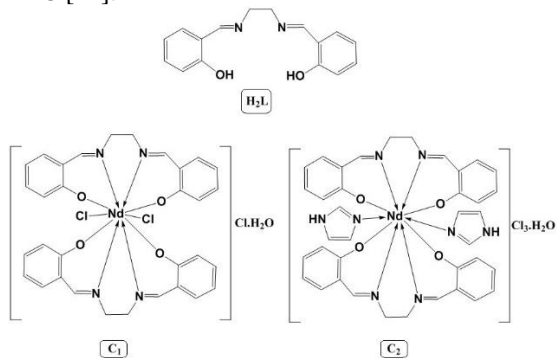
Detailed information on the chemicals, equipment, and procedures used for structural confirmation and application is included in the supplemental file (Section S1).

Synthesis of Ligand

In a round-bottomed flask, ethylenediamine (0.601 g, 0.01 mol) and salicylaldehyde (2.44 g, 0.02 mol) were mixed in absolute ethanol (50 mL) and refluxed for 60 min. The reaction mixture was concentrated to half of its volume, whereupon a yellow precipitate was formed. This was collected by filtration, washed with ethanol, and dried at laboratory temperature [22, 23].

Synthesis of two Neodymium(III) complexes

Binary and ternary complexes were obtained according to the following general procedure (Structures 1). The H₂L ligand (0.268 g, 1 mmol) was dissolved in ethanol (10 mL) and added to the metal salt (1 mmol; = 0.358 g, Nd(III)) in aqueous ethanol (10 mL). The reaction mixture was refluxed for 2 h at 70°C with constant stirring to produce binary complex, while to obtain ternary complex added 2 mmol of imidazole = 0.136 g, the resultant solution in the binary and ternary were let cool with solvent evaporation; the solid precipitate was filtered from the reaction mixture, thoroughly washed with ethanol to remove any traces of starting materials and dried under vacuum. The purity of the complexes was checked by TLC [24].



Structures 1. Suggested structures of ligand, binary and ternary neodymium (III) complexes

Antimicrobial potency

The biological potencies of the ligand, binary and ternary neodymium (III) complexes were evaluated against two Gram-positive bacteria (*Bacillus subtilis* (+ve) and *Staphylococcus aureus* (+ve)), two Gram-negative bacteria (*Escherichia coli* (-ve) and *Proteus vulgaris* (-ve)), and two fungi (*Candida albicans* and

Aspergillus flavus) using the agar diffusion test [25] (Section S2)

MTT assay for anticancer activity

The cytotoxicity of the synthesized ligand, binary and ternary neodymium (III) complexes against liver (Hep-G2) and breast (MDA-MB231) cancer cell lines were examined by MTT assay [MTT: 3(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] [23]. The cell suspension was diluted with complete medium to a concentration of 5×10^4 cell mL⁻¹, (Section S3).

Results and discussion

Characterization of ligand and Nd(III) complexes

Physicochemical properties

All metal chelates are colored and stable towards air and moisture [26]. The analytical results for the complexes are consistent with the proposed molecular formulas and confirm the formation of 1:2 (M:L) binary and ternary 1:2:2 (M:L:I) complexes [27]. The molar conductance values for the complexes in 10⁻³ M DMF solution are in the range 69.12 and 111.47 Ω⁻¹cm²mol⁻¹ for the binary and ternary Nd(III) complexes, respectively. The values of complexes reveal their electrolytic nature.

¹H and ¹³C NMR spectra of ligand

Figure S1 show the ¹H NMR spectra for H₂L ligand, where the multiplet signals in the 3.87 ppm region in the ligand spectrum may be assigned to the four protons of the ethane bridge (CH₂CH₂); The multiplet signals in the 6.69–7.37 ppm region in the ligand spectrum are assigned to aromatic protons; [28, 29]. The singlet signal observed at 8.54 ppm in the spectrum is assigned to the two imino protons. The singlet signal observed at 13.3 ppm in the ¹H NMR spectrum of ligand, which corresponds to the phenolic proton. Figure S2 shows the ¹³C NMR spectra for H₂L ligand [22].

IR spectra of ligand, and neodymium (III) complexes The FTIR spectra of H₂L, and Nd (III) complexes are depicted in table 2 and figure 1, 2, S3. The H₂L spectrum shows a band at 1608 cm⁻¹ which corresponds to the –C=N stretching vibration. On complexation, this band is shifted to a higher frequency for the binary and ternary complex (1642 and 1632 cm⁻¹), respectively. The red-shifts are evidence of the participation of the azomethine nitrogen atoms in complex formation. [30, 31]

H₂L ligand also presented a band at 1247 cm⁻¹, which was assigned to the ν(C–O) stretching vibration. The coordination of the phenolic oxygen atom was also confirmed by the red shift (1242–1241 cm⁻¹) of the ν(C–O) peak upon metal complex formation. This was supported by the appearance of bands at 531, 528 cm⁻¹ and bands at 453, 438 cm⁻¹ corresponding to the stretching vibrations of the M–O and M–N bonds [32-

34] for the binary and ternary Nd(III) complex. The appearance of a new band in ternary Nd(III) complex at 3131 cm^{-1} is due to the NH of imidazole. All the prepared metal chelates display broad 3316 and 3371

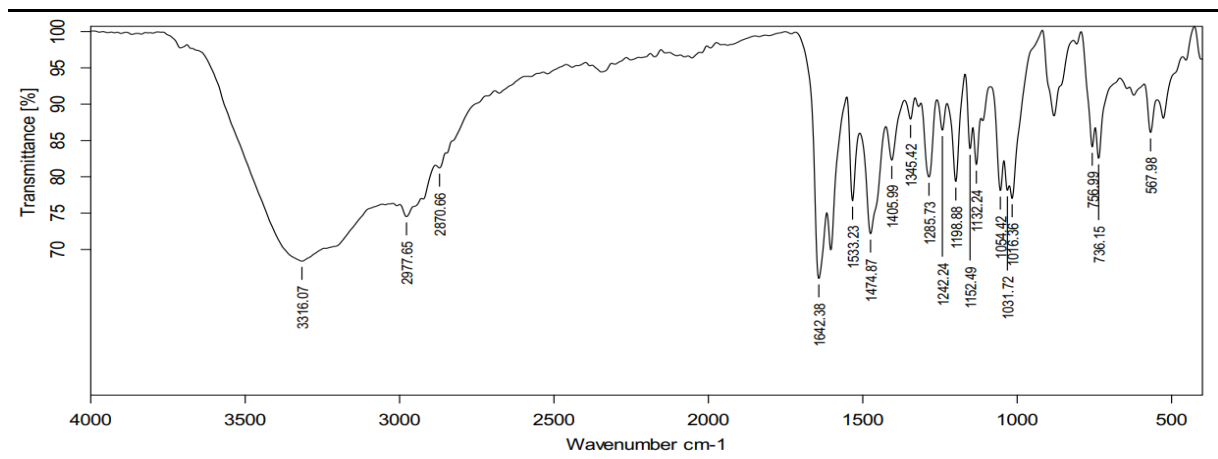
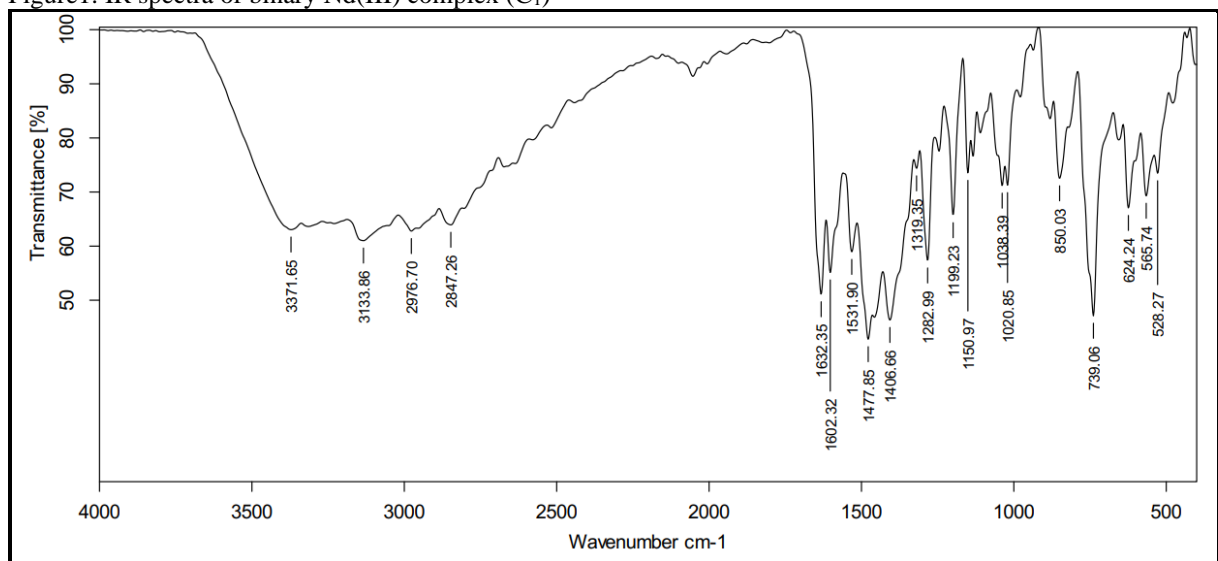
cm^{-1} , which are interpreted as the $\nu_{(\text{OH})}$ stretching vibrations of hydrated water molecules, in agreement with the elemental analysis and ^1H NMR results.

Table 1. Analytical data of ligand and Nd(III) complexes.

No.	Compounds Molecular Formula	Molecular Weight	Color Yield %	Conductivity μ s	M. P. $^{\circ}$ C	Found (cal.) %			
						C	H	N	M
	H ₂ L C ₁₆ H ₁₆ N ₂ O ₂	268.31	Yellow 95	0.83	127	71.32 (71.62)	5.95 (6.01)	10.28 (10.44)	-
	Imidazole C ₃ H ₄ N ₂	68.08	White	-	90	52.93	5.92	41.15	-
C ₁	[Nd(L) ₂ Cl ₂]Cl.H ₂ O C ₃₂ H ₃₀ Cl ₃ N ₄ NdO ₅	801.21	dark yellow	69.12	261	47.91 (47.97)	3.74(3.77)	6.89(6.99)	18.00
C ₂	[Nd(L) ₂ (I) ₂]Cl ₃ .H ₂ O C ₃₈ H ₃₈ Cl ₃ N ₈ NdO ₅	937.37	Pale yellow	111.47	273	48.63(48.69)	3.99 (4.09)	11.94(11.95)	15.39

Table 2. FTIR spectral data in cm⁻¹ for H₂L ligand and binary and ternary Nd (III) complexes.

Compound	$\nu(\text{OH})$	$\nu(\text{NH})$	$\nu(\text{CH})_{\text{arom}}$	$\nu(\text{CH})_{\text{aliph}}$	$\nu(\text{C}=\text{N})$	$\nu(\text{C}-\text{O})$	$\nu(\text{M}-\text{O})$	$\nu(\text{M}-\text{N})$
H ₂ L	3292	-	3049–3007	2899–2867	1608	1247	-	-
C ₁	3316	-	2977	2870	1642	1242	531	453
C ₂	3371	3133	2976	2847	1632	1241	528	438

Figure1. IR spectra of binary Nd(III) complex (C₁)Figure2. IR spectra of ternary Nd(III) complex (C₂)

Electronic spectra

The UV–Vis spectra of ligand, and neodymium (III) complexes were measured at room temperature in the region of 200–700 nm in DMSO. The absorption spectrum of H₂L shows three absorption bands. The first high intensity band at $\lambda_{\text{max}} = 280$ nm may be attributed to the $\pi \rightarrow \pi^*$ transition of the aromatic rings. The second and third absorption bands (at 320 and 409 nm) are attributed to the $n \rightarrow \pi^*$ transition of the azomethine group (C=N) and charge transfer, respectively.[35, 36] Compared to those of the free ligand, the electronic spectra of the binary and ternary Nd(III) complexes showed bands that were shifted to 273, 276 nm and 312, 314 nm for the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions, respectively, confirming the coordination of the azomethine nitrogen to the metal ions. The appearance band at 489, 504 nm in the Nd (III) complex corresponded to charge transfer from ligand to metal LMCT. Furthermore, absorption bands in the

visible region were considered to arise from d–d transitions [22].

ESI-MS spectra

MS has been increasingly used for the elucidation of ligand, and neodymium (III) complexes molecular structure. The ESI-MS spectra of the complexes are depicted in Figure 3 and S4, and the proposed fragmentation patterns for neodymium (III) complexes are shown in Scheme 1 and 2. The mass spectra of ligand, and neodymium (III) complexes display molecular ion peaks at m/z 269.07 amu for H₂L, m/z 802.21 and 937.82 amu for the respective binary and ternary Nd(III) complexes. These data are in good agreement with the proposed molecular formulas of ligand and Nd(III) complexes.

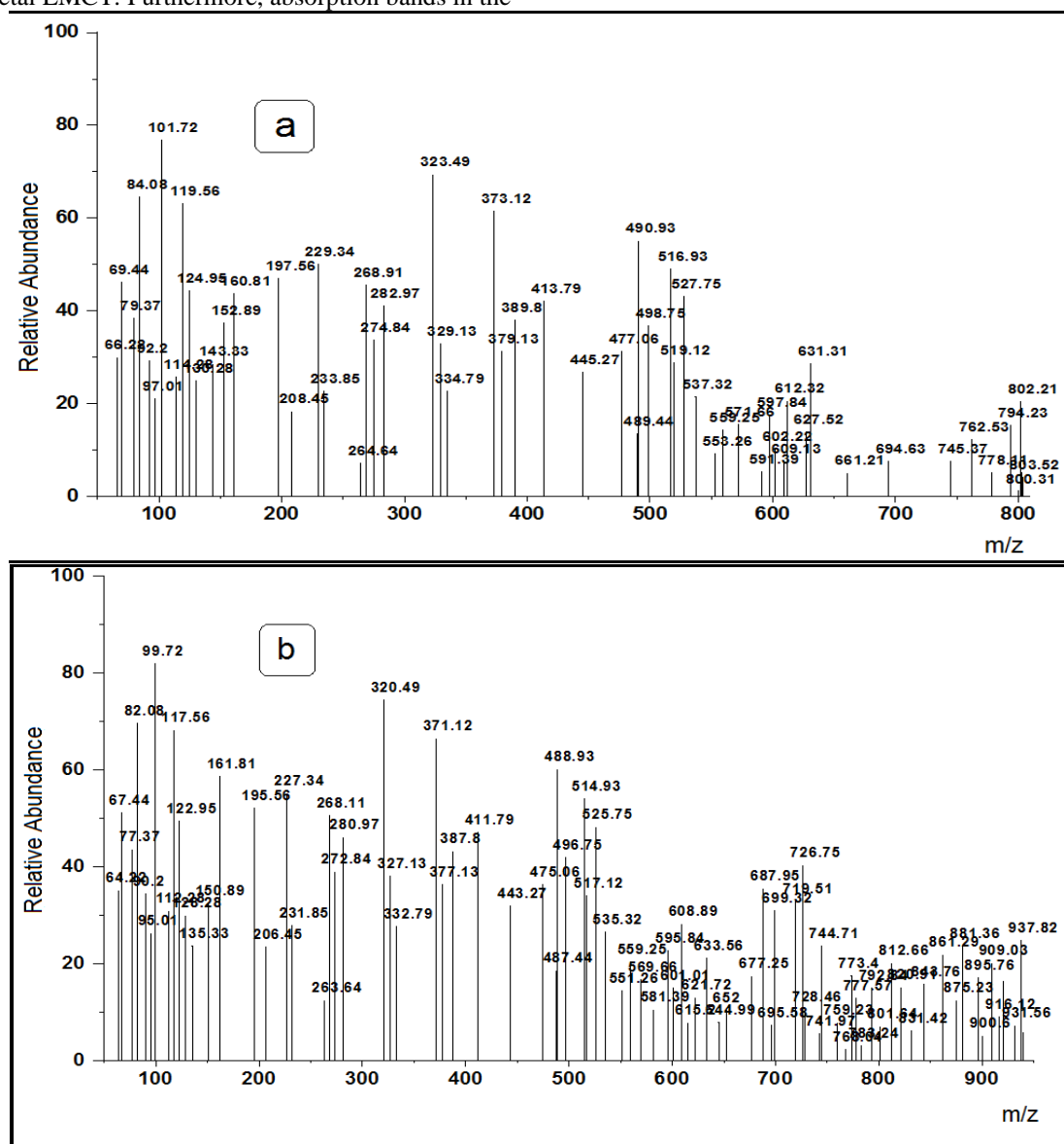
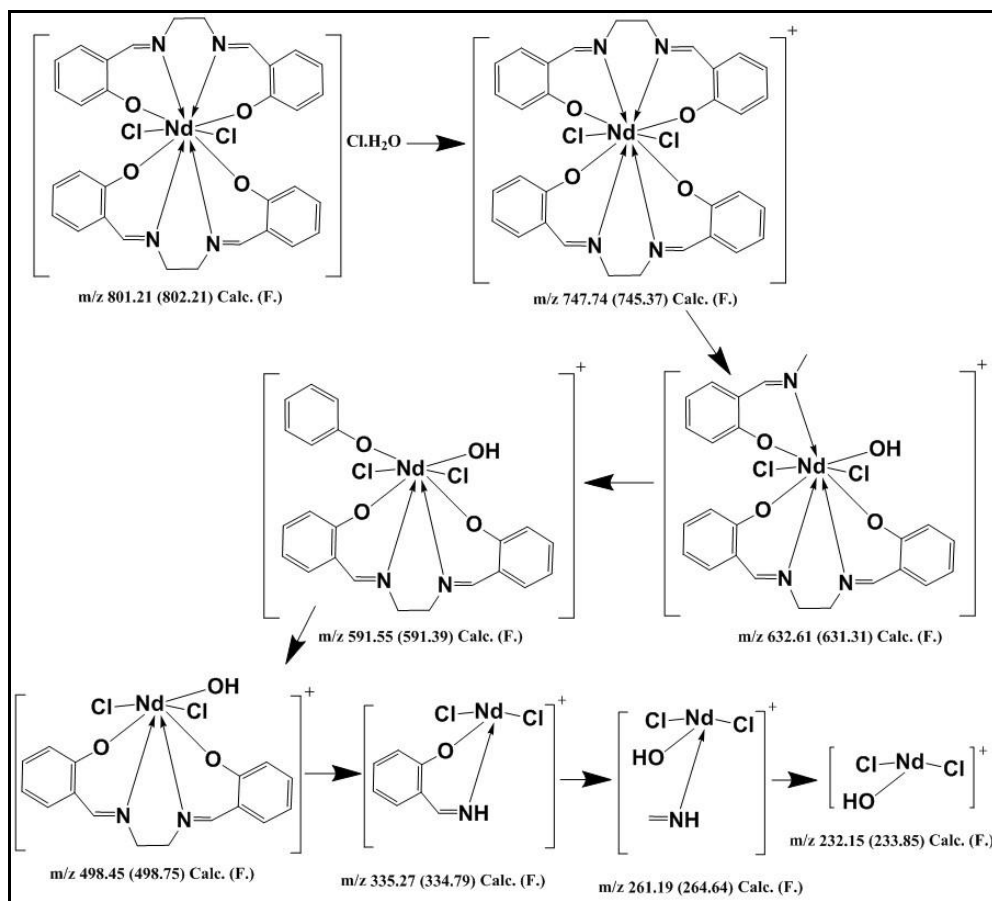
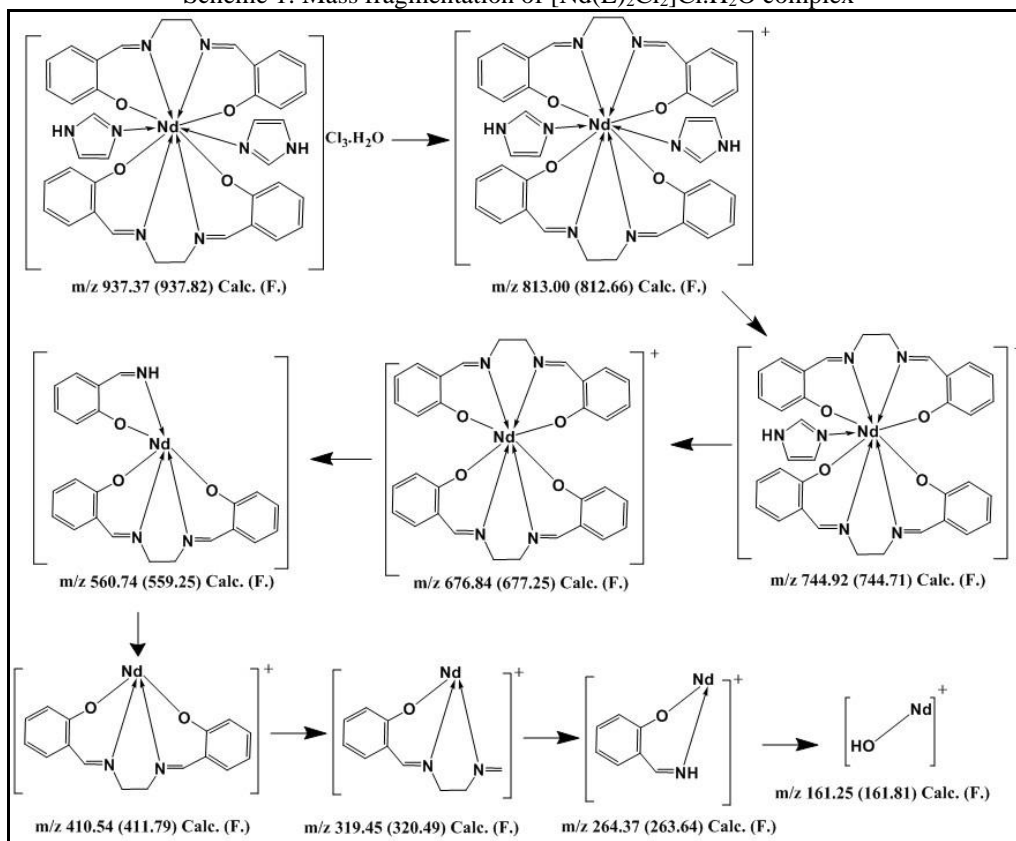


Figure 3. Mass spectra of the binary (a) and ternary (b) neodymium (III) complexes

Scheme 1: Mass fragmentation of $[\text{Nd}(\text{L})_2\text{Cl}_2]\text{Cl}\cdot\text{H}_2\text{O}$ complexScheme 2: Mass fragmentation of $[\text{Nd}(\text{L})_2(\text{I})_2]\text{Cl}_3\cdot\text{H}_2\text{O}$ complex

Thermogravimetric analysis

Thermogravimetric investigations were used to examine the thermal behavior of the binary and ternary neodymium (III) complexes in the temperature range of 25-800 °C. Table 3 and Figure 4 present the findings. The TG curves for the binary and ternary neodymium (III) complexes show four weight-loss events. The first decomposition step takes place between 25-93°C which is interpreted as the loss of the hydrated H_2O+HCl and H_2O which are accompanied by a weight loss of (Found/Calc. %; 6.61 (6.80) and 1.89 (1.92)). The second step occurs in the range 51-196 °C and corresponds to the losses of $C_{10}H_{10}N_2O$ and $C_{19}H_{14}Cl_3N_4O_2$ moieties with an estimated weight loss range of (Found/Calc. %; 21.52 (21.74) and 46.63 (46.59)). The third step occurs between 171-281 °C and are considered the loss of $C_6H_3Cl_2O$ and $C_{13}H_{17}N_4O$ moiety with an estimated mass reduction of (Found/Calc. %; 20.47 (20.21) and 26.19 (26.17)). The fourth step takes place at 241-461 °C with an estimated mass loss of (Found/Calc. %; 33.29 (33.24) and 9.96 (9.93)) and can be attributed to the loss of $C_{16}H_{14}N_2O_2$ and C_6H_5O from binary and ternary complex, respectively leaving neodymium metal as final residue.

Biological Activity

Antimicrobial bioassay

Practically, a number of designed pharmaceutical drugs have been found to be active against Gram-positive than Gram-negative bacteria [37]. In this study, H_2L and its binary and ternary neodymium (III) complexes were screened for their antibacterial activity against *E. coli* and *P. vulgaris* (Gram -ve bacteria) and *S. aureus* and *B. subtilis* (Gram +ve bacteria), as well as their antifungal activity against *C. albicans* and *A. flavus*, using the agar diffusion technique. Importantly, the obtained activities were compared with those of the standard drugs such as *gentamicin* (antibiotic) and *ketoconazole* (antifungal). The measured antibacterial and antifungal activities of ligand, binary and ternary neodymium (III) are listed in tables S1, S2 and Figure 5, S5. We observed the

activity of ligand against different strains of bacteria and fungi increased when chelate with neodymium (III) to form complexes, beside that the activity increased with addition of imidazole to first ligand where the activities of the follow the order: gentamicin, Ketoconazole > ternary complex > binary complex > H_2L . This enhancement in the antibacterial activity of complexes can be explained in terms of the partial sharing of the positive charge of the metal ion with the donor (N and O) atoms of ligand, leading to electron delocalization over the whole chelate ring system. Given that the geometrical structure of the complex, the type of the donor atoms, the metal ion, the overall charge on the complex ion, and the chelate action of the ligands all affect the biological activity of metal compounds [38].

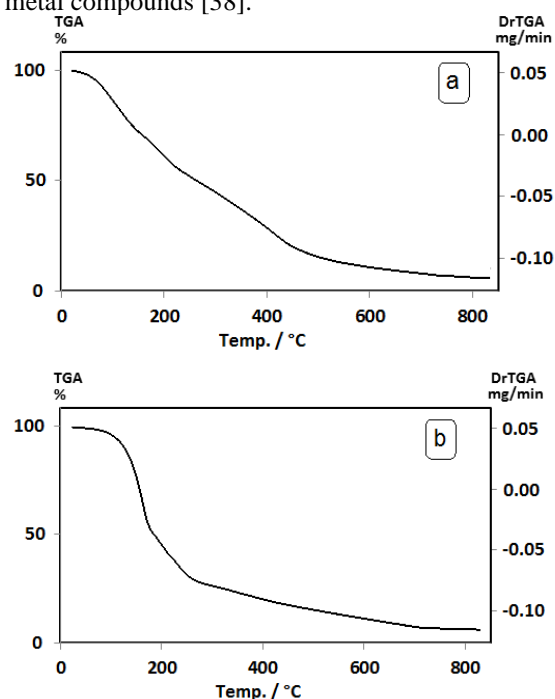


Figure 4. TGA/ DTG curves of the binary (a) and ternary (b) neodymium (III) complexes

Table 3. Thermal data of the binary and ternary neodymium (III) complexes

No	Compound	TAG(A)/°C	Wt. loss Found (Calc.) %	Leaving species	
C1	$C_{32}H_{30}Cl_3N_4NdO_5$	25-51	6.61 (6.80)	$HCl + H_2O$	Nd
		51-171	21.52 (21.74)	$C_{10}H_{10}N_2O$	
		171- 241	20.47 (20.21)	$C_6H_3Cl_2O$	
		241-443	33.29 (33.24)	$C_{16}H_{14}N_2O_2$	
Residue	> 443	18.09 (18.00)			
C2	$C_{38}H_{38}Cl_3N_8NdO_5$	25-93	1.89 (1.92)	H_2O	Nd
		93-196	46.63 (46.59)	$C_{19}H_{14}Cl_3N_4O_2$	
		196-281	26.19 (26.17)	$C_{13}H_{17}N_4O$	
		281-461	9.96 (9.93)	C_6H_5O	
Residue	>461	15.41 (15.39)	Nd		

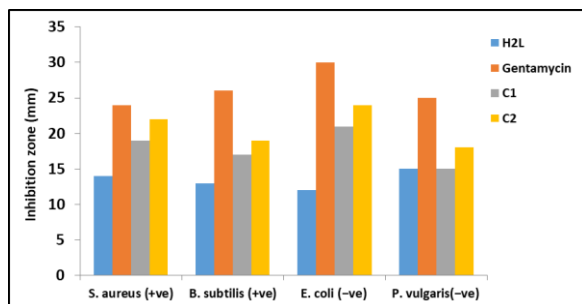


Figure 5: Graph showing antibacterial activity of ligand, binary and ternary neodymium (III) complex at concentration of 10 mg ml^{-1} against Gentamycin as reference drug

Cytotoxicity

The *in vitro* cytotoxicity of the binary and ternary neodymium (III) complex against human Hep-G2 liver and MDA-MB231 breast carcinoma cell lines was determined using the MTT assay, in which the mitochondrial dehydrogenase activity is measured as an indication of cell viability in terms of the optical density. In order to get the IC_{50} values for the investigated chemicals in both cancer cell lines, the absorbance values were assessed using non-linear regression techniques [22, 23]. The cytotoxicity results for ligand, binary and ternary neodymium (III) complex against HepG-2 and MDA-MB231 at concentrations of 0, 0.1, 1, 10, and 100 μM are shown in table S3 and figure 6. By comparison IC_{50} values for ligand, binary and ternary neodymium (III) complex at using cisplatin as standard reference, we found the activity of H_2L and neodymium (III) complex are follow the order: $H_2L > \text{cisplatin} > \text{ternary complex} > \text{binary complex}$ and $\text{cisplatin} > H_2L > \text{ternary complex} > \text{binary complex}$ against HepG-2 and MDA-MB23, respectively.

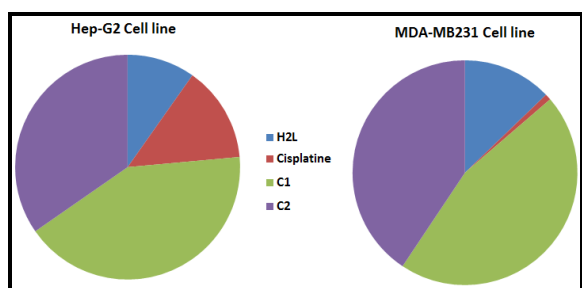


Figure 6. IC_{50} Values of ligand, binary and ternary neodymium (III) complex against Hep-G2 and MDA-MB23 carcinoma cell lines compared to cisplatin.

Conclusions

In this work, Two new binary and ternary Neodymium(III) complexes were synthesized and fully characterized using wide range of spectroscopic and structural tools. Their biological activities against different strain of microbial and human Hep-G2 liver and MDA-MB231 breast carcinoma cell lines were

evaluated. Where found that the activity of ternary Neodymium(III) complexes against different strain of bacteria and fungi is higher than the binary complex, also in the case of activity versus two cancer cell lines. Elemental analysis, molar conductivity, FTIR and thermal results showed that the binary complex has formed with a molar ratio (1:2) M:L with a general formula $[\text{Nd}(\text{L})_2\text{Cl}_2]\text{Cl}\cdot\text{H}_2\text{O}$, and ternary complex with a molar ratio (1:2:2) M:L:I having a general formula $[\text{Nd}(\text{L})_2(\text{I})_2]\text{Cl}_3\cdot\text{H}_2\text{O}$.

Conflicts of interest

There are no potential conflicts of interest.

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